THE DESIGN, CONSTRUCTION AND COMMISSION OF A FERMENTATION PLANT WITH GAS RECYCLE FOR THE PRODUCTION OF BIOMASS FROM NORTH SEA GAS AND SIMPLE NITROGENOUS MEDIUM.

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PART D: MATERIALS AND METHODS: COMMISSIONING EXPERIMENTS ON THE EQUIPMENT AND INSTRUMENTATION

SUMMARY

The purpose of this project was to design, build and commission a fermentation system for the production of biomass from north sea gas.

The need for unconventional scources of protein and the status of biomass in this context was assessed. The advantages of micro-organisms grown on different substrates as a scource of biomass was discussed with particular reference to the use of hydrocarbon substrates.

The extra cooling and oxygen requirements of hydrocarbon fermentations was noted and the influence of these factors on the overall process costs noted. The additional treatment of liquid and solid hydrocarbon grown biomass to remove potentially carcinogenic residues was compared unfavourably with biomass derived from gaseous hydrocarbons, where no such residues occur.

The historical background and present state of research into the microbiological aspects of natural gas fermentations was reported and especial reference made to the difficulty of isolating methane oxidisers in pure culture. The comparative success of mixed cultures of methane oxidisers grown in fermenters was considered as a significant feature of these organisms.

The rare use of gas recycle techniques in microbiological work was noted and the advantages of such a system in the reduction of substrate costs was discussed. The advantages of isolating methane oxidisers capable of growth at high temperatures (40^{o}C or over) was examined. It was noted that the higher costs of oxygen supply and cooling for natural gas fermentation may offset the cost advantage of using the cheapest widely available carbon substrate.

A fermentation plant was built comprising of two 5 litre fermenters used either as a multistage chemostat, or two single stage units, or as conventional batch fermenters, for the production of biomass from north sea gas at 40°C. The fermenters used were selected partially on the basis of an industrial standard of manufacture and were capable of high rates of gas transfer. This was considered essential to offset the relatively low solubilities of both oxygen and natural gas in water at the temperature used.

A gas recycle scheme was used and several control loops were incorporated so that the concentration of the gases in the circulating gas mixture was automatically kept constant. Since a closed cycle was in operation, only one of the two major constituents of the gas mixture - oxygen - was controlled on the basis of concentration changes. The other - methane - was controlled by means of pressure changes which occurred due to microbial growth on these gases. A special device was designed so that in the event of the ethane in natural gas not being consumed, any build up of the ethane was prevented by an automatically controlled purging arrangement. An integrator/photo cell device was used to record the total flow of gas through the ethane purge route.

Extensive use was made of recorders so that continuous traces of the various fermentation parameters could be compared. A new type of dissolved oxygen electrode employing a silicone nylon reinforced membrane was described.

At the high rates of agitation in the fermenters both vessels were completely full of a gas/liquid foam emulsion so that conventional volume control during continuous culture was impossible. A method of volume control was designed based on the differential measurement of current consumed by the fermenter stirrer motors at different volumes of liquid (i.e. at different foam densities). Changes in current consumption were detected by a photocell which operated flow inducers to remove culture fluid or deliver fresh medium.

The fermentation plant was situated in safety cubicles at a considerable distance from the nearest autoclave. Steam sterilisation of large quantities of liquid medium was therefore impractical and an automatic filtration rig was built which enabled automatic delivery of sterile medium to resevoirs in the vicinity of the fermenters. The transfer of medium from the resevoirs to the fermenters was automatically operated. Only bulk medium preparation was manual.

The explosive nature of methane/oxygen mixtures necessitated the consideration of safety features in detail. Several fail safe devices were incorporated into the plant and much use was made of intrinsically safe or flame proofed equipment.

A mixed culture of micro-organisms was isolated in the equipment described, which was able to oxidise both ethane and methane in batch culture at 40°C. The culture appeared to consist of a Gram$^-$ rod together with a Gram$^+$ rod and a Gram$^+$ yeast.

LIST OF ABBREVIATIONS USED IN THE TEXT

(Excluding those explained in the text)

F.A.O.	Food and Agriculture Organisation
S.C.P.	Single Cell Protein
CO_2	Carbon Dioxide
g.	Gram
^{o}C	Degrees Centigrade
O_2	Oxygen
%	Percentage
Hr (hr)	Hour
Btu	British thermol unit
Kwh	Killowatt hour
L	Litre
DOT	Dissolved Oxygen Tension
4_e	4 electrons
OH^-	Hydroxylions
H_2O	Water
H^+	Hydrogen ion
Cl^-	Chloride ion
Ag_2O	Silver oxide
AgCl	" chloride
FEP	Fluroethylene Propylene
PTFE	Polytetrafluroethylene
c.c.	cubic centimetre
V_A	Volumetric transfer rate of oxygen
H.E.	High Efficiency
V.H.E.	Ultra high efficiency
No	Number of organisms before filtration
N_1	" " after "
D	Dilution rate
u	Specific growth rate
Ks	Substrate concentration at $^1/_2$ u max.

P A R T (A) I N T R O D U C T I O N

P A G E S (1) - (27).

SECTION I.

(1) FOOD CRISIS IN GENERAL:

Much has been said about the subjects of increasing human populations, diminishing resources, widespread starvation and chronic malnutrition (Brown 1968). Yet no matter how familiar or distant and irrelevant the problems appear to an overfed Western world, no matter how often the statistics are quoted, the fact is inescapable that the world is becoming dangerously divided into those who can afford to be gluttons and those who cannot, or can barely afford to stay alive. The schism has many facets - ecological, economic and political but it is evident that the ability of the 'Developed World' to overconsume is subsidised by the natural resources of the so-called 'Under-developed World'. As a single but not isolated example, the U.S.A. has been increasing the permitted quota of imported oil steadily over the last four years because of inadequate home production.

Of the worlds population, 20% consume almost 80% of its natural assets to support and propagate a way of life that is regarded enviously by the remaining segment of the population. Without entering into political or ecological issues, the developed world has the power and the obligation to reduce the nutritional and economic disparity that is responsible for so much suffering. There are several ways in which this could be done and one is the subject of this thesis.

(2) POPULATION SIZE:

All possible avenues of protein production must be used to the full in order to give the world time to reach a state of education and awareness that will enable it to limit its population size.

Certainly the birth rates of many countries are beginning to fall but it is questionable whether it will be a rapid enough fall. In any case it is equally true that the death rates of most countries is also dropping much faster than their birth rates are. Ehrlich (1970) stated that 45% of the worlds population is still under 15 years old so that even if every family limited its size to two offspring, the world is almost certain to contain 6000×10^6 people by the year 2000 A.D.

Despite the pleas of Maddox (1973) to curb panic-inducing rhetoric, the problem is a real one which must be tackled vigourously. Thoday (1969) has pointed out that any comfort drawn from the fact that all population-trend predictions are inevitably inaccurate, must be tempered by the disturbing certainty that the error in all published statements so far have been on the low side. Reference to the U.N. 1960 prediction for the year 2000 A.D. shows this neatly. Fig (1) indicates the predicted trends in population growth and it can be seen that the actual 1973 world population figure of 3×10^9 exceeds even the pessimistic prediction by a large margin.

(3) PROTEIN DEFICIT:

When figures for world food production are perused the situation becomes even more alarming. The accompanying table I (FAO 1967) is a grim testimony that food production is not keeping face with population growth.

The major problem facing any poorly nourished population is one of protein deficiency (Brown 1966, Munro 1968) however derived. Despite great advances in conventional techniques of agriculture, genetic engineering, storage, land reclamation and fertilizer production and use,

it is clear that these procedures are not likely to be sufficient to solve the immense task of feeding a rapidly growing population. Naturally all of the above techniques have limits of expansion and extension, but at present it is apparent that our capacity to expand in population size far exceeds that of our ability to increase food potential. An index from the Food and Agriculture Organisation combined for agriculture, fishery and forestry products showed that world food production increased by 10% during the period of 1959 - 1964. During the same time the population of the world increased by 10%. Despite this effort the per capita food production remained static. (F.A.O. report 1965). The conclusion is manifest - the world must learn to limit its numbers. Given the exponential rate of population growth and the seemingly infinite capacity of the human race for self-deception, it is evident that there is not much time in which to learn. It is possible, as King (1969) pointed out, that to take advantage of all land classified by the FAO as "unused but potentially productive" would require the commitment of more and more technological resources to merely maintaining the per capita level of food production. It is unlikely that in the present world context, countries as poor as India for example, could ever meet this expenditure. If this fact is faced then surely the morality of the circumstances will dictate that the more affluent areas of the world will pursue a realistic and humane foreign aid programme. Concomittantly the catchword of highly industrialised countries - to maximise - must give way to a philosophy of optimisation. Recycling and conservation must become major issues at the political level in such a way that the directive and examples come from the developed world.

(4) METHODS OF PROTEIN SUPPLY:

GENERAL: Evidence has been published to show that the developing countries can no longer provide sufficient calories for their populations, partly because the export of grain has been reversed. Those countries which once produced excess grain have now become the chief importers (King 1969). It seems probable, therefore, that the most important methods of protein provision will be those which conserve calorie supplies to the greatest extent. Rice and cereals contribute most of the worlds protein, but unfortunately they also are the main sources of calories. (Abbott 1967). Conventional methods of food production already are limited in expansion by lack of adequate water supply in many parts of the world (Brown 1968). Thus it appears that some means of food provision which is not dependent on climate and which does not drain an already over-taxed calorie resevoir is vital to gain the time so greatly needed.

Non-conventional food sources will be of great importance in this respect but many workers feel that conventional agricultural practices rather than technology dependent methods of production, should be the main stay of any country. In those countries where a high percentage of the labour force is on the land this argument is even more valid. For those who believe that de-urbanisation and the step-down of industrialisation are essential for Mans' survival on this planet, the force of this point is clear.

Nevertheless the time scale of events is becoming more and more compacted yearly and some form of non-agriculturally derived food source must be developed to supplement conventional ones.

(5) SINGLE CELL PROTEIN: (BIOMASS)

The/

The United Nations Organisation support the view that alternative methods of protein supply must be found. In a report entitled "Increasing the Production and Use of Edible Protein", single cell protein is quoted as offering the best hope for major new protein supplies independent of agricultural land use. (Report of Advisory Committee 1967).

Single cell protein is a food source which has been used accidentally and deliberately in human consumption for as long as Man has been aware of fermentation procedures - certainly for centuries.

The real novelty of using micro-organisms in a nutritional capacity lies in the adoption of a single-minded philosophy of action to produce quantities of microbial cells with the specific intention of consuming them. It is probably true to say that the psychological barriers to the acceptance of such an unorthodox nutrient are collossal, but if only a small percentage of the talent and finance which is used to persuade people to acquire the trappings of a technologically "sophisticated" life were channeled into selling single cell protein at the right time and in the right manner, then the difficulties would be surmountable.

Assuming that the product from manipulating the hitherto latent food potential of micro-organisms will be marketable, the extent of the contribution that S.C.P. can make to the world protein pools must be examined.

(6) TYPES OF S.C.P:

GENERAL: The range of materials which can be utilised by micro-organisms as growth substrates is impressive. Almost every type of material known to man with the provisional exception of certain plastics,

can be attacked by some form of micro-organisms, given suitable conditions of operation. The costs of using any given substrate on a large scale and of providing suitable conditions of growth drastically restricts the range of substrates usable in S.C.P. context.

In terms of protein content all classes of micro-organisms compare favourably with more conventional food stuffs such as soya bean. Tables (2 - 5) give a comparison of the protein content and amino acid spectra of examples of the major classes of micro-organisms with those of some more orthodox foods.

Each class of micro-organism has specific advantages and disadvantages associated with it, but one feature of microbes in general which has led to their appeal to food producers as a possible source of protein is their rapid rate of growth. Bacteria can grow up to 500 times as fast as plants. For example E. coli is able to grow with a rate of division of once per twenty minutes, providing that toxic wastes do not accumulate and that all nutrients are in excess.

(7) ALGAE AS PROTEIN:

Algae, since they are photosynthetic, should be attractive as a form of protein because there is no requirement for a carbon source other than CO2. The present view is that they are uneconomic to produce in bulk, although the Czechoslovakian Institute of Micro-biology has an experimental set up using Scenedesmus quadricauda on a fairly large scale of up to 900 square metres (Mitsuda,1966; Oswald et al.,) (1959, 1960, 1963), reported that they felt the initial costing predictions were unduly pessimistic, especially if the utilization of algae in waste treatment was taken into account. Clement et al.,(1967) described/

described the production of 12g. of dry matter/square metre from a new type of alga - Spirulina Maxima which has 60-80% protein content.

It is recognised that algae are able to synthesise nearly all the important vitamins at fairly high levels. (Ghose 1969). A further attraction to using algae as S.C.P. is that there exists a potential for the rational use of land areas unsuitable for the cultivation of more usual plants. Political barriers or the high cost of material transport may well mean that to obtain complete use of local resources algae would necessarily be the choice for S.C.P production process. As part of a food chain leading to an acceptable protein source, local factors such as inadequate mineral resources or lack of technical expertise could well dictate the economics of the situation.

S.C.P. has two main avenues of use:-

i. Through direct human consumption,

ii. As part of an animal food stuff.

Vincent (1969) has stated that the present state of the art with regard to algae precludes direct human consumption but this should not prevent further investigation. Certainly workers such as Enebo (1969) and Oswald (1960, 1963) appear to be more optimistic that there is scope for further development with this class of organism.

There have been many technical problems associated with algal culture on a large scale and some of these were discussed by Mayer (1964). Mayer found it possible to operate a 2000 litre culture unit for several months on a continuous basis with the same nutrient medium. Apparently few "technical snags" were experienced and the unit was simple to operate. The algal species present in the culture varied

both/

both with temperature of growth and with light intensity but the bacterial spectrum remained low and relatively constant. The type and intensity of stirring affected the yield markedly.

Much more information on the functioning of deep mass cultures is essential before a critical evaluation of algal S.C.P. can be made, and studies of the type performed by Mayer et al., should be encouraged.

(8) FUNGI AS S.C.P.:

Compared with bacteria and yeasts, fungi have a relatively low growth rate; for edible fungi the most optimistic estimates of doubling times range from between 4 and 12 hours. Temperatures for growth tend to be lower than for other types of micro-organism, and this means that the cost of cooling large fermenters is higher particularly at high agitational rates. Information gleaned from relatively few feeding trials indicated that fungi are not as acceptable to animals as was hoped, in terms of taste and digestibility. (Vanderveen et al. 1963). It seems more likely that fungal S.C.P. will be used as an animal food additive rather than as a primary protein source. Reports have shown that supplementation with cystine and methionine (Skinner and Muller 1940) and also some vitamins is necessary (Vinson et al 1945) - (Litchfield 1967). Presumably fungal S.C.P. could stand as a protein source if addition of amino acids and vitamins were contemplated although this would further increase the cost of production.

In general the literature on the nutritional status of this variety of S.C.P. is conflicting and more investigation is needed to evaluate its potential correctly.

The list of fungal species being considered as sources of single cell/

cell protein grows daily, especially amongst those which are used in the antibiotic, citric acid and vitamin production industries where the cellular matter is largely wasted. If the nutritional deficiencies could be resolved then this approach will make a significant contribution to the worlds food resources.

(9) YEASTS AS S.C.P.:

The yeasts have received the most favour from workers engaged in S.C.P. studies. The reasons for this are partly historical and partly due to the inherent advantages of yeasts as a raw material for single cell protein. Johnson (1967) has quoted figures which indicate that the cheapest microbial protein produced in the U.S.A. was Torula yeast grown on sulphite liquour. Torula protein sold at 21 cents/kilo in comparison with soya protein which sold at 11 cents/kilo. The elevated content of B vitamins of the Torula accounts for some of this price differential and although Torula has never been produced at less than 17.6 cents/kilo, if the Torula was used as a major feed constituent, then this differential would disappear.

(10) CONSIDERATIONS OF SUBSTRATE COST:

In terms of production methods and gross chemical structure and composition there is not a great deal to choose between yeasts and bacteria as sources of S.C.P. At this point the subject of substrate type and economy must be considered. One of the key factors in the acceptance of a single cell protein process from an industrial point of view will be cost. The cheaper the raw materials costs are i.e. the substrate costs the cheaper will be the overall process costs. (Humphrey 1967).

There have been many publications relating to viable plants using cheap carbon sources, particularly petroleum products. Most of the major petroleum firms are engaged in such research. In the case of British Petroleum alone, 53 papers appeared up to January 1970 concerned with S.C.P. production from petroleum substrates. Notable publications were those of Shacklady (1969), Champagnat (1962) and Bennet et al (1969).

(11) HYDROCARBON SUBSTRATES:

It is evident that there is an excellent base for biomass production from hydrocarbon sources and similar success should be expected from other processes if equivalent amount of effort is expended.

Petroleum hydrocarbons are among the cheapest carbon sources available. Table (6) is a list of some common carbon substrate costs as quoted by Humphrey (1967). An added advantage of using petroleum fractions in an S.C.P. process is that the crude oil is often upgraded in the process. (Humphrey 1970).

(12) YEASTS GROWN ON HYDROCARBONS:

It appears that both bacteria and yeasts can be grown on hydrocarbons but with hydrocarbons of chain length (C_{10}) to (C_{20}) and above much more work has been done using yeasts rather than bacteria. Candida lipolytica was used in an investigation by Munk et al (1969). Gas oil contains hydrocarbons from (C_{10}) to (C_{25}) and apparently the lower paraffins were utilised most rapidly in continuous culture. The optimum dilution rate ranged from 0.11 to 0.33 and under these conditions the fermenter productivity was 17g. biomass/litre/hour at a yield coefficient of 0.92%.

Miller and Johnson (1966) studied the growth of a mixed culture of Candida lipolytica and Candida intermedia. The mixed culture grew more rapidly on n-alkanes than did C. intermedia. C. lipolytica did not grow at all on unsupplemented mineral salts/n-alkane medium. The results of growth of one of the cultures, Culture 4, is given in table 7. It can beseen that the (C_{22}) alkane gave the highest growth rates and cell yields. The crude protein content was highest when this substrate was used although the alkane-oxidising ability of C. intermedia seemed to be non-specific for alkane substrates.

(13) BACTERIA GROWN ON HYDROCARBONS.

Bacteria have also been observed to grow at the expense of n-alkanes. Wagner et al (1969) cultured Nocardia species and Mycobacterium phlei (as well as Candida lipolytica) on inorganic salts solution containing n-alkanes (C_{10}) to (C_{20}) as sole carbon and energy source. Cell yields on carbon were from 50 - 97% and the crude protein varied from 35% to 62% of dry weight depending on the organism and the substrate.

The mycobacterial group of organisms have been particularly well studied with respect to the oxidization of hydrocarbons (Lukina 1962). Although the ability to utilise a complete range of paraffins from the gaseous (C1-C4) alkanes to the solid (C15 and above) types is extremely rare in any one species, it can be said that all of the n-alkanes from methane (C1) to the higher alkanes such as octacosane (C28) can be oxidised by one species of bacteria or another.

(14) SUBSTRATE SPECIFICITY:

Substrate specificity is a confusing subject in the case of hydro-

carbon oxidising micro-organisms partly because very few papers have been published on this topic. Perry (1968) isolated three bacterial strains from soil by an elective culture method and tested them for their hydrocarbon oxidative capacity. Only one strain (Job 5) was able to grow on all alkanes tested from C1 - C22. Some interesting observations were made. The strains were grown on a variety of hydrocarbon substrates and the oxidative ability was greatest with those hydrocarbons closest in chain length to that of growth substrate. Secondly, cells which were grown on the gaseous alkanes (C1 - C4) could oxidise all of the alkanes in the homologous series but cells grown on n-alkanes from (C5- C8) oxidised n-butane well, propane poorly and methane and ethane hardly at all. Certainly such observations bear out a statement made by Johnson (1972) in which he declared that in order to obtain cultures with the required fermentative abilities for large scale processes, isolation under conditions simulating as closely as possible the actual conditions of operation in a fermentation vessel was essential.

Until recently no fungal species had been found which were able to grow on the lower alkanes such as (C1 to C5). Davies et al (1973) reported the growth of Hyphomycetes on ethane, propane and n-butane. Methane was co-oxidised but was not used as a sole carbon source. A batch process using Graphium species in a mixed culture which included a bacterium species, has been described (Volesky and Zajic 1971) in which the culture grew at the expense of natural gas. Ethane was chiefly oxidised but the bacterial strain seemed to use methane. A report by McLee (1972) confirmed the observation of Volesky and Zajic in that n-butane could be oxidised by a Graphium species. One facet of this work was that the Graphium species was able to grow in submerged culture - a prerequisite of industrial scale production of biomass.

The ability to oxidise the gaseous alkanes appears to be more common than at first appeared but even now few papers have been published dealing in more than a superficial way with the dynamics of growth on gases such as pentane or butane. A stoichiometric analysis of the consumption of n-pentane and oxygen was performed by Takahashi et al. (1970) but in this work no indication of culture purity was given.

It can be seen that isolation of organisms on a particular type of alkane is unlikely to produce a culture capable of efficient growth on other types of alkane. The nature of an isolate will depend on the nature of the substrate used in the isolation step, a point often overlooked by workers comparing kinetic data of organisms isolated under different conditions.

(15) IMPLICATIONS OF USING HYDROCARBONS AS SUBSTRATE FOR S.C.P. PRODUCTION:

Whilst hydrocarbons constitute the cheapest available carbon source, there are certain disadvantages associated with their use on an industrial scale. If hydrocarbons of any kind are used in preference to carbonhydrate substrates it is necessary to provide:-

i. A greater amount of oxygen per carbon molecule fixed as cellular material.

ii. A greater cooling capacity in the process.

Both the elevated oxygen requirement and the need for greater cooling increase the unit cost of producing S.C.P. When dealing with a process involving 100,000 tons per annum of biomass, for example, the costs of cooling and oxygen supply form a significant proportion of the overall running costs.

(16) OXYGEN REQUIREMENTS IN HYDROCARBON FERMENTATION:

Hydrocarbons/

Hydrocarbons have no oxygen in the molecule and therefore a supply of molecular oxygen must be provided in order for microbial cells to grow on such a substrate. Thus even though fermenter productivity may be the same using either carbohydrates or hydrocarbon substrates, oxygen transfer rates must be much higher on hydrocarbons. In large scale fermentation this is complicated by the relationship of the mass-transfer coefficient to power requirements for a given vessel. Table (8) gives theoretical 02 transfer rates for different substrates assuming a doubling time of three hours and a cell concentration of 15 grams of cells/litre.

Bartholomew (1960) has shown that the relationship of mass transfer coefficient (See section/on 21 Mass transfer partB) with power of agitation is highly influenced by vessel size. Table (9) outlines this correlation in the form of a scale-up index (After Wang 1968).

Thus the elevated oxygen requirement of hydrocarbon fermentations adds to the higher power in-put needed anyway in large scale operations. Wang has stated that the increase in operating costs due to the power increase could offset the economic gain of lower substrate cost.

(17) COOLING REQUIREMENT:

A highly agitated aerobic fermentation process generates heat in considerable quantities and this must be disposed of in order to preserve the optimum growth temperature. In some cases mechanical refrigeration is required particularly when the optimum temperatures are low. Cooling expenses can contribute to a large extent to process costs. In temperate areas of the world increased cooling is not a significant problem but in tropical areas, regrigeration is essential in fermentation work. Wang/1968 has published figures for heat evolution in some theoretical fermentations/

fermentations. See table (10).

It is known that yield factors are of importance in deciding the economic character of a fermentation process (Klass et al 1967). The higher the yield the lower the oxygen requirements are and the lower the heat evolution. Thus an absolute property of any isolate must be a high yield co-effecient.

(18) THERMOPHILIC ORGANISMS:

A further means of reducing the cost of cooling is to isolate thermophilic organisms so that the optimum growth temperature is relatively high. If the temperature of culture is set at 60°C. then according to Wang (1968) refrigeration would not be necessary. In fact, few studies attempting to isolate thermophiles have been described for hydrocarbon utilisers. Mateles et al (1967) reported the existence of a bacterium able to grow on hydrocarbons at 70°C and Sukatsch and Johnson (1972) have reported the growth of bacterial cells at high temperatures. Hexadecane was used as the only carbon source in mineral salts medium. Cell yields were determined for each of the cultures and an enrichment technique was employed to obtain cultures at 25°, 35°, 45°, 55°, and 65°. Each culture was grown at the enrichment temperature and in addition the 55° and 65° cultures were grown at various temperatures. It was found that in each case a stable mixed culture developed in the enrichment procedure starting from soil; in the case of the 55° culture three organisms were present and in the others only two. Table (11) summarises the results of Sukatsch and Johnson for all cultures grown at the enrichment temperature, and Table (12) gives the results of growth of the 55°c and 65°c cultures. The ratios of cells present changed when the culture grew at different temperatures from the enrichment/

enrichment temperature. Yields at lower temperatures were greater than those observed at higher temperatures irrespective of the temperature at which enrichment occurred. Cultures selected at low temperatures could not grow at higher temperatures and conversely cultures from high temperature enrichments performed better at lower temperatures. Johnson made the point that whilst the above experiments do not prove that high yielding thermophiles able to exidise hydrocarbons do not exist, they do indicate that such organisms may be difficult to isolate.

(19) NATURAL GAS AS A SUBSTRATE FOR S.C.P. PRODUCTION:

When organisms are grown on liquid or solid hydrocarbons, the resultant biomass usually has some residual hydrocarbon substrate dissolved in the lipid bodies of the cells. Before the biomass can be made acceptable, the remaining hydrocarbons must be removed in view of the potential carcinogenicity of the majority of such substances. Naturally, treatment procedures increase the process costs.

Methane, being a gaseous compound, does not suffer from the drawback of residual hydrocarbons since its volatility ensures its own complete removal at no extra cost.

Methane is the cheapest widely available carbon source (see Table 6) and although world stocks of natural gas are rapidly being consumed for energy purposes there are sufficient deposits of natural gas to last until the turn of the century. The nutritional status of methane oxidising bacteria has yet to be unequivocably established. Table (13) lists the amino acid compositions of several reported examples.

(20) GROWTH OF MICRO-ORGANISMS ON METHANE AND NATURAL GAS:

The natural/

The natural gas deposits of the world constitute a considerable potential source of good yet the implementation of the results of research into this form of S.C.P. has been slow. In part this has been due to the fact that there are several technical difficulties associated with the use of methane. Pure cultures have been especially hard to obtain and many of the earlier attempts to scale up cultures failed, apparently because of 02 toxicity. In addition methane and air or oxygen mixtures are highly explosive in certain conditions. Industrial production of methane-derived biomass has so far not been reported, and the object of this thesis has been to describe research into the techniques of methane fermentation with industrial criteria of operation.

(21) MICROBIOLOGICAL ASPECTS OF METHANE UTILISATION:

The existence of methane bacteria has been known for many years. Söhngen (1906) gave the earliest description of the isolation of an organism he called Bacillus methanicus which was able to oxidise methane. Since then, other workers have reported bacterial cultures capable of growth on methane. These workers include Aiyer (1920), Nechaeva (1949) and H utton and Zobell (1949). Repetition and extension of Söhngens' work by Dworkin and Foster (1956) and by Leadbetter and Foster (1958) produced approximately 30 methane oxidising strains similar to the pigmented strain of Söhngen. In 1958, Brown published on the discovery of a different type of organism producing very small colonies and able to use only methane or methanol as sole carbon source. This organism was named Methanomonas methano-oxidans by Brown.

Johnson and Temple/1962 isolated an obligate methylotroph similar to Pseudomonas methanica of Dworkin and Foster. The isolation of Methylococcus by Foster and Davies (1966) produced a third type of methane oxidiser/

oxidiser, and these three species have remained the best known and documented. Other less well known examples deserve mention. Davis et al, (1964) discovered a methane oxidiser which was also able to fix nitrogen although this characteristic was fragile and easily lost on sub-culture. Growth on nutrient agar was sparse. An extension of this work by Coty (1967) indicated that there is a widespread existence or organisms able to fix nitrogen at the same time as oxidising hydrocarbons. The existence of such bacteria was suggested as reason for the increased nitrogen content of soils through which natural gas percolates. Although many methane oxidisers do not grow well on agar media, the organism described by Bogdanova (1966) appeared to do so. A chlorella capable of methane oxidisation has been described by Enebo (1967) but this appears to have been an isolated example.

(22) PHYSIOLOGICAL REQUIREMENTS OF METHANE OXIDISING MICRO-ORGANISMS:

Studies on the physiological requirements of methane oxidising cultures have not been extensively reported with respect to fermentation experiments. Optimum requirements probably depend in some measure on the conditions imposed on the culture during isolation (Sukatsch and Johnson 1972), but this point is often overlooked when determining the best growth conditions. Different pH values and metallic ion concentrations have been reported to affect the growth of methane oxidising cultures and this is not surprising in view of the way such agents affect enzyme activity. Cell yields of culture HR grown on methane were measured by Vary and Johnson (1967) at different pH values. (Table 14 exhibits the results of this work). It can be seen from table (14) that the optimum pH for culture HR was about 6.3 but that fairly small pH changes away from this optimum caused significant drops in the yield co-efficients.

Dworkin and Foster (1956) found that nitrate was a better source of nitrogen than were ammonium ions, but in contrast to this finding Vary and Johnson reported that the reverse was true for culture HR. Experiments performed by Bewersdorff and Dostalek (1971) with culture TM10 indicated that this culture was relatively insensitive to pH. Optimal growth occurred between pH values 5.6 and 5.9, and growth ceased below pH 4.9 and above pH 7.0. In this study the nitrogen source was ammonium ion but no comparison with other nitrogen sources was reported.

It has been shown that ammonium ions are responsible for the activation of alcohol dehydrogenases, which catalyse the oxidation of methanol and formaldehyde in Methylococcus capsulatus (Patel and Hoare 1971) and if this property of ammonium ions is present in all methane oxidisers it might be expected to be an absolute requirement for growth.

Few papers have been published concerning the metallic ion requirements of methane utilising micro-organisms but the most complete have been those of Vary and Johnson (196 7) and Sheehan and Johnson (1971). Culture HR appeared to be unaffected by the addition of Zn^{++}, Mn^{++} and Co^{++} ions. Copper in the form of cupric sulphate apparently stimulated growth of culture HR. The mixed culture M45, described by Sheehan and Johnson, exhibited specific requirements for Ca^{++}, Cu^{++}, $MoO4^{--}$, Zn^{++}, Mn^{++}, Mg^{++} and Fe^{+++}. No conclusive evidence for a requirement for Co^{++} was found.

It is probably not possible to generalise accurately about the optimum growth conditions of methane oxiders and each newly isolated culture is likely to display special characteristics which can only be appreciated by a rational approach to isolation methods.

(23) MIXED CULTURE GROWTH ON NATURAL GAS:

As more work on methane oxidisers is completed the list of microbes known to be able to grow on natural gas becomes larger, but so often difficulty has been experienced in isolating truly pure cultures. It is this observation which has led to the suggestion that methane oxidation occurs in nature most efficiently in a mixed culture, possibly with one or more non-methane oxidisers being present. If this is the case, then as Whittenbury (1969) has pointed out the use of mixed cultures in basic studies on methane oxidation "would constitute a microbiological nightmare". Whittenbury himself has been able to isolate in pure culture over 100 species of micro-organism able to oxidise methanol and/or methane as sole carbon source. This was accomplished by ignoring the large colonies which appeared on first isolation and selecting micro colonies under a binocular microscope and sub-culturing only these (Whittenbury 1970). It can therefore be seen that methane oxidising cultures are able to exist in a state of pure culture but this does not mean that such a state is the most satisfactory one for industrial scale biomass production. The cultures obtained by Whittenbury grew slowly on agar plates and were very prone to invasion by protozoa and non-methane bacteria.

It is probably true to say that only in extreme environments such as hot sulphur springs or highly acidic situations does a pure culture persist, as Hobson (1969) has pointed out. It could further be argued that there is no such thing as a microbial pure culture unless it is comprised of a single cell.

Laboratory pure cultures are artefacts which are unlikely to behave in/

in the same way *in vitro* as they would in their natural environment. With organisms less fastidious than methane oxidising bacteria this might not be of such consequence in an industrial context but it was decided to adopt a deliberate policy of encouraging mixed culture in the work described in this thesis.

It seemed apparent in the early years of research on methane bacteria that all such organisms were obligate methylotrophs, i.e. were only able to grow on methane and/or methanol as a sole carbon source, but a few cases of less specific growth on hydrocarbons have been reported (see section 14); and it may be that the reason many of the cultures exhibit methylotrophy is connected with the method of isolation. In attempting to isolate cultures on C1 compounds from a miscellaneous mixed culture, it may well be that organisms capable of growth only on (C1) hydrocarbons are selected for. In the case of the gaseous hydrocarbons, the ability to use these compounds efficiently for growth may be a property of mixed culture.

The inhibitory effect of certain higher organic materials to some methane oxidising bacteria is puzzling in view of natural habitats of these organisms such as soil or sewage, (Eroshin, Harwood and Pirt 1968) and again it may be that this is a reflection of the method of culture. Perhaps it is only in mixed culture that methane oxidisers are tolerant of external organic matter. Harwood (1970) has shown that higher cell densities of *Methylococcus capsulatus* could be attained in pure culture if absorbents were present. Presumably the absorbents negate the action of inhibitory substances in the culture fluid. If this is a general phenomenon with methane oxidisers, then mixed cultures offer the opportunity of emulating the action of absorbents.

Strains of methane utilisers donated by Prof. Quayle (Sheffield University) and by the late J. W. Foster, although claimed to be pure cultures, were in all cases found to be mixed. Any attempts to separate the component organisms into pure cultures resulted in strains which were unable to survive in liquid medium, an observation supported by similar experiences in laboratories at I.C.I. Billingham (T.O. Owen, Personal communication). It must be emphasised that the ability of pure cultures to use methane is not excluded. As already noted, Whittenbury has been able to isolate over 100 cultures in a pure state. Johnson and Temple (1962) also appear to have developed a strain able to grow well in submerged culture, but their definition of culture purity was restricted to "a failure to obtain growth on any of the tested carbon sources except methane or methanol". They presumably did not exclude the possibility of the presence of methane oxidisers other than a rigidly defined Pseudomonas methanica or indeed of organisms able to use methanol produced by the methane oxidisers.

(24) METHANE BIOMASS IN FERMENTERS:

There have been relatively few papers published concerning large scale biomass production from gaseous hydrocarbons. Among the most notable was that of Vary and Johnson (1967), which described an attempt to isolate pure and mixed cultures with high yield factors on methane. It is extremely significant that all of the cultures which could utilise methane were mixed cultures of two or three types of gram-negative rod. Attempts to isolate fungi or yeasts on methane resulted in mixed bacterial cultures developing and not the yeast or fungi. Vary and Johnson reported that attempts to isolate pure cultures failed. Yeast extract agar or salts agar supported some pure cultures in the presence/

presence of methane but subcultures into liquid medium failed to grow. When growth did occur in liquid culture it was found to be mixed. Under optimum growth conditions, namely pH 6.5 at 30°C, and with NH_4^+ as nitrogen source, generation times were approximately 3 hours and cell yields on methane were between 65-70%.

A requirement for carbon dioxide and for cuprous ions was observed in the cultures described by Vary and Johnson and yeast or beef extracts were inhibitory to growth. These inhibitions presumably did not apply to pure cultures on agar/yeast extract since some growth under such conditions was observed.

A later paper by Sheehan and Johnson (1971) described a mixed culture (M45) which was capable of growth at 45°C in continuous culture, using methane as a sole carbon source. A doubling time of 2.29 hours and a yield factor on methane of 61% was reported. Significantly the productivity of 2.58 grams/litre/hour was limited only by the mass transfer rate of oxygen into the liquid phase. The productivity could have been a great deal higher if equipment with high has transfer capabilities had been used.

Bewersdorff and Dostalek (1971) using both batch and continuous systems, investigated biomass production on methane and found that the productivity was higher when continuous fermentation was used. Interestingly the culture which grew was a stable mixed culture yielding:

Y_{CH_4} 90%

Y_{O_2} 26%

One other important paper dealing with batch production of biomass from methane was published by Hamer, Heden and Carenberg (1967). A culture/

culture was produced which was considered as pure although "no nigorous criteria of purity" were established. Growth occured in baffled fermenters (see section 22 of Part C) capable of sufficient gas transfer to prevent growth limitation by the gaseous substrates.

A feature often quoted as a disadvantage of methane utilisers is the slow growth rates often observed with these organisms but the work described above by Bewersdorff, and Sheehan and Johnson proved that it is certainly possible to develop cultures with kinetic characteristics acceptable from a biomass point of view

(25) GAS RECYCLE IN METHANE FERMENTATION:

The work published by Hamer et al, (1967) concerned the application of industrial conditions of operation to the cultivation of methane oxidisers in that although initial isolation procedures were in shake flasks the culture once established was transferred to a one litre batch fermenter with magnetic stirring. One of the most important features of the Hamer system was that gas recycle was employed. In terms of gaseous substrates this technique has obvious economic significance in that there need be no wastage of unused methane or oxygen. Hamer pointed out that "the adoption of a re-circulatory system introduces complex and often inter-related factors into the system". Some of these problems have been investigated in the work reported here and described in part C of this thesis.

Surprisingly the concept of gas recycle has received only minor attention from microbiologists. Heden (1952) seems to have been the first to have proposed the technique for a pilot plant scale fermentation process. Clark and Lentz (1961) used gas recycle more recently in the production of citric acid at high oxygen concentrations in/

in the gas phase. A study concerning the growth of Pseudomonas aeruginosa by Owen and Hill (1965) made use of the technique but the most recent work with gas recycle involved the growth of Graphium species on ethane/methane gas mixtures. (Volesky and Zajic 1971). The findings of Volesky and Zajic were significant to the work reported in this thesis because natural gas contains approimately 3.25% (v/v) ethane. Growth of ethane utilisers as well as methane oxidisers on natural gas is desirable since the ethane would be wasted otherwise. At the same time biomass comprised of both ethane and methane utilisers each with specific amino acid patterns gives more chance to optimise the biomass from a nutritional point of view. (Hamer and Norris 1970).

(26) SUMMARY OF APPROACH ADOPTED IN THE WORK REPORTED HERE FOR THE PRODUCTION OF BIOMASS ON NATURAL GAS.

It was decided to investigate the techniques of producing biomass from natural gas as a useful contribution to the solving of the world protein crisis. Certain basic philosophies were adopted from the start of the project and these were:

(1). USE OF MIXED CULTURES GROWING ON MIXED SUBSTRATE ETHANE/METHANE.

(2). USE OF EQUIPMENT WITH HIGH GAS TRANSFER CAPABILITY.

(3). EMPLOYMENT OF GAS RECYCLE.

(4). USE OF CONTINUOUS CULTURE FERMENTATION.

(5). AUTOMATIC CONTROL OF THE GAS PHASE COMPOSITION.

(6). GROWTH AT TEMPERATURE OF 40° C.

(7). TO USE THE EQUIPMENT IN THE CONTINUOUS CULTURE MODE AS A SELECTIVE AGENT FOR ORGANISMS WITH HIGH YIELD FACTORS AND HIGH GROWTH RATES OF METHANE AT 40° WITH HIGH CELL DENSITIES.

The design of the plant described in this thesis was based on the above operational criteria and the details of design have been described in part (C).

(27) DESIRABLE CHARACTERISTICS OF MICRO-ORGANISMS USED FOR SINGLE CELL PROTEIN.

Whatever substrate and type of organism are used to produce biomass certain features of the process are essential to large scale operation:

a. Rapid growth on inexpensive media.

b. Simple nutritional requirements.

c. Capable of growth in continuous culture.

d. Resistant to contamination and have a stable, predictable culture behaviour.

e. High yield co-efficient.

f. Have a readily disposable effluent with little waste, preferably with water recycle.

g. An easily separated and harvested product (cells).

h. Non-toxic and non-allergenic in high concentrations.

i. Attractive organoleptic properties.

j. High nutritional values for protein fats and vitamins.

k. Simple storage and packaging requirements.

l. Economic to produce.

The fermentation system described in this thesis was designed with these features in mind and the broad rationale adopted to achieve this has been outlined in section (26).

From a biomass point of view, methane in the form of natural gas is only a short term substrate, since its chief use is likely to be as an industrial and domestic energy source. At present rates of energy consumption, stocks of natural gas are unlikely to last more than 50 years. It might appear that other substrates than natural gas would be more usefully explored as a source of biomass but methane is/

is obtainable from other origins. Domestic sewage is a plentiful source of methane when undergoing an anaerobic digestion treatment.

At standard temperature and pressure, 1 kilogram of B.O.D. removed by an aerobic oxidising yields greater than 0.35 cubic metres of methane (calorific value 35 KC/litre) or one cubic foot of gas per capita for 50% decomposition of total organic wastes. (Ainsworth 1967).

TABLE (1)

WORLD PRODUCTION AND CONSUMPTION OF THE MAJOR PROTEIN FOODS:

Protein Food	1961/63 Average		1975				1985 Demand	
			Low Estimate		High Estimate		Low Estimate	High Estimate
	Product.	Consump.	Prod.	Demand	Prod.	Demand		
	(10^6 Tons)		(10^6 Tons)				(10^6 Tons)	
All Grains	696.0	700.5	942.4	938.3	981.2	963.7	1,093.9	1,155.8
Milk, Milk (a) Products	14.004	13.960	18.312	18.669	18.704	19.795	22.878	25.917
Meat (a)	63.362	63.391	84.305	87.283	88.015	92.619	109.626	128.589
Eggs	14.050	14.039	18.825	18.863	19.648	19.971	23.832	26.914
Oil Seeds	63.00	63.00	95.00	95.00	102.00(b)	102.00	128.21	144.33
Fish	45.53	-	-	68.172	-	74.356	84.703	107.944

(a) Mainland China not included

(b) Assuming that oil seed production follows the same trend as Fats and Oils as a whole.

(Adapted from the Food and Agriculture Organisation, February, 1967).

TABLE (2)

PROTEIN CONTENT OF MAJOR CLASSES OF MICRO-ORGANISMS AND COMMON PLANT TISSUES.

SOURCE	% WATER	% PROTEIN (a)	TOTAL WORLD PRODUCTION MILLION TONS
BACTERIA	70 - 80	47 - 87	-
YEAST	75	45 - 50	0.238
MOULDS/FUNGI	-	19 - 57	-
ALGAE	70	24 - 80	-
PLANT TISSUE	75	15 - 30	-
SOYA BEAN	67	38 - 43	35.0
COTTON SEED	-	18 - 20	20.6
PEANUT	-	25 - 30	14.8

From Ghose 1969.

(a) % dry weight of cells.

TABLE 3.

AMINO ACID COMPOSITION OF SELECTED ALGE (From Lipinsky and Litchfield 1970).

AMINO ACID	Chlorella (a) pyrenoidosa	Chlorella (b) vulgaris	Spirulina (c) maxima	F.A.O.(d)
Alanine	5.9	-	6.8	-
arginine	5.6	8.1	6.5	-
aspartate	5.9	-	8.6	-
cystine	-	-	0.4	-
glutamate	9.3	-	12.6	-
glycine	4.8	-	4.8	-
histidine	1.4	1.9	1.8	-
isoleucine	3.4	5.2	6.0	4.2
leucine	4.0	9.1	8.0	4.8
lysine	7.9	8.6	4.6	4.2
methionine	1.8	2.4	1.4	2.2
phenylalanine	4.5	5.3	5.0	2.8
proline	4.0	-	3.9	-
serine	2.2	-	4.2	-
threonine	3.2	4.0	4.6	2.8
tryptophan	1.4	2.5	1.4	1.4
tyrosine	2.7	-	4.0	-
valine	5.1	7.4	6.5	4.2

Amino acid composition in g/16 Nitrogen

(a) Lubitz J.A. (1963)

(b) Fowden L. (1952)

(c) Clement et al, (1967)

(d) F.A.O. Nutritional Studies No.16, (1957)

\- not measured.

TABLE 4.

AMINO ACID COMPOSITION OF SELECTED FOOD AND FEED YEASTS

(From Lipinsky and Litchfield, 1970)

AMINO ACID	Candida lipolytica (a)		Candida tropicalis (b)	Candida utilis(c) (Torula)		F.A.O. Pattern (f)
	n paraffins	gas oil	glucose	xylose	hexadecane	sulfite liquor	
Alanine	7.4	5.8	6.6	5.0	6.8	3.4	-
Arginine	4.8	5.0	4.0	2.5	3.8	5.4	-
Aspartic acid	0.2	10.0	11.9	1.4	13.0	4.7	-
Cystine	1.1	0.9	0.2	0.1	0.7	0.7	-
Glutanic acid	11.3	12.1	8.3	6.9	12.3	15.0	-
Glycine	4.8	4.5	5.4	6.3	4.6	4.8	-
Histidine	2.0	2.1	2.4	1.7	2.2	1.9	-
Isoleucine	4.5	5.3	6.6	4.4	5.9	5.3	4.2
Leucine	7.0	7.8	8.3	5.3	7.1	7.0	4.8
Lysine	7.0	7.8	4.4	10.0	11.7	6.7	4.2
Methionine	1.8	1.6	1.4	0.9	1.3	1.2	2.2
Phenylalanine	4.4	4.8	4.8	3.2	4.0	4.3	2.8
Proline	4.4	3.7	4.0	2.9	3.7	3.5	-
Serine	4.8	5.1	9.5	7.2	11.1	5.5	-
Threonine	4.9	5.4	7.3	4.8	6.4	5.5	2.8
Tryptophan	1.4	1.3	0.7	0.5	0.7	1.2	1.4
Tyrosine	3.5	4.0	3.6	2.4	3.1	3.3	-
Valine	5.4	5.8	7.9	5.4	6.6	6.3	4.2

(a) Evans G.H. (1968)

(b) Yamasa K, et al; (1968)

(c) Powell M.E. et al. (1961)

(d) F.A.O. Nutritional Studies No.16 (1957)

TABLE 5.

AMINO ACID COMPOSITION OF SELECTED BACTERIA. (From Lipinsky and Litchfield, 1970).

AMINO ACID	(a) Hydrogenomonas eutrophia	(b) Micrococcus cerificans	(c) Pseudomonas No.5401	(d) Methane bacterium	(e) F.A.O.
Alanine	8.80	6.62	6.37	-	-
Arginine	8.00	4.51	4.68	-	-
Aspartic acid	9.57	8.92	8.47	-	-
Cystine	-	1.11	0.43	-	-
Glutanic acid	11.7	9.85	10.05	-	-
Glycine	5.47	3.36	4.26	-	-
Histidine	2.48	1.85	1.87	-	-
Isoleucine	4.58	4.08	4.33	5.0	4.2
Leucine	8.52	6.57	6.74	8.4	4.8
Lysine	8.61	5.70	9.06	6.4	4.2
Methionine	2.69	2.13	0.81	2.4	2.2
Phenylalanine	3.96	3.39	3.39	5.2	2.8
Proline	3.46	3.04	2.36	-	-
Serine	3.47	3.30	3.07	-	-
Threonine	4.52	4.50	3.77	5.0	2.8
Tryptophan	1.05	0.92	0.91	3.0	1.4
Tyrosine	3.26	2.83	2.30	-	-
Valine	7.13	5.12	5.08	6.8	4.2

Amino acid composition in g/16g Nitrogen.

(a) Galloway D.H. and Kumar A.M. (1969)

(b) Guenther K.R. (1965)

(c) Ko P.C. et al., (1968)

(d) Norris J.R. (1968)

(e) F.A.O. Nutritional Studies No.16 (1957)

\- not measured

TABLE 6

CARBON SUBSTRATE COSTS (After Humphrey 1967)

MATERIAL	COST IN c / lb.
REFINED HYDROCARBONS	2 - 4
SIMPLY SEPERATED HC.	1 - 2
CEREAL GRAINS	1.5 - 2.5
CEREAL GRAIN WASTES	I
CRUDE OIL	I
COAL	0.5 - 1
NATURAL GAS	0.25
FLUE GAS	0.04

TABLE (7)

GROWTH OF CULTURE 4 ON VARIOUS n-ALKANES (From Miller and Johnson 1966)

CARBON SOURCE.	GEN. TIME	CELL YIELD	CARBON (a) recovered as cells and CO2	CELL N2	CRUDE (b) protein	CELL lipid
		%	%	%	%	%
LIQUID:						
C15	4.0	86.5	89.2	7.77	42.0	4.6
C16	4.5	78.0	75.6	7.25	35.4	11.2
C17	5.5	74.2	76.0	7.42	35.4	9.2
C18	5.0	83.9	74.0	6.75	35.4	13.2
SOLID:						
C20	3.5	81.7	86.5	8.81	45.1	3.2
C22	3.0	89.5	86.4	8.48	47.6	1.9
C24	4.0	89.5	90.0	8.20	46.0	3.6
C28	8.0	88.4	93.0	8.19	45.2	3.7

(a) Assuming 48% Carbon in cells.

(b) As devised by Kjeldahl nitrogen x 6.25.

TABLE 8.

OXYGEN TRANSFER RATES FOR S.C.P. PRODUCTION ON DIFFERENT SUBSTRATES.

(From Wang 1968)

SUBSTRATE	DOUBLING TIME (Hr)	CELL CONCENTRATION (g.cell/litre)	02 REQUIRED (g.02/g.cell)	02 TRANSFER RATES (g.mole 02/L.Hr.)
CARBOHYDRATE	3	15	1.0	103
METHANE	3	15	5.0	540
n-ALKANE	3	15	3.3	356

TABLE 9.

INFLUENCE OF FERMENTER SIZE ON THE RELATIONSHIP BETWEEN MASS TRANSFER CO-EFFICIENT AND IMPELLER POWER. From Wang (1968)

SCALE OF OPERATION	FERMENTER VOLUME (GAL)	MASS TRANSFER DEPENDENCY ON (POWER/VOLUME)
LABORATORY	2.0	a = 0.95
PILOT PLANT	110	a = 0.67
PRODUCTION	6,000 - 12,000	a = 0.5

TABLE 10.

COSTS OF REFRIGERATION OF FERMENTATIONS ON DIFFERENT SUBSTRATES

(From Wang 1968)

SUBSTRATE	YIELD (g.cell/ g.subs.)	HEAT OF FERMENTATION (Kcal./100g. cell)	RATE OF HEAT EVOLUTION (Kcal) (L.Hr.)	POWER COST OF REFRIGERATION (¢ /lb.cells)
FROM EXISTING DATA				
CARBOHYDRATE	0.5	380	13.2	0.54
nPARAFFIN	1.0	780	27.0	1.03
METHANE	0.6	1,860	64.3	2.52
AFTER IMPROVEMENT				
METHANE	0.8	1,300	450	1.75
METHANE	1.0	970	33.6	1.31
n-PARAFFIN	1.2	590	20.4	0.79
n-PARAFFIN	1.4	453	15.7	0.61

Above table assumes: Doubling time - 3Hr., cell conc 15 g./l., refrigeration is 1.2hp/12000Btu/Hr, 1 Kwh is 1 ¢.

TABLE 11.

CELL YIELDS AND CELL COMPOSITION OF CULTURES GROWN AT ENRICHMENT TEMPERATURES. (From Sukatsch and Johnson 1972).

CULTURE	TEMP. OF ENRICH-MENT (°C.)	YIELD (g/cell/ g.hexade-cane).	MAX. DOUBLING TIME (HR)	% CELL COMPOSITION			POPULATION RATIO		
				C	H	N	ORG. A	ORG.B	ORG.C.
SI	25	1.12	1.1	49.2	7.1	11.9	40	60	0
S2	35	1.10	1.0	49.8	7.2	9.9	50	50	0
S3	45	1.00	1.2	49.6	7.1	9.8	40	60	0
S4	55	0.49	2.5	45.9	7.2	8.8	40	40	20
S5	65	0.26	4.0	46.5	7.1	10.6	70	0	30

TABLE 12.

CELL YIELDS FOR CULTURES S4 and S5 GROWN AT VARIOUS TEMP.

(From Sukatsch and Johnson 1972)

GROWTH TEMP.	CELL YIELD (g.cell/ g.alkane)	MAX. DOUBLING TIME (Hrs.)	POPULATION RATIO		
			ORG. A	ORG. B	ORG. C
(Culture S4)					
25	1.02	1.0	30	70	0
35	1.02	1.0	50	50	0
45	0.93	1.2	35	60	5
55	0.43	3.0	40	40	20
65	0.15	5.5	30	40	30
(Culture S5)					
25	1.06	1.0	30	70	0
35	1.08	1.0	50	50	0
45	1.06	1.5	60	40	0
55	0.36	3.0	90	0	10
65	0.23	4.5	70	0	30

TABLE 13.

AMINO ACID COMPOSITION OF SOME METHANE OXIDISING CULTURES.

AMINO ACID.	PERCENTAGE DRY WEIGHT OF PROTEIN CONTENT OF DIFFERENT CULTURE								
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
ALANINE	7.69	7.23	X	7.9	6.6	7.7	6.2	6.4	6.0
ARGININE	6.14	5.0	5.0	5.7	5.1	5.1	4.8	4.5	4.3
ASPARTATE	8.80	9.1	X	7.8	9.0	10.6	8.0	8.5	7.9
CYSTINE	0.28	0.35	0	0.9	1.3	1.0	0.7	0.8	0.7
GLUTAMATE	10.2	13.8	11.5	9.7	9.6	11.5	8.8	10.3	10.3
GLYCINE	5.60	5.8	7.7	5.5	4.6	5.4	4.5	4.8	4.4
HISTIDINE	1.87	1.6	1.9	2.2	1.8	2.5	1.7	2.0	2.0
ISOLEUCINE	4.94	5.0	6.1	4.8	4.5	4.9	4.0	4.7	4.0
LEUCINE	6.95	7.9	9.1	8.8	7.5	8.6	6.7	8.0	6.7
LYSINE	5.36	4.3	5.3	5.4	4.8	6.1	4.8	4.8	4.9
METHIONINE	1.88	3.0	3.4	1.3	2.3	2.2	1.9	1.5	1.9
PHENYLALANINE	4.06	5.2	6.2	4.8	4.3	4.6	4.0	4.7	3.9
PROLINE	1.95	3.8	X	X	X	X	X	X	X
SERINE	3.07	3.9	X	3.4	3.6	3.7	3.2	3.8	3.2
THREONINE	4.29	4.9	4.5	4.5	4.4	5.0	4.2	5.4	4.2
TYROSINE	3.51	3.8	4.1	3.5	3.6	4.1	3.5	3.7	3.6
VALINE	5.91	6.2	8.5	6.5	6.2	6.2	5.5	5.7	5.2
TRYPTOPHAN	trace	2.7	0	1.9	2.4	2.6	1.2	1.0	1.8

(1) From Bewersdorff and Dostalek (1971) Culture TM10
(2) From Sheehan and Johnson (1971) Culture M45
(3) From Vary and Johnson (1967) Culture HR
(4) From D'Mello (1972) Culture OB3b
(5) do. do. Culture BiSNO3
(6) do. do. Culture AZo
(7) do. do. Culture MC
(8) do. do. Culture BG8
(9) do. do. Culture 8

TABLE 14.

EFFECT OF pH ON GENERATION TIMES AND CELL YIELD OF CULTURE HR.

(From Vary and Johnson 1967).

pH	GENERATION Time (Hr.)	CELL YIELDS YCH4	Y02
5.42	0	0	0
5.68	5.4	0.61	0.19
6.30	3.5	0.67	0.22
6.40	3.6	0.64	0.21
6.83	4.3	0.57	0.18
7.10	6.3	0.55	0.17
7.69	0	0	0

TABLE (15)

SOLUBILITIES OF THE FERMENTATION GASES IN PURE WATER AT 40°C AND 760 m.m. Hg PRESSURE:

GAS	SOLUBILITY (VOLUME GAS AT S.T.P. IN 1 VOLUME WATER)
CARBON DIOXIDE	0.530
OXYGEN	0.02306
METHANE	0.02102
ETHANE	0.02897

TABLE 16.

PERCENTAGE COMPOSITIONS OF NATURAL GAS. (EASINGTON SAMPLE)

NITROGEN	1.16
HELIUM	0.03
CARBON DIOXIDE	0.48
METHANE	94.02
ETHANE	3.25
PROPANE	0.61
ISOBUTANE	0.11
n-BUTANE	0.13
PENTANES	0.08
HEXANES	0.04
HEPTANES	0.02
OCTANES	0.02
BENZENE	0.04
TOLUENE	0.01

PART (B) MATERIALS AND METHODS (I)

THEORETICAL BACKGROUND

TO THE DESIGN OF THE

NATURAL GAS FERMENTATION SYSTEM

P. (28) - (109).

(1) EFFECTS OF pH: GENERAL

It has been generally realised for some years that most species of micro-organisms grow optimally at fairly specific pH values, and methane oxidisers are no exception. (See section 22). Changes in pH can affect both the growth rate and chemical composition of cells, especially the emzymic patterns.

Growth of a culture causes the pH to change in the first place, so much so that the rate of change of pH as measured by the rate of addition of a pH control reagent, has been used as an indication of the rate of growth of cultures or of the rate of product formation (Kempe et al. 1956). This principle was of importance to the work described here since corroboration of growth rates (as set by the dilution rate) was possible. Also pH changes reflects culture behaviour closely, and gave the first indication of culture upsets.

In batch culture pH change may cause the inception of the stationary or death phase of growth. For these reasons it is essential to measure and control pH at selected levels reflecting the optimum values demanded by the culture.

The scientific literature contains many references to the effects of varying pH on growth in addition to those described in section (22). Gale and Epps (1942) reported that with Escherichia coli the enzymic content was influenced by pH. This was especially true of the deaminases and decarboxylases, even though growth did occur throughout the range pH 4.5 - 9.

Blackwood et al. (1956) demonstrated an interesting effect of pH, again using E. coli. Using automatic pH control, they showed that at pH 6.2, carbon dioxide and hydrogen were formed during the fermentation of glucose, whilst at pH 7.8, inactivation of formic hydrogen lyase prevented/

prevented these gases from being produced.

It has been shown that in Aerobacter cloacae, the yield drops and the generation time doubles by a decrease in pH of two units (Hernandez and Johnson 1967).

Morphological changes have been observed often after seemingly minor pH changes. For example, Pirt and Callow (1959) found that the hyphal length of Penicillium chrysogenum decreased when the pH was greater than 6.0. In addition, swellings on the hyphal tips occurred at pH 7.0 and over. Thus it can be seen that the pH of a culture has profound effects on cells. It is generally accepted that in fermentation work, accurate measurement and control of pH is essential, and several methods of achieving this have been described.

(2) MEASUREMENT OF pH.

An excellent discussion of pH measurements has been given by Munro (1970). This article contains background details of the principles of pH measurement, tables of standards and buffer solution recipes. Indicators and electronic measurement are included.

Indicator dyes and all non continuous measuring techniques were inconvenient for control purposes but electrodes capable of pH measurement have been developed by several firms. The Hydrogen electrode constitutes the fundamental indicator of pH, because this is the reference against which all glass electrodes are compared. The hydrogen electrode is a half cell and must be combined with a reference probe of fixed potential in order to measure pH. According to Munro the hydrogen electrode has many disadvantages for microbiological work and therefore the glass electrode is always used.

In essence the glass electrode consists of a pH sensitive glass membrane fused onto a stem, but the constructional details vary from

from model to model. The electrode used in the work described in this thesis was by Activion Ltd. (See part (C) section (3B)). but several other makes are suitable for fermentation work.

The potential of the glass electrode to the outer reference electrode is influenced by the temperature of operation, the exact magnitude of this effect being determined by the nature of the inner electrode and of the liquid in which it is immersed. Thus such electrodes must be used in conjunction with temperature control.

There are two basic types of glass electrode;

(a) Separate glass/reference electrodes

and

(b) Combined glass/reference electrodes

It was decided that the combined version would be most convenient for use in the work reported here, since only one mounting port was necessary and fewer electrical connections were needed. Since the electrodes were sterilized in situ, it was felt that the disadvantage of higher cost would be offset by the ease of use of the combined electrode.

Not all pH electrodes are steam sterilizable, but the existence of autoclavable electrodes completely eliminates the need to use any other type in fermentation work. Autoclavable electrodes are only marginally more expensive than ordinary ones and are time and labour saving. It is possible to sterilize electrodes by other means but such methods are less convenient and not as certain as autoclaving. Ethylene oxide as used by Callow and Pirt (1956) and by Tempest (1965[a]) seemed to work well, but the technique was somewhat dangerous since ethylene oxideforms an explosive mixture with air.

(3) CONTROL OF pH.

An/

An accurate electrode system is the basis of pH control, and linked to a pH meter/controller or to a pH meter connected to a recorder/controller operating valves or pumps constitutes the complete pH control loop.

The work described in this thesis employed an E.I.L. pH meter/controller linked to a slave recorder, mainly because this arrangement used up the least panel space. (See Figure 2). Other arrangements have been used. Callow and Pirt (1956) controlled pH my means of a pH meter linked to a recorder/controller, but according to Solomons (1969) this scheme consumed a considerable amount of mounting space. Practical details of pH measurement and control used in the project reported herein have been given in section (3B) of part (C).

(4) MEASUREMENT OF DISSOLVED OXYGEN; METABOLIC EFFECTS OF OXYGEN

The importance of maintaining an exact and constant gaseous environment is unquestionable, and the need to provide as constant a supply of dissolved gases as possible made this even more critical to the work reported here. Continuous culture was used and thus it was of prime importance to ensure a constant and reproducible supply of gaseous substrates so that the continued growth of a stable culture was possible.

(5) EFFECTS OF HYPERBARIC OXYGEN

Oxygen has been known to have many complex and obscure effects on micro-organisms for many years. Oxygen toxicity has been shown to be due to both excess oxygen and to oxygen depletion in a culture. For example Dalton and Postgate (1969) showed clearly that in continuous culture bacteria could be inhibited by high rates of oxygen transfer. It was noticed that very efficient agitation and aeration completely prevented the initiation of growth of Azotobacter chroococcum yet growth

Zobell and Hittle (1967) were able to distinguish between the effects of oxygen and hydrostatic pressure on E. coli, Bacillus subtilis and B. megatherium. Oxygen was found to be lethal at 100 atmospheres whereas nitrogen at the same pressure was not, according to Stuart et al. (1962), using baker's yeast.

Although unable to offer an explanation, Phillips and Johnson (1961) noticed that hyperbaric 02 reduced yields of bacteria; thus the simple axiom that the main problem associated with the culture of aerobes is the provision of sufficient oxygen is seen to be an oversimplification of the issue. The effects of hyperbaric oxygen on microbial cells has been the subject of an excellent review by Wimpenny (1969b). Excess oxygen as a regulator of metabolism was of interest because in the work described in this thesis, high gas transfer rates and pure oxygen in the gas phase were employed (See section 17) in an attempt to obtain high biomass levels. Any adverse effects of such a scheme were of great interest.

(6) CRITICAL LEVELS OF OXYGEN

Oxygen has extreme effects in certain cases, strict aerobes and anaerobes naturally are influenced in an extreme way by the presence or absence of oxygen in that no growth occurs or growth does occur depending on the relationship of the organism to oxygen. For most aerobic micro-organisms it seems to be generally true that oxygen is second only in importance to the carbon substrate in affecting growth. It has been shown that there is a critical level of dissolved oxygen above which the rate of respiration is independant of the concentration of dissolved oxygen. Harrison and Pirt (1967) found this critical level/

level to be about 10 m.m. Hg. A range of 10-5 m.m. Hg constituted a transition region leading to the limiting condition of oxygen depletion, and in this region complex oscillations in dissolved oxygen tension occurred. The critical level of oxygen seems to depend on the culture conditions and of course the nature of the organisms studied. Ferens and Squires (1969) indicated that in the case of cephalosporin C and capreomycin fermentation at least, there was a difference between the minimum oxygen tension allowable for maximum product formation and the critical dissolved level as defined above, which is related to 02 uptake and to the respiration rate. A difference of 5-15% for both fermentations was observed although in the case of the capreomycin, the minimum 02 tension was lower than the critical 02 level whilst the reverse was true for the cephalosporin C fermentation.

Gottlieb and Anderson (1948) studied the effect of the formation of Streptomycin and noted that the maximum 02 uptake occurred at circa 12 hours of maximum growth. The product formation commenced between 12-24 hours and none at all at the time of maximum respiratory activity. Maximum growth occurred at 36 hours. It can be concluded that there is no simple relationship between product formation and the uptake of oxygen.

Below the critical dissolved oxygen tension (DOT), the organisms investigated by Harrison and Pirt (1967), Harrison, MacLennan and Pirt (1969), Moss et al. (1969) and MacLennan ,Ousby et al. (1971) exhibited an adaptive response in that 02 uptake rate was stimulated or that the affinity of the cells for oxygen was stimulated.

(7) EFFECT OF REDUCED DOT ON CELL YIELD

In terms of biomass production, changes in growth characteristics as/

as described above have significant implications. When a culture is growing at DOT levels below the critical level, the yield drops because of inefficient assimilation of O2 and carbon into cellular material. Since it has generally been agreed that high yield factors are of the greatest importance in the economics of biomass production, such a state of inefficiency is clearly undesirable.

Yield has been shown to be affected both by high and low oxygen concentrations. Hirose et al (1966) studied the effect of aeration on glutamic acid fermentations. Extremely high and very low O2 levels were observed to reduce cell yield and productivity. Interestingly it has been noted that certain organisms display optimum cell yields at less than wholly aerobic conditions, for example Downey (1966) reported that with Bacillus stearothermophilus the highest yield was seen at a level of dissolved oxygen at which the organism could not grow aerobically.

(8) EFFECT OF O2 ON THE YIELD OF METHANE OXIDISERS:

Methane bacteria, since they have been shown to require more O2 per mole of methane consumed than carbohydrate oxidisers, (see Section 15, Part A) might be expected to exhibit drastic reductions in yield at low O2 levels. In fact Sheehan and Johnson (1971) found that the mixed culture they had isolated showed a higher yield when O2 limited than when CH_4 limited in continuous culture. Bewersdorff and Dostalek (1971) reported that the productivity of culture TM10 was higher with increased O2 levels in the gas phase. At an O2 flow rate of 0.4 litres/litre culture/hour the productivity was about 0.06 whilst at a flow rate of 1.0 L/L/Hour, the productivity rose to 0.14 litres/litre culture/hour Smith and Johnson (1954) showed clearly that there was a direct correlation between aeration/

aeration efficiency and cell yields/unit substrate consumed and also that the total fermenter productivity was increased at higher 02 flow rates.

It is certainly true that much more research into the reasons for reduced yields often seen in methane utilisers compared to yields of other hydrocarbon oxidisers. Johnson (1967) has suggested that the reason for this disparity may be that if the alkane is attacked at a terminal methyl group during oxidation by a mixed function oxidase as seems likely (Johnson 1964) then one mole of 02 is consumed and no adenosine triphosphate released. When a higher alkane is oxidised only a small percentage of the 02 used is consumed in the terminal methyl group oxidation. Methane, however, when oxidised consumes one mole 02 per mole CH_4 attacked by the oxidase so that the yield on oxygen is correspondingly less.

(9) DISSOLVED 02 ELECTRODES: GENERAL

It can be seen that dissolved 02 has profound and important effects on cells and to quote Wimpenny (1969b)"There is a fine distinction between oxygen toxicity and supra-optimal aeration conditions". The level of 02 in a culture has usually been taken for granted provided growth has occurred but it was felt that for the work reported in this thesis measurement of DOT was a prerequisite of successful management of the fermentation process. To this end dissolved 02 electrodes were considered in order to select the best type most suited to this particular application.

02 electrodes have been available in commercially produced forms for several years and most of these can be applied successfully to fermentation/

fermentation work in the short term. It has been the experience of workers in these laboratories that few if any could be guaranteed to give stable and predictable measurements over the considerable periods of time commonly required in continuous culture - circa 1000 hours. An electrode was designed and built here to overcome the problem of short term stability. Details of the electrode have been presented in section (5) part (C) of this thesis.

(10) TYPES OF O2 ELECTRODE

There are fundamentally two types of electrode used in biological work;

(1) the open electrode

and

(2) the membrane covered electrode

In fermentation work, only the latter type has found general application. This has been because although open electrodes measure the actual O2 concentration rather than tension, recalibration is necessary if the nature of the culture fluid is changed. In addition, poisoning of the electrode is usually very rapid, and thus can only be used for short term measurements. Finally they cannot be applied to the measurements of gases in the gas phase.

(11) OXYGEN TENSION

Beechey and Ribbons (1972) defined the O2 electrode as "A device that produces an electric current which is proportional to the concentration of oxygen in the medium in which the electrode is placed". In fact, as noted by Kinsey and Bottomley (1963), this is only true for open electrodes. Membrane covered electrodes measure the activity or tension of oxygen. Oxygen tension is proportional to concentration for any given/

given liquid system but if the characteristics of the liquid change such that the solubility of 02 is altered then the relationship of tension to concentration alters also. Strohm and Dale (1961) have also pointed out the distinction between 02 tension and concentration measurements and due to poor definition of terms, this aspect of dissolved oxygen electrodes is still a matter of confusion for many people.

Oxygen tension is the partial pressure of 02 in a gas which is required to maintain equilibrium at a certain level of dissolved 02 in a liquid over which the gas is held. 02 concentration is the absolute quantity of 02 present per unit volume of a gas or liquid (Kinsey and Bottomley 1963). Siegall and Gaden (1962) have defined 02 tension more accurately as being proportional to the 02 partial pressure in equilibrium with the 02 in solution. Apparently the output of DOT electrodes is now generally interpreted according to this definition, but with some reservations depending on both the actual design features of the electrode and the conditions of operation.

(12) DESIGN OF 02 ELECTRODES

In an excellent paper, Krebs and Haddard (1972) reported that for the correct operation of an amperometric electrode i.e. one which produces a current rather than a voltage, two conditions must be fulfilled:

(1) The applied potential to the indicating electrode be it anodic or cathodic should be of sufficient anodicity or cathodicity that only the species of molecule under analysis reacts, and that furthermore, the species reacts completely. This ensures that the concentration of the molecules at the electrode surface is essentially zero.

(2) There must be a fixed diffusion geometry between the electrode and the bulk of the measured sample.

With open electrodes the latter condition is difficult to achieve and sensitivity to flow effects around the electrode surface is high. The development of the first effective membrane covered electrode by Clark (1956) was probably the breakthrough needed in electrode technology. Clark's work led the way to the development of many successful electrode designs.

The membrane covered electrode can be used to measure O2 levels in both the liquid phase and the gas phase providing that the gas is kept saturated with water to prevent the membrane drying up. The covered electrode is almost independent of the environment in which it is placed but the output depends on its electrochemical identity. Electrodes requiring an applied potential are known as polarographic and those which can be used to drive an ammeter directly are referred to as galvanic; essentially their behaviour is identical.

(13) FEATURES OF AN IDEAL ELECTRODE

With O2 electrodes as with most fermentation equipment there are several ideal design criteria which should be incorporated as far as possible into a given design. Not all of these criteria can be fulfilled in practice. The features which would constitute an ideal electrode are:

(1) Mechanical strength.

(2) Shape and size appropriate to its application.

(3) Functional in the horizontal position if necessary.

(4) Autoclavable, preferably in situ, without alteration of the calibration characteristics.

(5)
/

(5) Stable calibration throughout long usage.

(6) The capacity of the electrolyte should be great enough to 'buffer' chemical changes due to 02 reaction.

(7) Simple to dismantle and replace components if required, preferably without interruption of the fermentation.

(8) The membrane should be standardised in such a way that it can be replaced and give reproducable readings.

(9) A rapid rate of response is preferable if a control function is required.

(10) High degree of accuracy, especially at low 02 tensions.

The choice of electrochemical system, type of membrane, thickness of the membrane, electrode geometry and whether to use temperature compensation are still largely matters of experience and judicious guesswork. Fortunately a review of the literature highlighted some of the complexities and contradictions which have arisen in the gestation of practical electrodes and this information was used to gain an understanding of the effects of the conditions of operation on the response of electrodes of a given design, and to undertake the design of a workable electrode.

(14) IMPROVEMENTS TO BASIC ELECTRODE DESIGN

Under conditions of continuous culture, according to Harrison (1965) galvanic probes seem to be better suited to such work than are polargraphic electrodes. Whether this can be considered as a general rule is not yet clear and no work on this aspect 02 electrode design has been carried out in these laboratories.

The viscosity of the fermentation broth has been shown to affect electrode operation. Aiba and Huang (1969) studied the effects of liquid/

liquid viscosity on the electrode current and found that when the liquid viscosity was greater than 10 centi poises then the value of the current decreased. This was attributed to the fact that the rate of O2 diffusion was limited by the liquid film resistance and not by the membrane resistance. Aiba and Huang concluded that for viscous fluids, a thicker membrane and/or one of lower permeability should be used. In this event the rate of response will be reduced. Both Mackereth and Beckman electrodes were used in the study and it was noted that the Mackereth version gave a smoother output and did not suffer from bubble interference as the Beckman model did. The effect of gas bubbles impinging on the membrane surface and distorting the relationship between output/DOT in the case of the Beckman electrode may have been due to the mounting position.

The original Mackereth design (Mackereth 1964) was the most sensitive of the early galvanic electrodes but at that time suffered from the disadvantage of not being autoclavable. Since 1964 several improvements to the Mackereth electrode have been made.

Flynn *et al* (1967) replaced the polythene membrane with a silicone one which was thicker and stronger but had the same permeability. Flynn reported poor stability of output from the Mackereth model and felt that this was due to loss of membrane diffusion control at high O2 tensions. In effect this meant that the reaction rate at the anode varied as the lead of the cathode was consumed and therefore the output varied correspondingly. To obviate this effect, they reduced the cathode area relative to the anode and thus reduced the current density of the anode.

A later modification by Harrison and Melbourne (1970) followed from/

from the innovation of a new hydrocarbon resistant membrane by MacLennan and Pirt (1965) called fluroethylene propylene (FEP). FEP had the added advantage of being resistant to temperatures as high as 250°C and could therefore be autoclaved. Harrison and Melbourne applied the new membrane material to the basic Mackereth electrode and by changing the electrolyte to one of potassium carbonate in potassium bicarbonate and glycerol were able to autoclave the probe without damage to the membrane and without the electrolyte boiling off. At the same time the electrode was clearly suitable for hydrocarbon fermentations.

(15) FACTORS WHICH INFLUENCE ELECTRODE PERFORMANCE

Since Clark (1956) published on the first practical membrane covered probe the state of the art of electrode design has improved manifold, but to appreciate the suitability of a given design it is necessary to understand the factors influencing electrode performance.

(1) DIFFUSION RESISTANCES

There are essentially three diffusion gradients to consider when a membrane covered electrode is covered. Two are concentration gradients and one is a pressure gradient across the membrane. It is usual to consider the concentration of oxygen in the electrode to be zero. Depending on the flow rate of liquid past the membrane the pattern of the oxygen diffusion path may be different.

If the velocity of liquid is high then the membrane pressure gradient will be the significant variable. In the case of the flow rate being low or static, it is possible for a concentration gradient build up around the membrane on the outside. When this occurs, the electrode measures some intermediate function lying between tension and concentration (Kinsey and Bottomley 1962). It has been recommended by Kinsey and Bottomley that covered electrodes be used in conjunction with some form/

form of agitation in the sample liquid. The thicker the membrane the higher the likelihood that the electrode will measure tension since the resistance to passage of oxygen is mostly in the membrane. At the same time the measuring sensitivity of the electrode is reduced by a thicker membrane, and this must be balanced against flow sensitivity effects.

In fermentation systems where high rates of agitation and aeration are employed flow sensitivity is unlikely to prove to be troublesome providing that the pattern of flow around the electrode remains constant. Calibration of an electrode for use in a particular aeration/agitation system may give erroneous readings when used at lower gas flow rates and lower rates of agitation (C.C. Williams pers. communication).

(2) ELECTROCHEMICAL SYSTEM

The choice of electrochemical system affects the performance of an electrode, especially in long term usage. Platinum has been used in cathode construction because it can be fused into a glass body without damage to give effective insulation. Poisoning from hydrogen sulphide appears to be more pronounced with platinum. Gold and silver have also been used in cathodes. Platinum, silver, lead and aluminium have all been used to construct anodes and as far as is apparent there are no particular advantages with any of the above systems compared with any other arrangement. Table \ (17) has been presented to summarise some electrochemical systems published in the literature.

The electrolyte most commonly used has been potassium chloride although Carritt and Kanwisher (1959) have advocated the use of potassium hydroxide solution as an electrolyte. This was because the reaction of the/

the reaction of the electrolyte;

$$O_2 + 4_e + 2H^+ = 2OH^-$$

produced OH^- ions at the cathode and Carritt and Kanwisher claimed that the replacement of Cl^- ions by OH^- ions caused a gradual change in the electrode output. It is probable that this phenomenon is more extreme with electroded of small volumes of electrolyte. Certainly the electrode of Carritt and Kanwisher used minute quantities of electrolyte. According to Phillips and Johnson (1961), the use of potassium hydroxide caused deposits of silver oxide Ag20 to form and this had a tendency to fall off into the bulk of the electrolyte. The Ag20 accumulated on the membrane surface and reduced the sensitivity of the electrode. At the same time the solubility of Ag20 was higher than that of AgCl and thus the base current was greater. Phillips and Johnson recommended that a high concentration of potassium chloride be used since the life of an electrode is determined by the availability of Cl^- ions, as well as silver ions. One problem which saturated KCl electrolyte has been reported by Phillips and Johnson and that was the fact that complex ions formed with the AgCl. The result was severe electroplating and an elevated base current. It was noticed that the electroplating can be so severe as to cause short circuiting of the electrode. The optimum concentration of KCl was stated as about 0.75 molar.

A variation on the KCl electrolyte system was reported by Gore and Phillips (1964). Essentially the actual electrolyte was KCl/water but a 5% addition of ethylene glycol was used. This arrangement had the advantage that the electrode could be autoclaved providing that there was some means to equilibrate pressures in the body of the electrode during autoclaving.

(3) MEMBRANES

Most of the membranes used in early electrodes were made of polyethylene and therefore were unsuitable for both hydrocarbon fermentation work and for in situ autoclaving. Materials used more recently have included Teflon (FEP), polypropylene, silicone, polytetrafluroethylene, Mylar, natural rubber and polyvinylchloride. Not all have been successful innovations. According to Severinghaus and Bradley (1958), Mylar was too impermeable. Natural rubbers tend to be tooo permeable to substances other than oxygen. To date Teflon has been the material of choice for autoclavable electrodes. The use of FEP by MacLennan and Pirt (1965) and by Harrison and Melbourne (1970) in hydrocarbin fermentations has already been noted.

A nylon-reinforced silicone membrane has been used on the electrodes in the work reported in this thesis. (See section 5 of part C).

(4) TEMPERATURE EFFECTS

Oxygen electrodes have been shown to have very high temperature coefficients which can significantly affect the measurements (Mancy and Westgarth 1962). There have been several published accounts of electrodes using some form of temperature compensation circuit. One of the most simple was that of Morisi (1965). The work reported in this thesis involved fermenter temperature control and it was decided that temperature compensation was unnecessary, although had the control point of 40°C been changed then recalibration of the electrode would have been required. An alternative method of overcoming temperature effects was proposed by Mancy and Westgarth (1962): They proposed the use of nomographic charts from which the amount of temperature compensation needed to accommodate the temperature coefficient could be estimated. Equations to facilitate this procedure were derived.

According/

According to Brown (1970) some indication of the degree to which temperature coefficients can be gained by "Consideration of the basic design of the device" but little supporting information was given. It was stated that the temperature effect noted by Mancy and Westgarth (1962) was due to changes in the permeability of the membrane. These workers have shown that essentially all the resistance to 02 transfer resided in the membrane and presumably Brown alluded to the fact that information on resistances of a particular electrode could allow the reasons for a given temperature effects to be determined.

(16) OXYGEN TENSION MEASUREMENT BY MEANS OF THE COIL METHOD

One other technique of O_2 tension measurement has been investigated in these laboratories and that is known as the tubing or coil method.

Relatively few workers have published papers on the coil method perhaps because of the fact that more extensive and expensive equipment is necessary to measure O_2 tension by this method.

The technique of the tubing method is simple in principle; a coil of suitable permeable material, e.g. teflon or P.T.F.E. is immersed in the fermentation broth and a stream of very pure nitrogen allowed to pass into the coil at a constant flow rate. The stream of nitrogen then passes into some form of O_2 analyser. Oxygen, present in the broth solution, diffuses into the coil in the same manner as for an electrochemical membrane electrode and its concentration is measured by the O_2 analyser.

Phillips and Johnson (1961) used a teflon coil of $^1/_8$" internal diameter, 40' in length and 0.012 thick. Nitrogen passed through at a flow rate of 20c.c. a minute. It was found by these workers that the sensitivity of the coil method was dependant on several factors, namely:

/

namely:

(1) Coil length,
(2) Coil thickness,
and (3) Coil diameter.

The sensitivity of the coil used by Phillips and Johnson was low with the physical dimensions employed. In addition, the Beckman O_2 analyser was not sufficiently sensitive itself for the extremely low O_2 tensions involved.

Roberts and Shepherd (1968), Solomons (1966) and Solomons (1969), made use of the Hersch meter, an instrument capable of measurements of O_2 in the parts per million range. These workers reported that greater sensitivity and accuracy was possible with this instrument. The comparison of the coil method and the electrode method of O_2 tension measurement performed by Phillips and Johnson (1961) was illuminating and has been presented below for ease of reference:

(1) The tubing method was not affected by autoclaving. This was in direct contrast to the effect such treatment had on the electrochemical probe, although both were autoclavable.

(2) More accuracy was noted with the tubing method and calibration seemed to be more stable. Calibration was easier than with the electrode.

(3) The tubing had an indefinite life span, which was certainly not true of the electrochemical probe.

(4) A measure average of both time and space was given by the coil method whereas the electrode gave only a time average.

(5) The coil was more robust.

(6) A slower initial response was observed with the coil than with the electrode, but the output reached a steady state value after step changes/

changes in the O_2 level sooner than did the electrode.

(7) More expensive ancilliary equipment was needed for the coil method than for the electrode.

Perhaps the major advantages of the coil method were:

(A) Its extreme long life, and

(B) That it could be used for the measurement of gases other than O_2 at the same time. For example carbon dioxide generated during growth and methane supplied as a substrate for growth may be measured by means of a coil insert.

The work described in this thesis has not included results of experiments using the coil method as these will be reported elsewhere.

(17) IMPORTANCE OF THE EFFICIENT MASS TRANSFER OF MATERIALS.

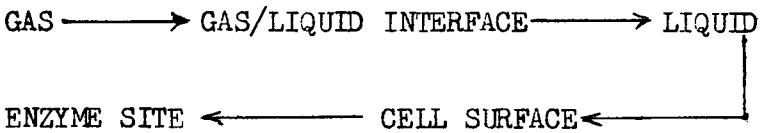
The term mass transfer coefficient has been used to describe fermenter performance in terms of gas transfer mixing ability. In methane fermentation work the ability to transfer as much methane and oxygen into solution as possible in as short a time as possible is essential in a vessel. Mass transfer is a function of both agitation and aeration and the two terms are usually considered together. In view of the importance of high mass transfer coefficients it is surprising that little serious attention was made to the subjects of aeration and agitation until the publication of an excellent review by Finn (1954). This paper sparked off much greater interest in the gas requirements of cultures and even today forms the basis for an understanding of the problems of transfering materials into solution in fermenters. In continuous culture of methane utilisers where high concentrations of biomass are required an understanding of gas transfer and mixing is essential especially where methane limited cultures are used/

used, in view of the poor solubilities of both O_2 and methane in water. (See Table 15).

(18) FACTORS INFLUENCING THE MASS TRANSFER COEFFICIENT.

In the fermentation system described in this thesis nitrogenous substrates and minerals were provided in the liquid feed (See section 6, part C). The importance of efficient mass transfer to this was that adequate mixing was essential to conform to the requirements of continuous culture theory (See section 53) but the most important aspect of mass transfer was the need to dissolve the carbon and oxygen substrates.

As oxygen and methane (and to a lesser extent ethane) passed into the culture fluid, the transfer pathway of gases into cells could be visualised in the following manner;



(After Solomons 1966)

Calderbank (1958) showed clearly that the gas liquid interface constitutes the barrier of greatest resistance to gas transfer. Agitation systems are designed to reduce the effect of this barrier as much as possible. This can be understood more readily by reference to the equation describing the rate of gas transfer; Elsworth et al (1957)

$$\frac{dC}{dT} = 3.6\ D.A.\ (C_e - C)g \qquad (1).$$

D (cm^2/sec. Fluid diffusion coefficient, a constant)

A (cm^2 area of gas/liquid interface.)

g (cm Thickness of stationary film at the gas liquid interface.)

C_e (m.moles/litre Saturation concentration of dissolved gas)

C (m.moles/litre concentration of dissolved gas in the liquid)

dC/dT (Rate of mass transfer)

3.6D/

3.6D was equivalent to Kl the mass transfer coefficient often quoted in reference to mass transfer efficiency. The rate of mass transfer is controlled by factors described in the expressions A/g and (C_e-C) . (C_e-C) is effectively the concentration gradient driving force.

An agitational system which reduces (A) by reducing the size of gas bubbles and g by increasing shear has an increased efficiency of gas transfer. In the case of oxygen the oxygen transfer rate (OTR) increases. The rate of gas transfer can be affected by several factors which are characteristics of either the agitational system or the aeration system:

Agitation (i) Impeller type
(ii) Stirrer speed
(iii) Liquid height
(iv) Rheology of fermentation fluid

Aeration (i) Sparger type
(ii) Gas velocity
(iii) Gas partial pressure
(iv) solubility of the gas; this is affected in turn by temperature and the presence of solutes.

Finn (1954) pointed out that it was not possible to completely aerate a culture vessel without employing some form of agitational system but the rate of gas transfer can be increased by several methods.

(i) Increased aeration rate. This true only until concentarion driving force has become to great so as to no longer affect the rate. At this point the gas/liquid interface constitutes the rate limiting resistance.

(ii) Increased agitation rate. This has three effects.

a) The bubble size is reduced and thus the area available for transfer is increased.

b)/

b) Greater shear forces reduce the liquid film thickness.

c) Increases the residence time of the gas bubbles in the vessel by increasing the length of the escape pathway.

(19) VOLUMETRIC MASS TRANSFER COEFFICIENT:

Wang and Humphrey (1968) represented the oxygen transfer rate by the equation

$$N_A = K_L A \quad (Cg - C_L) \qquad (2)$$

where $K_L A$ is the volumetric mass transfer coefficient. C_g and C_L are the gas concentrations in equilibrium with the gas partial pressure at the interface and the liquid gas concentartion respectively. KL and A must be considered together since it is extremely difficult to measure the area for mass transfer accurately nor indeed is it easy to estimate the mass transfer coefficient for gas transfer from the gas bubble interface to the bulk liquid i.e. the KL value. The expression $(C_g - C_L)$ represents the mean driving force and if perfect mixing is assumed then,

$$C_g - C_L \text{ mean} = C \text{ outlet gas} - C_L \qquad (3)$$

Hanhart et al (1963) reported that the above relationship was valid for small dimension equipment and if perfect mixing was produced. It was suggested by Wang and Humphrey (1968) that the actual driving force was between $C_g - C_L$ (outlet gas) and the opposite extreme case where the bubbles rise through the liquid without being dispersed but whilst the liquid itself is mixed thoroughly. In this case the driving force is a logarithmic mean.

$$(C_g - C_L) = \frac{C_{g\,in.} - C_{g\,out.}}{2.3 \text{ Log.} \dfrac{(C_g \text{ in} - C_L)}{(C_g \text{ out} - C_L)}}. \qquad (4).$$

The/

The actual condition of operation is such that neither of the two limit cases is effective but that neither the gas bubbles nor the liquid phase is perfectly mixed. The expression $(C_g - C_L)$ is much simpler to use but is subject to experimental error especially if the rate of gas uptake is great and C_g approaches C_L. (Wang and Humphrey 1968).

In the work reported in this thesis, the expression;

$$N_A = (C_g - C_L)$$

has been used to evaluate the performance of the fermenters. (See section 1 Part D).

Several agitational/aeration systems for fermentation work have been designed in the last twenty years but the exact design depends on the application. The work reported here employed a working temperature of 40°C thus the solubilities of both oxygen and methane were lower than at ambient temperature and to generate high biomass levels fermenters of exceptionally high masstransfer capability were used (see section 4 Part C).

(20) AGITATIONAL AND AERATION SYSTEMS:

The shake flask type of apparatus as used generally in microbiology and first described by Kluyver and Perquin (1933) are impractical on an industrial scale because of volume considerations. The mass transfer capability of such equipment is such as to allow the development of only low biomass levels. It has been claimed by McDaniel and Bailey (1969) that by operating a baffled shake flask at 230-385 revs. per minute, masstransfer rates in the region of those obtained in agitated fermenters. Figures quoted were in the region of 1 to 8 m.moles oxygen per litre per minute, as determined by the sulphite oxidation method of Cooper et al (1944).

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Work done in these labs. indicated that most fermenters were capable of much higher rates of transfer even at the low revs. used by McDaniel and Bailey.

The basic stirred tank fermenter is in essence a vessel with an impeller shaft possessing one or more blades which rotates. Gas bubbles entering the vessel via a sparge pipe are reduced in size and dispersed. At the same time the effects of agitation and aeration mentioned above take place.

(21) THE POWER/VOLUME AND TURBULENCE RELATIONSHIP.

Cooper Fernstrom and Miller (1944) reported that for a well designed fermenter the K_LA value is almost directly proportional to the stirring power applied per unit volume, i.e.

$$K_LA \qquad (\text{power/Volume})^{0\cdot95} \qquad (5)$$

A publication by Karow, Bartholomew and Sfat (1950) was in agreement with this statement.

According to Finn (1954) the power input was related to the degree of agitation only if there was complete turbulence and if there was no power loss through swirling or vortexing of the culture fluid.

Turbulence as defined by the Reynolds number is a dimension less term, i.e.

$$N_Re = \frac{L^2 N_p}{\mu} \qquad (6)$$

L is the impeller diameter,

P is the fluid density

u is the fluid viscosity,

N is the revs./unit time of the impeller shaft.

If the Reynolds number is above $N_Re = 10^5$ then the

$$\text{Power/Volume} = cN^3L^5p \qquad (7)$$

where/

where c is a constant depending on the impeller design. Equation (7) is valid for all N_Re values where turbulence is fully developed. In this situation the power uptake is independent of the fluid viscosity. Below regions of fully developed turbulence the power consumption is defined by the expression;

Power/Volume = $cN^3L^5p \div (L^2Np)$. As soon as turbulence becomes fully developed then the function (L^2Np) becomes zero (Bartholomew et al, 1950a).

(22) THE EFFECT OF BAFFLES ON MASS TRANSFER AND POWER UPTAKE:

It has been shown that agitation of a fermenter by means of a rotating impeller requires the presence of baffles to prevent swirling and vortexing, so that the impeller is able to create turbulence and dispersion in the culture. (Rushton et al 1950).

Some fully baffled arrangements were described by Mack and Kroll (1948). A standard version was of 4 baffles extending the whole length of the vessel and each were of about 1/10 the tank diameter. Elsworth (1957) described A 20 litre fermenter employing 4 baffles and noted that the sulphite oxidation rate reached a maximum when the baffles were $1/1/2"$ wide. Any further increase in width did not increase the oxidation rate.

(23) IMPELLER ARRANGEMENTS:

Impellers which give radial flow patterns rather than axial flows are more efficient in breaking up gas streams although it is true that propeller shaped blades giving axial flows induce higher flow rates than do radial impellers. (Finn 1954).

A combination of disc turbine (radial) at the shaft base and a propeller/

propeller blade above was described by Jensen (1966). The rationale of this unique arrangement was that the disc turbine allowed high gas transfer rates to occur whilst the propeller acted as an efficient mixer. Regrettably no empirical data for this system was given. Many forms of impeller have been used but the greatest amount of information has been derived from the use of 4 or 6 blade disc turbine impeller. The fermenters used in the work reported here used three 4 blade disc turbines coupled with an emulgator for increased emulsifying effect. (See secion 4 Part C).

Solomons (1969) stated that "Until such time as more sophisticated impellers are available it is recommended that the standard 6 blade disc turbine be used." When multi-impeller systems are used each disc should be situated at about one impeller diameter from each other.

The degree of agitation is often defined by the power uptake alone but this is usually inadequate because no information on how the power is distributed within a system can be derived. Reference to equation (7) illustrates this point.

$P/V = c\, N^3 D^5 p$ it can be seen that for any given power uptake a small impeller at high speeds could give the same degree of agitation as a large impeller at low speeds. The result is that the former system may be able to break up a gas stream into small bubbles but is then unable to disperse them throughout the vessel. The latter arrangement may well act as a good mixing pump but yet be unable to impose enough shear the gas stream to disperse it into bubbles. The actual compromise between these two extremes is usually arrived at by a combination of experience guesswork and empirical experiment. A rational approach to the design of agitational systems awaits a better understanding of gas transfer resistance and bubble mechanics, (Finn 1954).

(24) THE INFLUENCE OF LIQUID HEIGHT ON MASS TRANSFER.

Maxon and Johnson (1953) reported on the effect of liquid height on oxygen transfer efficiency. They found at a constant power input the OTR decreased with increasing liquid volume. The lower the gas flow rate there was the greater the effect appeared.

A depth factor was considered by Cooper et al (1944) in an attempt to relate the OTR for a given set of conditions with for a ratio of liquid height (Z) to vessel diameter (D). As Z/D increased the aeration efficiency increased correspondingly such that at a Z/D ratio of 2, the efficiency of gas transfer was 1.4 times as great as when the ratio was unity. This kind of information was of great importance in scale up of processes to production scale.

The fermenter working Z/D ratio in the work described in this thesis was 2.3 but other ratios were not tried.

(25) EFFECT OF AIR OR GAS VELOCITY:

When conditions of operation in fermentation work have been reported, the values of gas flow rates as an index of aeration has usually been quoted as volumes of gas per volume of liquid per minute (VVM). In fact Wise (1951) showed clearly that there was no relationship between gas holdup and K_LA. He plotted the K_LA/Flow rate against liquid volume and found that the two parameters were unrelated.

Cooper et al did find that there was a relationship between the K_LA value and the superficial or linear gas velocity. The relationship noted by Cooper et al was;

$$K_LA = \text{Constant.} \quad VS^{0.67} \qquad (8)$$

(VS is the linear gas velocity.)

Cooper et al reported further that flooding of the impeller at high flow rates (Circa 400ft /hr.) could occur and aeration efficiency did not increase/

increase with greater flow rates. The magnitude of the flooding was a function of the nature of the impeller type and speed of rotation. In this context, the disc turbine impellers were less susceptible to flooding than those connected directly to the shaft.

According to Bartholomew et al (1950b), aeration efficiency increased with superficial gas velocity only until the loading or flooding point. It is generally recognised that only the superficial gas velocity is an appropriate method of describing gas supply rates. Several other methods have been tried and these were listed by Lee (1950).

An additional feature of increased gas velocity was pointed out by Elsworth et al (1957). Increased entrainment gas in culture fluid tends to reduce the overall density of the liquid and thus power consumption is reduced. In the case of the work reported in this thesis it should be noted that changes in gas velocity can affect the control of liquid level in the fermenters. Level control was accomplished by measuring power consumption (See section 4 part C) and changes in power drawn due to changes in gas flow rates necessitated recalibration of the measuring element.

Elsworth remarked on the effect of gas flow on the concentration driving force. Using the sulphite oxidation method of Cooper et al (1944) to measure transfer rates Elsworth et al (1957) found that for a given gas flow rate the concentration driving force decreased as the OTR increased. These workers recommended that the gas velocity be adjusted in such a way as to preserve the concentration driving force at its maximum value.

(26) EFFECT OF SPARGER DESIGN ON AERATION EFFICIENCY

Most industrial fermenters have been designed so that gaseous substrates enter the vessel via a single orifice centrally placed below the impellers or via a series of orifices in a ring sparger or manifold.

Non-uniform gas dispersion has been observed for spargers of the pipe manifold type because of progressively reduced pressure drops along the ring. The closer the individual holes were to the gas source the less this effect was evident. (Dow 1950).

It has been noted that multi holed spargers are susceptible to plugging. Fortune (1950) reported that almost half of the holes of a ring sparger tended to block after a relatively short time of operation. It is probably true to say that the modern tendency is to use an open or slightly constricted pipe and to depend on high rates of agitation to produce high rates of mass transfer. In fact, a ring sparger has been used throughout the work reported in this thesis.

Chain and Gualandi (1954) showed that an open pipe sparger beneath the impeller was twice as efficient as a ring sparger which was 4 times the impeller diameter. Apparently this was due to the escape of gas along the fermenter walls. The system used in the work described here prevented escape of gas both due to entrainment along the vessel walls and along the impeller shaft by means of an efficient emulsifying agent - the emulgator (See section C Fig. 3).

An alternative to the ring or pipe sparger was investigated by Hixson and Gaden (1950). These workers employed a fine bubble aerator and noted that this form of aeration gave a greater aeration efficiency at/

at low gas velocities than a single orifice sparger but as the flow rate of gas increased this differential disappeared. Presumably this was due to an increased turbulence with the sparger at high flow rates. There is no reason why fine bubble aerators should not be used in conjunction with intense agitation, although blockage would be more likely to occur than with an open pipe or manifold sparger.

(27) THE EFFECT OF AGITATION AND AERATION ON MIXING:

Although the primary objective of agitation and aeration is to transfer gases into solution, both forces contribute to fluid mixing in a vessel. Oldshue (1960) suggested that in addition to those features of a mixing system which increase mass transfer;

(a) Blending of liquid/solids/gases throughout the entire vessel.

(b) Maintenance of a constant dissolved oxygen tension at all points in a fermenter,

and (c) The transfer of heat and distribution of it were all functions of a mixing system.

It can be added that perfect mixing is a prerequisite of continuous culture (see section 53 Part B), since the theory of the chemostat and turbidostat is based on it. Homogeneity of the vessel contents is vital when a product such as biomass is the end result of fermentation to ensure controlled quality and reproducible material. For a mixed culture of methane oxidising organisms, adequate mixing is necessary for culture stability.

Good mixing is important when automatic control of the fermentation process is undertaken. Transfer lags in control loops can be so great as to prevent automatic control (see section 32 Part B) and the/

the better the mixing the smaller the magnitude of transfer lags in the control of such parameters as pH, and temperature.

(28) SUMMARY OF IMPORTANT FACTORS IN IMPROVING MASS TRANSFER AND MIXING IN FERMENTERS:

Increased mass transfer and mixing can be obtained by a combination of one or more of the following;

(1). The presence of baffles.

(2) Increased speed of rotation of impeller shaft.

(3) Larger impellers.

(4) Higher gas velocity.

(5) Elevated system pressure and/or increased partial pressure of component gases.

(29) GENERAL AUTOMATIC CONTROL THEORY:

Reference to section (3) in Part C of this thesis shows that several fermentation parameters were controlled in an automatic fashion;

(i) pH

(ii) Temperature

(iii) Liquid level in the fermenters

(iv) Gas phase Methane
Ethane
Carbon dioxide
Oxygen concentrations

were all controlled automatically. Account must be taken of the factors which influence the operation of control loops before the most economic and effective loop can be applied to a given control function. Every control loop has unique features associated with it but there are general aspects of control loops which are common to all. An adequate understanding/

understanding of automatic control principles is essential to sensible design of an automatically controlled fermentation process.

A useful and detailed text on the subject of automatic process design was written by Young (1960) and an introduction to process control aimed at microbiologists was presented by MacLennan (1970).

(30) DESIGN OF CONTROL LOOPS:

From the beginning of the work described in this thesis a basic philosophy was to provide as constant a growth environment as possible. Automatic measurement/recording/control equipment were used extensively in part fulfillment of a controlled environment. The operational details of the control loops used in the work reported here have been presented in sections (3 , 4) in Part (C) of this thesis.

Equipment choice for the control loops was a compromise between;

(a) Economy and

(b) Effectiveness.

When a control loop is set up it should be remembered that;

(1) The controller need be only as sophisticated as a given application requires. For example there is no point in using a controller capable of proportional control (see section 35B) if adequate control of a parameter can be achieved by means of simple ON/OFF control. This was true of the control of pH and temperature in the work reported in this thesis.

(2) The sensitivity of the primary or measuring element need not be greater than the efficiency of control required warrants. If temperature can be controlled to within a degree without upset to the culture then it is extravagence to buy a temperature probe able to measure in fractions of a degree.

It must be added however, that some thought to future applications should be made when obtaining instruments, since it may well be more economical in the long term to buy more sophisticated equipment at the outset if it is likely to be required at a future date.

As instrumentation becomes more complex it often becomes more specialised in function. Should the project concept change slightly then much of the equipment becomes redundant. This was true of the methane biomass project reported here. For example a whole series of transducers analysers and flow meters would have become obsolete had it been decided that the gas phase composition was an unnecessary complication to the production of biomass from north sea gas. For this reason equipment was chosen that could be used in several applications or which could be modified at little cost. The Fischer-Porter differential pressure cells were used to measure flow of methane and oxygen into the recycle system (See section 7D, Part C and Figure 4). The accuracy of the instrument depended on it being used at specific pressures and temperatures and had been calibrated at these values. Had it become necessary to change the operating conditions then all that would have been necessary would be recalibration at the working values.

In some cases the use of specialised equipment was unavoidable. Obviously an oxygen analyser was essential to control the gas phase composition of oxygen.

(31) FEATURES OF A BASIC CONTROL LOOP:

All control loops can be considered as "closed loops" consisting of the following elements:

(a)

/

a) A sensory element, e.g. pH electrode

b) A measuring unit, e.g. gas analysers

c) A controlling element,

d) A regulating unit, e.g. valves or pumps.

e) The process which is controlled. The temperature of the fermenters for example.

f) The process variable by means of which the process is controlled. Cooling water or alkali for example. (See fig. 4).

To a large extent the effectiveness of a given control loop depends on the quality of the sensory/measuring element. A control loop is only as efficient as its measuring unit, and sensing and measuring elements can be assessed on the following features.

(i) Accuracy,

(ii) Reproducibility of output

(iii) Stability

(iv) Robustness

(v) Reliability

(32) FACTORS WHICH INFLUENCE CONTROL LOOP ACTION:

Every control loop has certain response lags associated with it, some of which are due to the physical limitations of the components of the loop, and others are due to the nature of the process, or the manner of action of the process variable.

The magnitude of the lags in the process and the associated instrumentation can be so great as to completely prevent automatic control from being accomplished. When a load change or process change occurs there is a delay before the process regains the value at which it is being/

being controlled, and a fermentation process should be designed in such a way as to minimise these delays. Each of the elements in a control loop is an integral part of that loop and the behaviour of each individual component contributes to the overall control loop performance.

(33) TYPES OF RESPONSE LAGS; TRANSFER LAGS:

Transfer lags are those delays due to time needed to transfer the effect of a control agent to the process. The largest lags of a system are usually the transfer lags. Transfer lags can be divided into two categories;

(1) Capacitance lags and

(2) Resistance lags.

(1) Capacitance lags; Basically in a control situation the problem is to transfer energy from a supply side to a demand side. The rate of transfer affects the time lags contributed by the process to the overall lag. The ratio of supply to demand capacities affects the rate of transfer; thus the supply capacity is defined by the ability of the supply to meet the demands of the fermentation process expressed through the control loop, e.g. the rate of cooling water supply. The demand side capacity is the ability of the process to store or consume energy supplied to it. The majority of the lags occur here.

If the Demand capacity/Supply capacity ratio is high, lags are great. On the other hand should the supply capacity/demand capacity be high then the response of a given loop is rapid, but if this ratio is too high then instability and cycling of the controlled parameter may develop.

(2) Resistance lags; A good example of resistance lags occurs in temperature/

temperature control. Resistance to flow along pipework, the thermal conductivity, and physical dimensions of the cooling system contributes to resistance lags.

The rates of transfer of control agents such as pH and temperature can be reduced by adequate mixing in the fermenter vessel (see section 27, Part B) and the fermenters used in the project described here were capable of excellent mixing. (See section 1 Part B). Good mixing reduces both capacitance and resistance lags but lags due to flow resistance remain and the thermal conductivity of the materials used in temperature control are normally inherent characteristics of that control loop.

(34) TIME CONSTANTS FOR CONTROL LOOPS:

In most cases it is not possible to entirely remove response lags from a control loop, and each loop has a time constant associated with it. Engineers define the time constant of a control loop as; that time taken for a process to change by 63.2% under control action after a load change has occurred. The rate of change is asymptotic so that the rate of change progressively decreases, as can be seen from fig. (6).

The effect of the time constant is that the indicated value is not the actual value. Thus for a time constant of 1 minute, with a process change in temperature of $3^{o}C$ per minute as an example, then the indicated temperature is $3^{o}C$ lower than the real temperature. Following from this, it is true that the greater the rate of process change, the greater the error is. Reference to Fig. (7) indicates this well. Fig.(7 a) shows a greater rate of process change than does Fig.(7 b). It is evident that the value difference between the true and indicated values/

values is larger for (a) than for (b). At the same time the slower rate of change seen in (b), whilst causing smaller error in the measured value means that the time constant is now greater. Some compromise between speed of response and error must be made.

Since the controller acts only on the measured value, problems arise when the process variable cycles. In the case of temperature control, although the indicated value cycles with the actual temperature, the time constant imposes a phase lag. Fig. (8) illustrates this point. The phase lag may become so great as to cause the regulating valve to open when it should close, or vice versa, and thereby exacerbate the cycling instead of reducing it.

The sensitivity of the sensing/measuring elements influences the lag effect since the more rapidly a process change can be sensed and measured the more quickly can control be achieved.

Perhaps more important to the functioning of a control loop than sheer accuracy and sensitivity in the sensing element is the stability and reproducibility of the sensing/measuring elements. It is essential that the output of the measuring station remains stable and proportional to the process parameter controlled. When applied to continuous culture, stability of calibration is vital to prolonged fermentation.

(35) TYPES OF CONTROLLER:

Automatic control can be achieved by means of several different forms of control loop, the nature of the process determines which is the most suitable.

(A) ON/OFF OR TWO POSITION CONTROL

Essentially ON/OFF control constitutes the simplest form of control involving the movement of the regulating to one of two extreme positions, e.g. open or closed.

ON/OFF control is cheap and usually applicable to cases where there is a large demand capacitance and low transfer lags. For instance pH and temperature controlled on the work presented in this thesis by ON/OFF control (See section 3 Part C).

The main disadvantage of ON/OFF control is that only small and slow load changes can be accommodated. In continuous culture pH can be effectively controlled by this means but in batch fermentation the load change may become too large for efficient control. Temperature can be reasonably well controlled in both batch and continuous cultures. Dissolved oxygen tension having a low demand side capacitance could not easily be controlled with an ON/OFF controller.

To partially compensate for time lags occurring in ON/OFF control a period timer can be incorporated into the basic loop. The net effect of the timer is that the controller can only operate the regulating unit at certain intervals of time. This tends to reduce overshoot of the process variable. A timer was used as part of the pH control loops in the work reported for this thesis.

In all ON/OFF control applications the measured value continuously cycles due to the nature of the control action. In the case where a valve forms the regulating element correct valve sizing can reduce the degree of cycling to the minimum and where a pump is in operation the cycling may be adjustable by changing the pump delivery speed.

(B) PROPORTIONAL CONTROL

In this form of control the output from the controller is proportional to the measured process change for a given variable. Theoretically the regulating action is equal and opposite to the deviation causing the movement of that regulating element. Thus it is possible for/

for the relationship between the movement or action of the regulating element and the deviation in the controlled parameter to vary; the relationship is referred to as the proportional band. Engineers define proportional band as that range of values of controlled condition which operates the regulating unit over 100% of its movement. The range of values is usually expressed as a percentage of the process scale range so that for instance if during a fermentation the pH changed by 75% and caused a regulating valve to move through 100% then the proportional band would be 75%. By changing the band setting the degree through which a valve opens for a given process load change can be altered.

The chief disadvantage of proportional control is that if a sustained load change occurs then the setting of the proportional band prevents the process variable from returning to the set point. A new setting of the proportional band is needed so that a new ratio of process change to corrective action more appropriate to the new conditions is obtained. If this is not done then a situation known as offset arises and persists so that the new value of the controlled parameter is always above or below the desired value. In effect, the controller controls about a new mean value. The larger the band setting the larger the offset is and vice versa, but if the setting is too low then cycling may result, because a small process change causes a relatively large movement in the regulating element, a situation which may lead to instability.

Under normal conditions of operation it is not possible to continuously adjust the proportional band setting and therefore proportional action alone is inappropriate where rapid and sustained load changes can be expected.

(C) /

(C) INTEGRAL OR RESET ACTION

Although smoother than ON/OFF control, the offset occuring with proportional control precludes its use for all control applications, and reset or integral action is used in conjunction with proportional control to remove the effect of permanent offset.

Reset action produces a signal transmitted to the regulating element which is determined both by the magnitude of the deviation and by the duration of the deviation from a set value. As long as the load change persists the signal from the controller increases until the set point is regained or until the regulating member is at the limit of its operation. Reset is considered to be a repeat of the action due to proportional control, and American instruments measure reset rate in terms of the number of times that reset action repeats proportional action per minute. Instruments made in this country describe reset action as a time in minutes needed to repeat proportional action, i.e. one is the reciprocal of the other.

Integral action should be used thoughtfully because its operation depends on a process change and must always follow on behind the action of the proportional control element. In effect reset action can be considered as contributing an additional lag to the system and the correct settings of reset action must be made. Too high a reset action setting may cause too great a corrective action and may even become so far out of phase as to exacerbate cycling rather than eliminate it. Often long recovery times are noted after process upsets when reset controllers are used.

Reset action has been used in the work presented in this thesis to control the gas phase compositions of ethane methane and oxygen (See section 3 Part C).

(D) DERIVATIVE CONTROL ACTION:

This form of control is defined as action.which is proportional to the rate of change of a measured process variable. It is not related to the ratio between the deviation and the set value. Corrective action due to derivative control does not alter if the rate of process change is constant but only when the rate changes does derivative action alter.

When derivative or so-called preact was first introduced it was hailed as the solution to all control problems but it is now evident that only in about $^{1}/_{20}{}^{th}$ of control applications is derivative action workable. Processes where load changes occur very slowly accept preact control readily but for fermentation work where measured parameters tend to have a high rate of change preact is virtually useless. Flow, pressure and level cannot usually be controlled by means of a derivative controller. It can be used with proportional or with proportional and reset action but never alone.

The work described here in fact employed a three term controller. This was the oxygen controller (see section 3/ Part C.) but although the derivative component of this instrument was switched off it was bought with other applications in mind, in addition to methane biomass production.

(36) SUMMARY OF THE CONTROL APPLICATIONS USED IN THIS THESIS:

1)	pH	ON/OFF plus timer and adjustable pump speed
2)	Temperature	ON/OFF with regulating valve
3)	Level	ON/OFF plus adjustable pump speeds
4)	Gas phase composition of:	
	methane ethane	proportional plus reset via regulating valve
	Oxygen Carbon dioxide	ON/OFF plus solenoid valve.

(37) METHODS OF STERILIZATION OF GAS SUPPLY:

Most aerobic submerged fermentation processes require a clean dry sterile supply of gases and north sea gas fermentations are no exception. A sterile gas supply can be provided in several ways, all of which are technically possible but only a few of which are economically feasible. For instance Humphrey (1960) listed:

a) Dry heat
b) Adiabatic compression
c) Irradiation
d) Electrostatic option
e) Seiving
f) Filtration

as being mechanisms of gas sterilization but as Cherry and Kemp (1963) pointed out in an excellent paper, only (a) and (f) have had any widespread application. In fullscale operations dry heat is regarded as being economically inviable. Heating whilst highly effective too has an added disadvantage in that a power failure will alter the discharge of contaminated gases into the atmosphere. This is a problem which can be solved by means of suitable valves and alarm trips. Certainly it is true that virtually all fermentation equipment designed in recent years have had filtration units as a means of sterilising the input and exit gases required for the process. In the early days of fermentation history the theory and techniques of filtration were incompletely understood. Not surprisingly poor design and maintenance led to failure of the fermentation. Gaden stated that in 1956 the air sterilisation units were still the weakest links in long term prevention of contamination. A paper by Ross (1958) pointed out that there was a need for a rational basis for the design of efficient air sterilising equipment. Now that this has been achieved it is a relatively simple procedure to design/

design and make a filter unit suitable for normal fermenter requirements. Unfortunately it remains true that many of the mechanisms of particle trapping in filters are still obscure, and until these are clarified it will not be possible to design filters from basic principles. Test programmes contribute the only valid method of filter design. Naturally testing procedures entail a degree of experience and judicious guess work.

(38) METHODS OF FILTRATION:

Filters are essentially one of two kinds. 1. Absolute and 2. non-absolute. The former are capable of retaining 100% of microbes as long as the filter remains in an undamaged state. Non-absolute filters prevent ingress of some high proportion of organisms, the exact proportion depending on its design characteristics. This is commonly in the region of 99.99% or better. Absolute filters are often referred to as membrane filters and non-absolute as depth or bed filters, and both types have entirely different modes of action. Depth filters are still the most favoured agents for air sterilisation partly through habit and partly because the early types of membrane filters were made of unsuitable materials, indeed as pointed out by Humphrey and Gaden (1956) "They are mechanically fragile, unable to stand heat sterilisation, and have a small capacity before serious plugging occurs". However, that was in 1956 and the situation is entirely changed now.

(39) MEMBRANE FILTERS

Cellulose esters are used to make modern membrane filters and these are capable of being autoclaved. It is still true that membrane filters have a low dirt handling capacity and this is a result of the nature of their/

their mode of action. A typical membrane filter is about 150 um thick and has a regular pore structure whose diameter is smaller than the microbes it is designed to hold back. Thus effectively there is 100% surface retention but equally true its dirt handling capacity is confined to a single layer. The use of membrane filters for the sterilisation has been described in section (6) of Part C of this thesis.

(40) DEPTH FILTERS: GENERAL

Depth filters act by forcing the gas stream plus any entrained particles through a mesh of fibres arranged in such a way that there is a high probability that such particles will be trapped in the mesh. This means that the statistical chance of trapping will be affected by changes in both the density and depth of the packing.

(41) MODE OF ACTION OF DEPTH FILTERS:

Normally many of the channels in the mat have diameters larger than those of the particles so the mechanism of retention is different from that of membrane filters and thus 100% retention cannot be guaranteed. Whilst depth filtration is not a simple process to understand it is generally agreed that the following factors are instrumental in causing entrappment.

1) Diffusion. Random motion of particles due to molecular bombardment enabling collision of fibre and particle.

2) Electrostatics. This is most significant in materials which are charged e.g. resins particularly with low flow rates and small particles.

3) Inertial interception. A particle will impinge on the fibres because its momentun is sufficient to take the particle out of the flow stream. Humphrey (1960) stated that different forces acted on particles at

at/

at different flow rates and this results in a flow rate for a given filter which gave minimum filter efficiency.

Depending on the size of the particle there will tendency to bounce off unless the forces of adhesion are great enough to prevent this. Van der Waals forces contribute significantly to adhesion (see Stenhouse (1969). In terms of performance, depth filtration can be expressed as functions of particle size, permeability/unit area, dirt handling capacity and unit area to give an index of retention efficiency. High retentivity and high permeability tend to be mutually exclusive, as noted by Russell (1971) and attempts to increase one result in a reduction of the other.

(42) TESTING OF DEPTH FILTERS:

Firman (1969) distinguished between High efficiency filters and Ultra high efficiency filters. For applications where sterility must be of a high standard, Ultra high efficiency filters were recommended. Firman characterised Highland Ultra High efficiency filtration as being capable of retaining;

H.E. 95-99.95% of particles and

U.H.E. Above 99.997% of particles.

In essence two general methods of filter testing have been developed.

(i) Physical

and (ii) Microbiological.

The former include the sodium penetration test as developed by Dorman (1965) in which the penetration of atomised sodium chloride is measured with a flame photometer, and the Methylene blue test (BS2831 1957) during which methylene blue (British standard test dust No.1) is drawn through a filter at the rated flow rate. Samples are collected on esparto/

esparto paper and the staining densities compared with known or derived standards. In fact it has been pointed out that although the methylene blue test requires less complicated equipment than the sodium penetration test, it is only suitable for testing of up to 60% penetration of methylene blue and in addition is usually time consuming.

In these laboratories microbiological testing methods have been exclusively used. (See Part D, Section 12 , of this thesis).

(43) FACTORS INFLUENCING FILTER PERFORMANCE:

It has been shown (Firman 1970) that penetration increases with increasing flow rate and that resistance to flow also increases with flow rate. Figure (9) has been presented to illustrate the relationship between penetration and flow rate and the pressure drop across both U.H.E. and H.E. filters. It can be seen that a filter should be used at a flow rate less than the maximum possible to increase filter life significantly. Firman suggested that flow rates in the order of 20/30% of maximum was ideal.

Since the plugging rate is proportional to the volume of gas passing through the filter, the life of the filter is therefore inversely proportional to the cercentage of maximum rated flow. Thus any filter has a maximum rated gas flow which should not be exceeded and lower flow rates giving efficient sterilisation over long periods (Elsworth 1969). It is generally accepted that most fibrous depth filters have an intermediate flow rate where the filter efficiency is lowest and testing procedures should be comprehensive enough to determine whether this flow rate is in the region of the working flow rate, (Humphrey and Gaden 1955).

To /

To make full use of the qualities of a depth filter, it is advisable to use a filter which has been rated for a flow rate greater than the actual required working flow rates so that both long life and maximum throughput can be obtained.

A study of the factors influencing fibrous filters by Humphrey and Gaden (1956) led to several general conclusions about the behaviour of different depth filter designs;

(a) The penetration of individual cells was logarithmic.

(b) At high velocities, inertial impaction was the major means of particle collection and filter efficiency increased with a decrease of filter fibre diameter.

(c) In low velocity regions an inverse flow relationship obtained for collection action - possibly an electrostatic action.

(d) An intermediate velocity region was noted where collection was least efficient because the two opposing velocity relationships mentioned in (b) and (c) above operated at the same time. (See Figure 10).

(e) The density of fibres and their distribution was more critical to filter efficiency than was the nature of the fibre material.

(44) CALCULATION OF FILTER EFFICIENCY:

Humphrey and Gaden (1956) pointed out that if the logarithmic relationship held then it was possible to derive a simplified equation from which filter efficiency could be calculated.

Thus according to Humphrey and Gaden;

$$\text{Efficiency} = \frac{100\ (N_o - N_1)}{N_o} \qquad (9)$$

This/

This could be expressed alternatively as a percentage penetration value. Thus;

$$\text{Penetration} = \frac{100\ N_1}{N_o} \qquad (10)$$

These relationships have been used in conjunction with microbiological testing methods to calculate the efficiency of the filters used in the work reported in this thesis. (See section 12 Part D).

The calculation of filter efficiencies has been the subject of several excellent articles and these should be referred to for a more detailed treatment of the subject. In addition to those papers already cited, those by Hale and Daniels (1961), Cherry et al (1951) and Grace (1956) gave good accounts of the calculation of filter efficiency.

(45) APPLICATIONS OF DEPTH FILTERS:

Although both membrane and depth filters have been used in these laboratories, depth filters were found to be most convenient for sterilisation of gases. Depth filters were employed in the following positions:

(1) On media resevoirs to allow pressure equilibration under sterile conditions during emptying and filling.

(2) On the fermenter inlet and exhaust ports. The filters used here were a specially provided ceramic type designed by the makers of the fermenters Chemap of Zurich (See Fig. 11). According to studies performed here they appeared to function mainly as depth filters but were quite different to those supplied for batch fermentation. These filters were used in preference to self-constructed ones because the stainless/

stainless steel housing enabled quick release couplings to be attached. (See section 2 Part C) and also because they were known to be safe in oxygen/methane service.

(3) On culture effluent collection vessels. In the same way that culture aerosols were generated in the fermenter and were removed by the ceramic filters on the exhaust ports, so the aerosols produced in the collection vessels had to be filtered to prevent contamination of the fermenter environs. Similarly to prevent deposition of cells in instruments filtration was necessary before the gases from the collection vessels were returned to the main recycle system. (See section 1A part C).

There was no condenser on the collection vessels and it was therefore possible for the gases to become saturated with liquid. Under these conditions filters behaved differently. According to Elsworth (1969) the penetration is maximum at low flow rates and falls off with increasing flow rates. Elsworth recommended that to accommodate these observations filters with large areas and at low flow rates with a low pressure drop or a filter of small area at high flow rates but of high pressure drop.

Tests were carried out on various filters and the results of these tests have been presented in section (12) part E. of this thesis.

(46) STERILIZATION OF LIQUID MEDIUM:

Advantages of Filtration

Sterilization of gaseous substrates by filtration is by now a widely accepted practice, and has been the usual method of sterilization in fermentation for some years (See section 37). However, for the sterilization of liquid media, filtration as a routine procedure has generally been completely ignored in the fermentation industry. In part this has been due to lack of familiarity with the technique and partly/

partly to the existence of established alternative methods, especially heat processing.

The applicability of heat as a sterilizing agent is restricted to those media which do not contain heat labile components. Whilst this exclusion does not apply to filtration, it is equally true that not all media may be sterilized by filtration. Liquids containing a high solids content or of high viscosity can only be filter sterilized in large quantities with great difficulty and at great expense.

Consideration of the fermentation system described in this thesis explains the adoption of filtration as the means of sterilizing liquid substrates.

(a) Continuous culture of the methane oxidisers necessitated the provision of large quantities of medium.

(b) The nearest large capacity autoclave was situated in a different building to the safety laborotories in which the fermenters had been placed.

(c) The liquid medium contained little particulate matter. (See Part C Section (6)).

(d) Use of the only large scale autoclave would mean that it was used on a full time basis exclusively for the sterilization of medium for the methane fermentation project. This was not feasible in a teaching department.

The maximum probable rate of medium consumption was calculated in the following manner;

Fermenter capacity	5 litres (x 2) = 10 litres
Maximum dilution rate	1.0 n^{-1}
Total medium required/Hr.	10 litres/hour
Total medium required/day	240 litres/24 hours.

The/

The capacity of the autoclave using 20 litre resevoir vessels was 40 litres with a minimum turnround cycle of 3 hours. Assuming a 12 hour shift, medium could have been produced at the rate of only 160 litres/day, maximum. In addition, autoclaving would have involved the use of at least 6 20 litre vessels. Filtration required the involvement of only 2 such resevoirs (See section 6/Part C.). Circumstances determined the choice of filtration as a sterilizing agent for the liquid medium.

(47) METHODS OF FILTRATION:

Both depth filtration and membrane filtration have been used to sterilize liquids, but it must be remembered that with depth filters no guarantee of absolute sterility is possible, and there are no degrees of sterility. Only a probability value of microbial penetration of the filter can be determined (See section 41 part B), but if used properly, there is no reason why depth filtration cannot be used to sterilize liquid media. Varying success has been achieved with depth filtration as a sterilizing agent and it is probably only with the development of the autocavable membrane filter that reliable and predictable sterilization could be achieved. Membrane filters have certain inherent advantages over depth filters when applied correctly; amongst these are the following:

(48) ADVANTAGES OF MEMBRANE FILTERS:

(1) High porosity. Up to 80% of the total volume of a membrane is void space, thus allowing a high rate of flow through (Mulvany 1969).

(2) A highly standardised technique of manufacture ensures that the specified pore size for any quoted range of flows will be accurate to a high degree. Thus reproducibility of desired flow rates is guaranteed. This/

This has implications in automatic filtration techniques (See section 6 part C).

(3) Since the pore size is standard, the membrane prevents the passage of any particle of larger diameter than the pore. Thus a membrane with a pore size of 0.22 (smaller than the smallest bacteria) is selected, such a membrane used at a differential pressure of 70 cm. Hg. can filter sterilize at a rate of 22ml./minute (Mulvany 1970). In some applications a pore size of 0.45_{um} can be used, but this does not ensure sterility because certain organisms have a shape with a smaller dimension than this pore size. Chandler and Clark (1970) examined the passage of Treponema pallidum through membranes of different pore size. Only the 0.22_{um} was capable of completely preventing passage of the Treponemas. To use the 0.45_{um} size a knowledge of the nature of likely contaminants in a liquid to be sterilized. It has been reported (Bowman et al 1967) that many of the organisms which are able to pass through 0.45_{um} pores are of the obligate parasite type, or have been isolated from tissue. Weston (1972) stated that Pseudomonads appear to be able to pass such filters and it seems that pseudomonads are more persistent in water supplies than was hitherto believed (Hooper 1971). Even after chlorination plate counts of 10^2 and 10^3 were observed. Thus 0.45_{um} filters should not be used for the sterilization of water supplies.

(4) Those filters made of cellulose esters may be autoclaved at $121^{o}C$ for 20 minutes, either loose or in holders.

(5) Microbial grow through does not seem to occur. Therefore long sterilization runs are possible. Depth filters when used for long periods exhibit microbial grow through especially if mycelial organisms are present in the filtrate.

(6)/

(6) Membranes impart no particulate matter nor ionic contamination to the filtrate. Similarly they are not affected solvents common in microbiological labs; solvent resistant versions can be obtained.

(7) A simple test of filter integrity can be performed using a filter, before use so that sepsis of containers and transfer lines is prevented in the event that the membrane has become damaged. The form of this test is the bubble point test (Schaufus (1968) (See section 6 part C). Another feature of this test is that it can be carried out any time during the filtration cycle if there is any doubt as to the filter integrity.

(49) DISADVANTAGE OF MEMBRANE FILTERS:

Perhaps the chief disadvantage of membrane filters is their low dirt handling capacity, and in practice neither the membrane type of filter nor the depth variety are completely adequate for long term high volume liquid sterilization. It has been found that a combination of the two types produces satisfactory results (Richards 1968). If a depth filter rated at approximately same retention capacity as the membrane to be used is placed in line prior to the membrane, it will then act as an efficient prefilter with a comparatively large dirt handling capacity. The membrane filter is then employed exclusively in a sterilizing function. This arrangement prolongs the life of the membrane greatly. Richards had pointed out that even relatively clear and clean fluids contain a certain amount of fibrous and particulate matter, and recommended that prefiltering be included in filtration sterilization.

(50) RELATIVE ADVANTAGE OF POSITIVE PRESSURE FILTRATION

Membrane/

Membrane filtration can be performed using either positive pressure or withvacuum as the driving force and both have been used successfully in this laboratory. It has been found that in practice, of the two methods, pressure filtration has several distinct advantages over the vacuum system (Weston 1972). These advantages have been listed by Weston as follows:

(a) Higher flow rates are possible since flow is proportional to the applied pressure. A vacuum system is limited to a differential of one atmosphere.

(b) Any leakage from the filtration takes place in an outward direction rather than inwards, so that the chance of contamination from outside is less than under a vacuum where leakage occurs inwards. (In fact it has been found in these labs. that a leak of any type was disastrous. Once a leak developed, sterility was lost in a short time.)

(c) No intermediate vessel is required so that filtration directly into resevoirs on site is possible. This procedure eliminates the hazards of "aseptic" transfer operations.

(d) No contamination can occur due to 'blow-back' as may occur in a vacuum pump.

(e) Positive pressure filtration allows the simple procedure of 'bubble point testing' to be carried out, to confirm filter integrity.

The advantages of membrane filters as a means of liquid sterilization as outlined above have long been known in the pharmaceutical industry. Both at research and production level membrane filters have been used on virtually every type of biological and pharmaceutical products (Schaufus 1968), yet in the majority of microbiological laboratores they have found little application. In view of the versatility of membrane filters, this is surprising. Ehrlich (1960) has reviewed many of the applications of membrane filters.

An account of the filtration rig used to sterilize the liquid medium for the methane biomass project described in this thesis has been set out in part (C) section (6).

(51) BUBBLE POINT TESTING:

The bubble point test has been described by Mulvany (1970) as a "simple, rapid, non-destructive test". In essence the test measures the size of the largest pore, and the procedure was described first by Schaufus (1968). The largest pore size is measured indirectly by determining the gas pressure required to overcome the forces of capillarity of the liquid retained in the pores of the membrane.

The pressure (P) needed is obtained from the "capillary rise formula";

$$P = \frac{4a.\cos\theta}{d} \qquad (11)$$

a = liquid surface tension

θ = angle of contact

d = diameter of pore.

For a given liquid, a and θ are constant and thus d can be derived easily. In practice the bubble point test is performed so that some indication of the bubble point can be quickly obtained. If the outlet side of the filter is submerged in water a continuous stream of gas bubbles appears at the bubble point. The pressure required to cause the emmision of a continuous stream of bubbles is the bubble point. The results of the test can be interpreted as an indication of filter integrity because tears or pinprick holes in the membrane reduce the bubble point drastically (See results of commissioning the filtration rig, section (17) part B). An undamaged membrane should give at least the/

the same pressure reading each time the test is carried out.

The bubble point testing procedure for the filtration rig used in the work reported here has been given in section (18) part D.

(52) CONTINUOUS CULTURE AS A MEANS OF BIOMASS PRODUCTION FROM NORTH SEA GAS:

Of the two basic methods of fermentation on a large scale namely batch and continuous culture, the latter appeared to have the greatest potential for the work presented in this thesis. Almost all of the design work relating to this project was done with continuous application in mind and this entailed a more rigorously planned approach since the specifications for equipment must be more exacting than would be the case for batch fermentation.

It was decided that continuous culture offered the only means of selecting rapidly strains of organisms either in mixed culture or pure, which would be capable of growth under the conditions imposed in an industrial context. For a biomass production process the characteristics of a culture, difficult to attain in batch culture, are in fact selected for in continuous culture. The features of a biomass culture considered as important have been outlined in section (27) Part A of this thesis.

(53) THE THEORETICAL BACKGROUND TO CONTINUOUS CULTURE:

The major difference between batch and continuous culture is that the latter are 'open' and the former are 'closed', this concept first being introduced by Herbert (1961). Since the fundamental principles of continuous culture were first propounded by Monod (1950) and Novick and Szilard (1950), several modifications have been innovated. The experiments described in Herbert Elsworth and Telling (1956) and

in/

in Powell (1965) gave an excellent illustration of the principles of continuous culture, but perhaps the most complete account of the techniques of continuous culture was published by Malek and Fencl (1966).

Although continuous culture techniques have been well understood for some years, the adoption of the technique by industry has not been extensive. This applies also to many fundamental research laboratories, a somewhat surprising state of affairs in view of the distinct advantages possessed by continuous culture over batch methods (Maxon 1960).

(54) ADVANTAGES OF CONTINUOUS CULTURE:

A constant culture volume with continuous addition of fresh medium at a rate which supports growth of the culture in such a way that the rate of growth is dependent on the rate of supply of fresh nutrient is the basis of all continuous culture. A continuous removal of culture at the same rate as fresh medium is supplied is inherent the need for a constant volume. In practice only one nutrient is limiting i.e. the growth of the culture is governed by the rate of supply of one nutrient. This rate is the dilution rate and is a function of the ratio of flow/ volume.

(a) DEPENDENCE OF GROWTH RATE ON D. The importance of continuous culture is that when the culture attains a steady state the rate of growth is equal to the rate of nutrient addition;

$$\text{i.e.} \quad D = u \qquad (12)$$

Thus it can be seen that between the limits of maximum growth rate and minimum growth rate the actual growth rate can be varied mechanically via alterations in the dilution rate. Naturally the highest growth rate,

u_{max}/

u_{max} cannot be exceeded but according to Tempest (1967) an absolute minimum growth rate is difficult to determine.

Manipulation of the growth rate (u) was one of the selection pressures used in this work to develop a strain from natural sources, and only with continuous culture was this possible.

(b) RELATIONSHIP OF CELL CONCENTRATION AND SUBSTRATE CONCENTRATION

The steady state concentration of cells in the culture vessel is determined only by the substrate concentration in the resevoir or input medium and not by the concentration of medium in the culture vessel itself. Thus cell concentration (X) can be varied independently of the growth rate in continuous culture.

(c) THE SELF REGULATING NATURE OF STEADY STATE CONTINUOUS CULTURES:

The substrate concentration in the fermenter is independent of the cell concentration but dependent on (D). The concentration of the inflowing medium does not affect either (u) or the fermenter substrate concentration (s); therefore such a system is inherently self-regulating. For example the dilution rate and effluent removal rate determine the volume of culture in the vessel but such devices which control level are notoriously inaccurate, Solomons (1969), therefore volume fluctuations occur. Assuming that at one instant of time too much culture is removed. In effect the dilution rate has increased because of the ratio:

Flow/Volume = Dilution rate.

Since (D) is greater the growth rate (u) is increased so that the concentration of cells (X) becomes greater until almost the original steady state level of (u) is regained. The contrary situation can arise where the dilution rate decreases because too little of the culture is removed. In this event the cell concentration goes down since the cells are/

are washed out of the vessel. The reason for this is that the growth rate has been reduced and in effect there are too many cells than can be supported by the rate of fresh medium addition (D).

(d) THE CONSTANCY OF ENVIRONMENT: In a batch culture, as (X) increases due to growth, the substrate level (S) decreases so that the relationship between cell concentration and substrate concentration is never constant. Since in continuous culture, the substrate concentration and the cell concentration are constant, cellular material has the same environment throughout growth. This has implications from a biomass point of view because it has been known for some time that the chemical and physical environment of cells can influence their biochemical compositions (Tempest 1966), Neidhart 1963). For the purposes of quality control a constant composition of the cellular material is desirable, when edible products are produced.

An implication of a constant environment is that automatic control procedures are easier to implement than when constantly changing conditions arise. (Sher 1961). Several automatic control loops were incorporated into the fermentation system described in this thesis, so that continuous culture facilitated their operation.

(55) THE ECONOMIC ADVANTAGES OF CONTINUOUS CULTURE:

Deindorfer and Humphrey (1959) calculated the unit costs for a hypothetical process involving up to 6 stages with various yields and productivities. Apparently a two stage continuous process had the least cost per unit of production. This was about 75% of the batch figure and the continuous fermenter productivity was 5 times as great as the batch culture. Assuming a 16 hour process and a down time of

10/

10 hours the yield of the continuous fermentation was calculated to be almost as high as the batch process.

Using the conversion of sorbitol to sorbose by Acetobacter suboxydans, a growth dependent process, Elsworth et al (1959) calculated unit costs in a small experiment involving both batch and continuous cultures. At maximum output the continuous process gave 85.6% yield and the batch culture gave 95.4%. At the same time the product cost was 1.3d/kg greater in the continuous process than with the batch. When the dilution rate was decreased by increasing the vessel size, the yield in continuous culture increased to 89.2% and the productivity was now greater than that of the batch process. In these conditions the product cost became 3.59d/Kg cheaper than for the batch culture, the largest savings being in labour.

It can be seen that continuous culture has distinct advantages over other forms of fermentation, but as Maxon (1955) pointed out the major disadvantage of continuous culture where all ancilliary processes are continuous too is that shut down of any one of these processes causes a total shut down of the overall process, unless one is prepared to waste product until repairs are effected.

(56) THE ADVANTAGES OF A MULTI STAGE CONTINUOUS CULTURE PROCESS:

The single reactor chemostat, whilst possessing unique advantages over batch methods has a disability which is derived from its very mode of operation; growth rates of a culture in single stage chemostat are restricted to those regions below u_{max} and above u_{min}. In other words maximum growth rates cannot be achieved in a single stage chemostat. Similarly in fermentations where a metabolite is the end product and which is growth dependent, batch processes are preferable to single stage continuous fermentations.

One/

One of the major innovations in continuous culture technique was the development of the multistage chemostat in which a primary stage feeds one or more subsequent stages, each of which may or may not have independent substrates supplies. Such an arrangement means that the complexity of the process increases and that inevitably the chances of shut down due to equipment failure are increased concommitantly. In practice rarely are more than 3 stages used and in most cases two suffice.

Multistage equipment provides the user with all of the advantages of a single stage chemostat together with the facilities to grow cells at u_{max} and above, and to enable growth dependent metabolite production. In addition to combining the features of a batch fermenter with a single stage chemostat, multistage reactors possess some useful and surprising properties;

(1) The second stage is fed continuously with the effluent culture fluid from the first stage, thus if stage 2 has a smaller volume than stage 1, or has an indepent supply of medium then the dilution rate of the second stage and hence the growth rate is much greater than that of stage 1. Thus;

$$FI/V2 = D2 \quad \text{or}$$

$$\frac{FI + F2}{V2} = D2$$

It can be arranged that D2 is equivalent to u_{max} or greater. Since the second stage is fed continuously by fermenter I, there can never be wash out of the culture in stage 2. The implication of this is that stage 1 can be used to grow cells at maximum productivity and stage 2 to allow these cells to reach the physiological state needed for product formation, the point being that certain metabolites are only produced in such conditions of substrate excess.

(2)
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(2) Complex media with more than one utilizable nutrient (e.g. ethane/methane in north sea gas). The first stage allows complete consumption of the most easily assimilated nutrient whilst stage 2 enables the second nutrient to be used, at least when a mixed culture is involved. (Fencl and Burger 1958).

(3) In mixed cultures with a dependency relationship where organism A produces a metabolite essential to the growth of organism B, organism B can be successfully grown in stage 2 as this receives the effluent from culture A.

(4) The conditions of operation in each reactor can be varied separately. This includes both the physical as well as the nutrient conditions. Thus as a selection agent, the multistage chemostat is able to apply more conditions of selection in different combinations than either batch or single stage reactors.

(5) The use of multistage continuous culture has been found to have some unexpected effects on the culture characteristics. Fencl (1966) noted that the average value of u_{max} of a Candida utilis strain in batch culture was $0.65hr^{-1}$ and therefore the culture doubling time was slightly over 1 hour. In the second stage of a multistage chemostat at a dilution rate of $1.4\ hr^{-1}$ the doubling time became less than 1 hour and u was $0.7hr^{-1}$.

In the dehydrogenation of sorbitol to sorbose, by Acetobacter suboxydans in multistage equipment, the specific rate of product synthesis was 2.7 times as high as the rate predicted from batch culture experiments. The increases appeared to be related to the specific growth rate and reached a maximum close to the observed u_{max}.

The ability of multistage chemostats to increase u_{max} and to reduce the culture doubling time was of great relevance to the project presented/

presented in this thesis in that in some cases methane oxidising organisms have been observed to have prohibitively long generation times (See section 24 part A) and any means of reducing this was of great interest.

The versatility of multistage equipment influenced the decision to use such a system in preference to batch or single stage apparatus to a great extent. The operational details of the methane biomass production system have been presented in Part C of this thesis.

(57) SUMMARY OF FEATURES OF MULTISTAGE REACTORS:

(1) It is possible to arrive at substrate limitation early on so that selection of cultures adapted to continuous culture occurs as soon as possible.

(2) The washout capability of the first stage is useful in removing non-methane/ethane substrates from isolation material in the fermentation.

(3) Improved kinetic characteristics of cultures.

(4) Final stability greater in the second stage since washout cannot occur.

(5) It is possible to grow cultures at maximum growth rate.

(6) Manipulation of the chemical and physical environment is possible to a greater extent in a multistage operation.

(58) CONTAMINATION OF CONTINUOUS CULTURES:

One disadvantage of continuous culture which has often been quoted is that of contamination of the culture by either mutants or extraneous organisms. Olsen (1961) suggested that in the long term contamination of the culture is probably inevitable unless expensive procedures are taken/

taken to prevent it and although Bartlett and Gerhardt (1959) considered contamination to be the most important factor limiting the exploitation of continuous culture, it must be added that Herbert et al (1961) reported several months of continuous culture without shutdown. Reusser (1961) reported similar success. Perhaps the reason for poor performance has been that too often equipment not designed for continuous runs of say 1000 hours has been used. Solomons (1969) advised against the use of modified batch equipment and chemical glassware adapted to continuous culture.

(59) THE SELECTIVE ACTION OF THE CHEMOSTAT:

To some extent the steady state culture of a chemostat has an inherent resistance to many types of extraneous contaminants and mutant forms of the original strain, because at the steady state the conditions of growth are specific to the original culture. An especially lucid account of the growth of contaminants has been given by Powell (1958) who made the point that the destiny of an intruder can be predicted from the theory of continuous culture.

The fate of a contaminant depends initially on its preferences for growth conditions such as pH. temperature and nutritional requirements and this applies both to batch and continuous cultures. Powell (1958) has shown that when a culture in a chemostat is in the steady state and the limiting nutrient is essential for the growth of a contaminant which does not otherwise influence the original culture, then the contaminant either completely supercedes the original or it is entirely washed out of the vessel. Powell calculated that:

(a) /

(a) Dilution rate

(b) u_{max}

and (c) K_s are the factors determining the success of an invasion by a contaminant, assuming no interrelationship between the original organism and the contaminant. Thus it can be seen that contaminating or mutant organisms will replace the original culture depending on the nature of the two growth curves. Fig. (12a) illustrates this point well. Assuming that the original organism (B) is growing at a rate corresponding to a dilution rate of (D) when contamination by organism (A) occurs. Since the saturation constant for (A) is lower than that of (B) and in addition the maximum growth rate of (A) is greater the contaminant completely supersedes (B). Thus at a dilution rate of (D) organism (A) grows at a rate of u_A since the growth limiting nutrient concentration at the time of contamination is S_B. Organism (A) increases in numbers and as a consequence the concentration of limiting substrate decreases so that u_A equals (D). At this point the limiting nutrient concentration has reached S_A and organism (B) can only grow at u_B which is less than the dilution rate. Therefore organism (B) is washed from the vessel.

It can be seen equally that had the saturation curves for the two organisms been reversed then the contaminating organism could never have become established.

It is possible that the curves could have the form such that an organism could have a lower K_s value and a higher u_{max} i.e. the curves cross over. The fate of the contaminating organism then depends on whether the dilution rate is set at a point above or below the cross over point (Tempest 1970). Figure 12b).

It is probably true to say that no interaction other than substrate competition/

competition is an ideal state of affairs, and is unlikely to pertain in an actual culture, especially one which was isolated from a source such as sewage or soil. The point to be taken is that the chemostat offers a tool for selection of strains best suited to growth in the conditions existing in continuous culture. This selective force is outwith the selection due to imposing certain desired chemical and physical conditions on a culture.

(60) SELECTION AND MIXED CULTURES IN THE CHEMOSTAT:

In many mixed cultures inhibition and/or mutual stimulation of growth, over even interdependency relationships have been observed (Meers and Tempest 1968; Tempest , Dicks and Meers 1967, Megee et al 1972, Chao and Reilly, 1972). Combinations of interactions in mixed cultures have been noted frequently (Yeoh et al, 1968). Any of the relationships mentioned alter the survival chances and behaviour patterns a given culture. In complex media supporting mixed cultures, more than one limiting nutrient is possible so that culture conditions are extremely complex.

The use of the chemostat as a strain selection tool has not been widely used as a deliberate method of strain improvement, except in some cases where a species has been enriched from an area where that species was present in very low quantities. (Jannasch 1967, Jannasch 1968). It was shown by Powell (1958) that the chemostat selects organisms with the lowest K_s value and the highest growth rate and thus forms the basis of a selection tool which makes obsolete the more traditional screening methods and "training" programmes.

(61)

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(61) FACTORS IMPORTANT IN THE CHOICE OF GAS ANALYSERS:

(A) OXYGEN ANALYSER Oxygen can be measured in one of two general ways;

(i) Intermittent analysis. A fixed volume of gas is sampled and the oxygen is chemically removed. The volume change, corrected to standard temperature and pressure, gives the concentration of oxygen present in the sample. Accuracy varies between different operators and according to quality of chemicals used. Equipment is inexpensive and can be used to measure gases other than oxygen. They cannot be used in a continuous recording or control capacity and therefore were useless for the purposes of the work reported here.

One form of intermittent gas analysis - gas/liquid chromatography - can be adapted to automatic measurement but G.L.C. is an extremely expensive method of doing so.

(ii) Continuous analysis. Since gas analysis was applied to control procedures in the work described in this thesis, only those instruments able to give a continuous measurement were of interest. Of these again two general classes are available.

(1) ELECTROLYTIC CELL TYPE Oxygen level is measured in an electrolytic cell which although slow in response, can be used to measure dissolved oxygen. (Roberts and Sheperd 1968). One disadvantage is that such instruments require the scrubbing of acidic gases, particularly sulphur dioxide, hydrogen sulphide and carbon dioxide.

The oxygen present in a sample diffuses across a membrane and causes a current to flow between two electrodes. The current is related to the oxygen concentration. The Hersch meter (Hersch 1962, 1964) is of this type using a silver cathode and a cadmium anode. The galvanic/

galvanic cell form of instrument has been successfully developed by Westinghouse using a calcium stabilised zirconium electrolyte and porous platinum electrodes. The Westinghouse instrument responds quickly to changes in oxygen concentration and is specific to oxygen, and CO_2 and H_2O do not affect the reading. The cell operates at 850° and so hydrocarbons present in the sample gas burn consuming O_2 thus giving an erroneous result. Both the Hersch meter and Westinghouse analyser are sophisticated instruments, capable of measuring very low levels of oxygen. Consequently they are very expensive.

(2) PARAMAGNETIC ANALYSERS Oxygen has an almost unique property which can be exploited in measuring instruments. This property is known as paramagnetism. The two unpaired electrons in the outer shell of the oxygen molecule are responsible for the paramagnetism. Usually two paired electrons in the same shell, according to the Pauli exlusion principle have opposite directions of spin and thus neutralise the magnetic moment of each other. Having two unpaired electrons oxygen is therefore strongly paramagnetic. Among common gases only Nitric oxide and Nitrogen dioxide are also free radicals but their paramagnetism is much weaker than that of oxygen.

Some paramagnetic oxygen analysers operate on the principle of "magnetic wind". This basically involves measuring the changes in thermal conductivity according to the concentration of oxygen in a sample. Magnetic wind instruments suffer from several disadvantages with respect to the work reported here.

(i) Only a specific gas mixture to which the instrument has been calibrated can be measured on any given instrument. The filament temperature/

temperature is affected to different extents by different gas mixtures and thus the thermal conductivity reading can only be specific for the calibrating mixture.

(ii) Unless the instrument is correctly alligned and mounted gravitational flow effects may cause erroneous measurements.

The analyser designed by Servomex Controls Ltd. is based on the original measuring cell of Pauling (1954) modified by Munday, 19 Two diamagnetic glass spheres are filled with nitrogen and arranged at each end of a rod in dumb-bell fashion. This is mounted horizontally on a platinum torsion suspension thread. A strong non-uniform magnetic field surrounds the whole measuring cell. When the magnetic susceptibility of the gas surrounding the dumb-bell is the same as that of the nitrogen in the spheres, there will be no displacing force. If the oxygen is present, the magnetic susceptibility of the gas is greatly increased. The nitrogen glass filled spheres are diamagnetic relative to the surrounding gas and the dumb-bell tends to rotate to lie in the area of weakest magnetic force. The analyser by Servomex Controls Ltd. uses an electromagnetic feed back mechanism which maintains the dumb-bell in its original position, i.e. at zero. The current required to do this is a measure of the magnetic susceptibility of the gas and the instrument is calibrated to convert this to percentage oxygen read out.

A Servomex instrument was chosen after due deliberation for the project described in this thesis. Model O.A. 137 has the following features which contributed to the choice.

a) Readings were not affected by carrier gas composition, other than that this should be dry.

b) The range was linear from 0-100% oxygen.

c) /

c) Calibration required only nitrogen for zeroing and air to span the instrument.

d) Response to concentration changes was 90% complete in 8-9 seconds.

e) The model O.A. 137 was extremely robust. Application in other laboratories covering some 25 years of production had shown that the instrument is stable over long periods and requires little maintenance. (R. Hutchinson pers. communication).

f) It was intrinsically safe in use with explosive gas mixtures. A safety purge is fitted as standard.

g) Accuracy was 0.05% O_2 or 1% full scale deflection whichever was greater. This was adequate for the need of the work reported here.

h) Relatively cheap. It compared favourably with other analysers. (See appendix VII p.).

Model O.A. 137 was chosen for the above features and because of favourable reports from other workers using the instrument. (Nunn et al 1964, Roberts and Shepherd, 1968).

(B) METHANE
ETHANE
CARBON DIOXIDE ANALYSERS: Under normal running conditions the only gases present in significant quantities in the recycle lines will be

O_2
CH_4
C_2H6
CO2

Intermittent/

Intermittent means of measuring CO_2, CH_4, C_2H_6 were ruled out for the same reasons that they were for oxygen measurement. Thus equipment such as the Orsat, Kitagawa detection tubes or the Draeger Normalair detectors were not considered in this respect.

Concentrations of gases other than oxygen are usually measured continuously by one of two general methods;

a) Thermal conductivity measurements.

or b) Infra-red radiation absorbtio n.

(A) Thermal conductivity; The basic unit of instruments using changes in thermal conductivity as a means of estimating gas concentration is the Katherometer. The instrument offered by Cambridge has several points of merit in that;

i) A wide range of gases can be monitored because thermal conductivities differ appreciably between different gases.

ii) Gases can be returned to source after monitoring because there is normally no chemical alteration of the gases with type of equipment.

iii) Accuracy is only slightly affected by changes in

a) Flow. (gases enter by molecular diffusion)

b) Pressure.

c) Ambient temperature. The Katherometer is electrically and thermally symetrical.

iv) The analyser can be made intrinsically safe. This feature is of the utmost importance when dealing with gas mixtures such as O_2/CH_4.

On the debit side the instrument is not so attractive in that;

a) It is relatively expensive.

b) Strictly speaking it is only designed to handle binary gas mixtures and thus was unsuitable for the purposes of the present work, unless/

unless special devices were used. Lack of experience with this form of analyser led to its rejection in favour of the infra red variety.

(B) Infa-red analysers. Two instruments generally available in Britain are the M.S.A. LIRA 300 and the IRGASB2 by Grubb Parsons. Both work in essentially the same way. Reference to fig. 61. on p. illustrates the mechanism by which these analysers work. A beam of infa-red radiation is emitted from a heated nichrome filament and passes into each of two cells alternately. In one cell is sealed a reference gas and through the other passes the sample gas. The radiation leaving both cells enters a single detector unit. As the radiation passes through the gas mixture differential absorbtion of energy occurs. This in turn causes a differential expansion and contraction of the detector gas in the detector cell. The pressure changes in the detector cell results in movement of a membrane which changes the capacity of the condenser microphone. Finally the capacity changes alters the electric signal from the detector in proportion to the difference between the degrees of absorbtion. After amplification the signal is recorded. In terms of principle of operation there is no difference between the two instruments and a choice was made on the basis of price. As usual a compromise between quality and economy had to be made. The LIRA was approximately half of the cost of the IRGA and when one considers that a separate analyser for each gas to be monitored is necessary, the pricing is highly significant. The advantages of the LIRA are;

i) It was small and easy to mount in a panel.

ii) It was easy to operate and calibrate. (See section 3 Part D)

iii) Accuracy is plus/minus 1% fullscale deflection

iv) Fast in response. 90% of full reading in 5-9 secs.

The disadvantages of this instrument were;

i) Less accurate and sensitive than the IRGA.

ii) Required frequent recalibration, once per day. (See calibration/

calibration procedure p.144) Stability was poor.

iii) The components were not very reliable. A great deal of trouble was experienced with several parts in all the instruments used in this project.

(62) FACTORS INFLUENCING FERMENTOR CHOICE:

Methane as a constituent of natural gas is considered to be the cheapest widely available carbon source. (See Table 6). Whilst this was attractive from a fermentation point of view, the limited solubility of methane in water presented problems of fermenter design. The concentrations and solubilities of the component gases in Natural gas have been given in Table (15), and (16).

(1) To compensate for the poor solubility of both Methane and Oxygen a fermenter had to be found which was capable of high rates of mass transfer so that gases would be passed into solution at a sufficiently high degree of efficiency. (See section (18) on mass transfer, Part B).

(2) A high rate of mass transfer is useful in that it ensures excellent mixing. Thus automatic control procedures could be adopted more readily since operational time lags are reduced to a minimum with adequate mixing. (See section automatic control (33), Part B.

At the same time good mixing is important to continuous culture operation. The theory of continuous culture is based on the concept of perfect mixing. (See section continuous culture (53). In practice such high gas transfer rates were found to generate a homogeneous gas/ liquid foam, completely filling the fermentor. The foaming made conventional wier devices inoperable for level control in continuous culture and therefore provision of an alternative level control device was essential.

(3)/

(3) The fermenter was required to reproduce industrial conditions of operation as far as possible and thus many laborotory scale fermenters were eliminated from the start. The working volume necessarily had to be at least 5 litres with a gas head space of 2 litres, in order that industrial grade control and measuring equipment such as differential pressure transmitters and proportional valves could be used. (See equipment list App. VI). A 5 litre working volume is at the limit of the control abilities of the equipment and a larger volume would have been preferable. A 5 litre fermenter was chosen to reduce the requirements for liquid medium throughput to a minimum.

(4) Attention had to be paid to the safety aspects of operation and so a flame proof stirrer motor was an absolute requirement. Similarly all materials of construction in contact with the gas mixture had to be non-inmflammable. (See section safety aspects Part B).

(5) The fermenters were fixed in situ by the nature of the gas recycle lines. It was therefore essential that the fermenters were autoclavable in situ.

(6) A sufficient number of ports on the head plate to accept all the electrodes and probes necessary for measurement and control of the various fermentation parameters was required. At least 10 ports were needed (see Fig.63) and a bottom stirred fermenter was felt to be more functional in this respect. This was because a mechanical defoamer was incorporated as an integral part of the system design and had a top stirred fermenter been employed a separate defoamer and gas exit port would have been needed. With bottom stirred vessels the defoamer can be mounted on a hollow central shaft through which the effluent gases pass into cooling condensers.

(63) /

(63) THE USE OF CHEMICAL ANTIFOAMS:

Although each of the fermenters was fitted with the means to break down foams mechanically, it was not known at the time of equipment choice what the intensity of foaming in methane/north sea gas fermentation would be. It was therefore necessary to consider chemical methods of foam destruction as a possible supplement to the mechanical method.

(64) FACTORS INFLUENCING CHOICE OF A CHEMICAL ANTIFOAM:

(1) TOXICITY; One of the most important features of an anti foam is its toxicity both to the fermentation culture and in the case of biomass, to the recipient of such material in a feeding capacity. Of those antifoams considred, the silicone based ones were the least toxic and indeed may be considered as virtually non-toxic. Sillicone antifoam reagents have been used therapeutically (Rider and Moeller 1960) and Rowe Spencer and Bass (1950) reported no adverse effects on rats fed with foodstuffs containing silicona antifoam materials.

(2) EFFECT ON MASS TRANSFER; Several reports have appeared concerning the effect of antifoams on mass transfer in fermenters, and certainly it is true that any reduction of the rate of gas transfer and mixing efficiency would be wasteful of power. In conditions were a gaseous substrate was limiting such and effect could be disastrous for the culture.

Antifoams have been shown to reduce the gas transfer rate by as much as 50% (Solomons 1961 and 1967). Similarly Deindorfer and Gaden (1961) noted that alkaterge C in lard oil decreased oxygen transfer rates by over 50%. In a review of anti foams (Evans and Hall, 1971) it was noted that the effect of antifoams on gas transfer was not clear because in/

in certain cases silicone antifoams do not reduce transfer rates. These authors called for more critical experiments on the effects of antifoams in fermentation.

Some types of antifoam to be effective, must be used with a carrier species such as lard or cotton seed oil but as Rolinson and Lumb (1953) pointed out the carrier itself may be used as a growth substrate with considerable effect on the growth of the culture.

Phillips et al (1960) made single additions of each of three antifoams

i) Silicone based DCA

ii) Lard oil

iii) Hodey R4

and noted the effect on oxygen consumption. A marked depression of the consumption of oxygen occurred along with a drop in cell productivity. When, however, incremental additions of antifoams were made, the effect was less severe.

A more recent investigation on the effect of additives on mass transfer rates was published by Bendek and Heidegger (1971). It was evident that different effects could be obtained with different antifoam materials. For example it was observed that Nor-dodecyl sulphate appeared to increase the rate of mass transfer whilst Dow-Corning antifoam caused a reduction in mass transfer. The actual mass transfer coefficient in both cases was the same function of bubble size so that the different rates of mass transfer were due to different effects on interfacial area. Clearly the study of the effects of antifoams and additives on mass transfer requires a deeper and more critical investigation than has been given so far.

The/

The preliminary results of tests on the mechanical defoamers indicated that no additional means of foam destruction would be necessary and therefore provision for the addition of chemical antifoams was not included in the final fermentation system. As a precautionary measure MS silicone antifoam emulsion was kept ready to be added manually in the event of the formation of unusually intense foaming. Addition of anti foam could be effected via the sampling lines and sampling pump.

(65) SAFETY ASPECTS OF NORTH SEA GAS FERMENTATION:

Any system which involves the handling of explosive gas mixtures including methane/air or methane/oxygen combinations requires consideration of safety features which reduce the effect of an explosion or prevent it entirely. In the plant described in this thesis there was a high risk of ignition due to the highly inflammable nature of both methane and oxygen. The presence of small amounts of ethane in north sea gas was not considered to affect the risk of ignition to a significant extent.

Particular attention was paid to safety aspects in the design of the fermentation system described in this thesis. The features relating to safety have been given in section (8) of Part C.

(66) EXPLOSIVE LIMITS OF METHANE/OXYGEN MIXTURES:

A gas such as methane has, when mixed with oxygen or air explosive limits beyond which explosion can not occur. These limits define the range of methane content in the gas mixture and are as follows:

GAS MIXTURE	UPPER EXPLOSIVE LIMIT	LOWER EXPLOSIVE LIMIT
METHANE/AIR	15% by volume	5.0 % by volume
METHANE/OXYGEN	59.2% by volume	5.4% by volume

If/

If the concentration of methane falls between these limits then the gas mixture is potentially explosive. It is likely that other gases will be present during fermentation, particularly carbon dioxide and water vapour, which reduce the explosive range of such mixtures. According to Lewis and Von Elbe (1951) a mixture of Methane/Oxygen/Carbon dioxide can not be ignited if the oxygen content is less than 14.6%. If nitrogen instead of carbon bioxide is present the oxygen content must be less than 12.1%.

The figures quoted above were also cited by Hamer and Heden (1967) who pointed out that as a safety factor, operating conditions should be in the non-explosive range, i.e. if the oxygen concentration in the gas phase is always below 12.1% by volume then no ignition can occur. In fermentation work where methane/air or oxygen mixtures have been used with no consideration of the danger even though no mishaps have been reported, to ignore elementary safety procedures is inexcusable.

Hamer and Heden found that when operating in the non-explosive range the cell density of the resultant biomass was relatively low but they felt that this was attributable to the inefficiency of gas transfer in the fermenter used in the study.

(67) GENERAL SAFETY PRECAUTIONS:

The first rule of the design of a north sea gas fermentation plant must be to assume that an ignition source is present and that an explosion will occur. On this assumption it is possible to incorporate suitable design features to minimise the effect and extent of an explosion.

The/

The first step was to remove as many scources of ignition as possible. This was not as easy as it may seem because not all scources of ignition can be identified or isolated.

Ignition has been caused in the past by;

1) Compression of gases. This may occur in tight pipe corners at 'T' joints as well as in blocked passages.

2) Heat, either external to the ignition point or internal and localised, e.g. the frictional heat generated in pumps or meters or caused by particulate matter striking vessel walls.

3) The presence of inflammable materials in the plant construction in contact with explosive gases, e.g. packing, stoppers, filters gaskets. Many materials are spontaneously combustible in pure oxygen so care was excercised in the choice of equipment. As far as was practicable flame proofed versions of the necessary instruments and equipment and only non-combustible materials were used. (See section 8 Part C).

4) Greases used on gaskets 'O' rings diapragms and connections. These were of the non-inflammable variety. It was found that Silicone based oils and greases were permissible.

5) Filings and particles in new piping. All new equipment of this sort was thoroughly cleaned before being incorporated into the plant.

(Points 1-5 personal communication from R. Hutchinson 1971).

6) According to Sprenger (1956) a less common scource of ignition is a 'hot spot' in pipes and vessels caused by the passage of a gas past branches and junctions. This phenomenon is referred to as a 'whistle'.

7)/

7) Static electricity has been described as a scource of ignition and only 0.1 Joule is required to cause ignition, however such instances are extremely rare. (Personal communication R.Hutchinson 1970). All metalwork should be properly earthed, especially the fermenter paddle.

Discussions on safety features in fermentation systems with R. H utchinson, I.C.I., Billingham indicated that contamination of oxygen lines by rust and scale particles is unavoidable. It was recommended that;

a) Reducing valves be of bronze, preferably tin plated to prevent season-cracking. In fact the small sizes of valve used were not generally available in bronze and so stainless steel alternatives were used.

b) Pipe bends should have a minimum radius of 5 times the external diameter of the pipe.

c) Efficient degreasing and decontamination of water scale, welding residues should be performed wherever possible.

d) Sintered bronze or stainless steel filters and flame traps should be used in preference to other types.

It was pointed out by R. Hutchinson (Pers. communication 1970) that no reliance on non-return valves can be placed, since they cannot stop the travel of an explosion. The shock wave is able to pass through the valve before it can close and the detonation wave may reach 6 times the speed of sound. The subsequent combustion wave can cause ignition on the other side of the valve.

The British Oxygen Company recommended that oxygen used should be filtered better than 25 microns. It was also recommended that the following/

following flow rates of gas should not be exceeded;

below 50p.s.i working pressure

..... 150 feet/second in pipelines and

..... 55 feet/second in distribution

systems with control valves 'T' joints and similar constrictions.

TABLE (17)

ELECTROCHEMICAL SYSTEMS OF SOME PUBLISHED DISSOLVED OXYGEN ELECTRODE DESIGNS:

MEMBRANE	ANODE	CATHODE	ELECTROLYTE	SOURCE
POLYTHENE	SILVER	PLATINUM	KCl	Clark (1956)
POLYTHENE	LEAD	SILVER	KCl	Mackereth (1964)
SILICONE	LEAD	SILVER	KCl	Flynn *et al* (1967)
F.E.P.	LEAD	SILVER	KCl/ glycerol	Harrison and Melbourne (1970)
F.E.P.	LEAD	SILVER	KCl	MacLennan and Pirt (1965)
TEFLON	GOLD	SILVER	KCl	Kinsey and Bottomley (1962)
POLYTHENE	SILVER	PLATINUM	KOH	Carritt and Kanwisher (1959)
TEFLON	ALUMINIUM	PLATINUM	KCl/ glycerol	Gore and Phillips (1964)
TEFLON	SILVER	SILVER	KCl	Phillips and Johnson (1961)

TABLE (18)

THE CHEMICAL COMPOSITION OF THE NITROGENOUS SALTS MEDIUM:

(A)	PHOSPHORIC ACID (H_3PO_4). Wet process phosphoric acid liquid portion only used.	0.0165 Molar
	AMMONIUM SULPHATE ($(NH_4)_2SO_4$) Spanish, 96% purity.	9.00 grams
	MAGNESIUM SULPHATE ($MgSO_4$) Anhydrous.	0.30 grams
	ZINC SULPHATE ($ZnSO_4.7H_2O$)	0.5 milligrams.
(B)	All present as impurities to the following levels;	
	FERROUS SULPHATE ($FeSO_4.7H_2O$)	5.0 milligrams
	COPPER SULPHATE ($CuSO_4.5H_2O$)	0.1 "
	BORIC ACID (H_3BO_4)	0.07 "
	MAGANESE SULPHATE ($MnSO_4.4H_2O$)	0.5 "
	SODIUM MOLYBDATE (Na_2MoO_4)	0.1 "
	CALCIUM CHLORIDE ($CaCl_2.2H_2O$)	13.24 "
	COBALTOUS CHLORIDE ($CoCl_2.6H_2O$)	0.1 "
(C)	Tap water to 1000 c.c.	

(pH at make up = 2.6)

PART (C) PRACTICAL ASPECTS OF THE DESIGN OF THE DESIGN OF THE NATURAL GAS FERMENTATION SYSTEM

PAGES: (110) TO (141)

(1) THE LIQUID LINE SYSTEM

(A) MEDIUM SUPPLY:

A schematic flow sheet of the liquid lines has been given in Fig.(13). Medium was stored in 20 litre glass resevoirs (R1, R2, MR1 and MR2. Each resevoir had a refill attachment (A, B. W.) and was provided with a filter to allow sterile equilibration of pressure during filling and emptying. Normally, resevoirs R1 and R2 were re-charged with sterile medium by an automatic filtration process (Section 6). Integral with filtration into an empty resevoir, the full resevoir then automatically became the supplier of medium to both fermenters via solenoid valves (P or Q) on the outlet lines serving peristaltic pumps 5 and 6. In the case of failure of automatic filtration, standby resevoirs (MR1 and MR2) were supplied from a manually controlled filtration rig, or were autoclaved with medium inside, and are connected in parallel to one outlet line which branches to serve both fermenters via solenoid valve R (manually switched).

(B) CULTURE OVERFLOW

Control of the volume of liquid in the fermenters has already been dealt with (See section 4). Culture fluid effluent was fed into 20 litre collection vessels (CV1) and (CV2). The fluid contained both gas and liquid and the gas was returned to the gas recycle line through solenoid valve (R2). A non-return valve (N) prevented backflow of main system gas into the cellection vessels. The vessels could be emptied manually by pressurising the vessel and forcing fluid out through a dip tube in the vessel.

It was possible to use the fermenters in the following combinations;

(i) /

(i) Two single stage reactors; Line (D) was clamped off at (T2) with (T1) open.

(ii) Multistage function; Line (D) Fed Fermenter (F1), clamp (T1) was closed off and (T2) opened.

(iii) Cell recycle; Fermenter (F2) only; Line (D) to (F2) with clamps (T1) and (T2) adjusted to give required rate of cell recycle

(2) THE GAS RECYCLE SYSTEM:

In section (26) part A, it was concluded that biomass production from Natural gas should employ a multistage chemostat with automatic control of the gaseous environment via a gas recycle system. A flow diagram for the system has been given in fig. (4). The following is an account of the rationale of this particular arrangement.

PIPEWORK: Pipework ($^{3}/_{8}$" O.D. stainless steel) extended from the high pressure side of the gas circulation pump (N) to one way valves (D, A1, A2, A3, C) and continued through the gas delivery and effluent pathways of both fermenters to a 'T' junction (J). For the low pressure side of the system, for the pathways leading directly to the gas analysers (11, 18, 20, 21) and from the gas cylinders (1, 8, 15) to one way valves (A1, A2, A3) $^{1}/_{4}$" O.D. stainless steel tubing was fitted. All branches from the recycle lines to the water lute (V) or to the rotary gas meter (26) and to atmosphere, were comprised of $^{1}/_{4}$" O.D. copper tubing. Solenoid valves (R_1, S_2, R_2, S_2, N2, Mx) were made of brass whilst all other valves had stainless steel bodies and neoprene seals. Compression couplings were used throughout.

Each of the gas inlet filters and condensers were fitted with stainless/

stainless steel quick release couplings (male). A corresponding female coupling was fitted to the ends of the inlet and exhaust pipes. These couplings facilitated maintenance work on the fermenters.

GAS FLOW: The pipes and valves were positioned to provide a choice of operational systems. Single, series or parallel delivery of gas was possible.

(i) SINGLE PASS OPERATION FERMENTER (I)

Valves: B, C, F6, H1 K : Opened

Valves: D, E, H2, H3, F6 : Closed

(ii) SERIES OPERATION:

B, C, F5, H3, F6, K : Opened

D, E. H1, H2 : Closed

(iii) PARALLEL OPERATION:

B, C, F5, H1, H2, F6, K : Opened

D, H3, E : Closed

The composition of the exhaust gas from each fermenter could be monitored separately by opening valve (E) to sample fermenter (1) effluent gas (valve (C) closed). Subtraction of these valves from those of the mixed exit gases from (F1) and (F2) gave the composition of gas leaving (F2).

SINGLE PASS OPERATION: FERMENTER 11

B, C, H2, F6, K : Opened

F5, E, H1, H3, D : Closed

Under normal conditions of operation the gases were sampled after make up due to control action i.e. by passing through valve (C) into the analysers. If desired, gas concentrations could be measured before make up through (D) instead of valve (C).

Whilst/

Whilst analysis was proceeding all valves on the analyser (F1-F4) (G1-G4) were left open.

The rate of flow of gases into the fermenters was regulated by rotameters (29) and (30) into fermenters (I) and (II) respectively. Sample gases flowed continually into the anlysers via valves (C) or (D) at a constant rate regulated by rota meters (53-56). Sampled gas was returned to the main system through solenoid valve (R1). Spent gas passing through the culture collection vessels re-entered the main recycle system via solenoid valve (S1). (See description of liquid flow system Section (1) above.

(3) CONTROL LOOPS

(A) CONTROL OF GASEOUS ENVIRONMENT:

(1) OXYGEN: Consumption of oxygen by growing on natural gas as a carbon scource caused a fall in the concentration of oxygen in the closed system. The information was relayed by, the oxygen analyser (II) to a recorder/controller (12). A deviation from the set point caused the controller to transmit a signal to the electro-pneumatic transducer (13) which converted this electrical signal to a pneumatic one. The pneumatic signal controlled a proportional valve (14) in such a way as to regulate the flow of oxygen into the system according to the deviation from the controller set point. The oxygen was supplied from a cylinder (8) and passed through a shunt-tube differential pressure cell (10). The rate of oxygen flow through the D.P. cell was relayed to a pneumatic recorder (4). The D.P. cell was calibrated at 20 p.s.i upstream pressure which was maintained by regulation of the gas cylinder valve and observed on pressure gauge (9). The pressure downstream of the D.P. cell was maintained at 10 p.s.i. by the manostat (27) indicated on gauge 28. Had the upstream pressure been reduced for any reason, backflow from/

from the recycle lines into the gas delivery lines was prevented by one-way valves (A1, A2, A3).

(2) METHANE: When methane was consumed it could not be restored to the system by measurement and control of concentration changes. In attempting to control two or more gases in a closed system by concentration control, dilution of one gas by the other when both gases are demanded could prohibit a return to the set point values of both gases. A large pressure build up would then ensue. For this reason consumption of methane was detected and controlled by sensing pressure changes in the system with a pressure transducer (5). Pressure changes were transmitted to a pressure/indicator/controller (6) which governed a proportional valve (7) on the methane feed line. The flow rate of methane into the system was measured by the shunt-tube D.P. cell (3) and recorded on a multipoint recorder (4). Upstream pressure was indicated on a gauge (2) and regulated at the cylinder (1). The methane concentration was measured (19) and recorded (22).

(3) CARBON DIOXIDE: Carbon dioxide concentration was measured with an infa-red analyser (21) and the level recorded (22) on a multi-point recorded fitted with a microswitch control facility in circuit with the CO2 point. When the level of CO2 rose above a preset value the microswitch was actuated and a solenoid valve (23) opened. This action allowed a preset proportion of the circulating gas to pass through a CO2 scrubber (T) until such time as the CO2 level returned to the set point - when the solenoid valve closed. The rate of flow through the scrubber was regulated by the needle valve (B) and indicated on rotameter (24).

(4) ETHANE: Natural gas contains approximately 3% ethane (See Table 16 of gas composition. Unless ethane was consumed together with/

with methane and oxygen, ethane concentration tends to build up as the system controls the concentrations of the other gases, with the resulting pressure increase, the flow of natural gas into the system decreases and instability in the control operations occurs. Therefore changes in ethane concentration were relayed from an infa-red analyser (20) to an electropneumatic transducer (20A) which sent a pneumatic signal to the pneumatic recorder controller (20B). A proportional valve (25) was governed by the output from this control station so that the ethane was purged at the expense of the system gas mixture. The purge rate was measured by means of a rotary gas meter (26). A special device was designed to give a remote continuous integration of gas flow. A recorder output from the integrator enabled flow trends to be followed with time. (See circuit diagram of integrator and purge measurement arrangement: Fig (14) and (15).

The two-term controllers used in the work reported here possessed proportional and integral facilities. This enabled the concentrations of the gases to be adjusted to set points easily. Such adjustments were equivalent to load changes. Two-term control restored process conditions most satisfactorily following deviations from the set points.

(B) CONTROL OF pH:

(i) MEASUREMENT OF pH; A steam sterilisable electrode was used to measure pH. The combination electrode (See section 2 part B) consisted of a glass body containing a coated silver reference wire and a reference element in a separate chamber. The element in the central chamber had a dielectric covering on the cable and it was essential that this was kept clean and dry to prevent electrical 'noise' affecting the output.

The/

The elements could be withdrawn before autoclaving the electrode did not require recalibration before use. The withdrawn elements were stored in distilled water to prevent contamination.

A side arm was provided so that electrolyte could be added easily. The outer chamber received an electrolyte supplied by the manufacturers of the electrode - Activion Glass Ltd., (See appendix V) and the central chamber was filled with saturated potassium chloride.

The terminals were connected to the appropriate leads from the pH/meter/controller (38, Fig. 16) and the meter output was recorded on recorder (41).

The desired value of pH was set on the controller which possessed two control positions - one below and the other above the set point. Only falling pH values were encountered in the work described.

The salts medium used had a make up pH of 2.6 and the fermentation pH was set at 6.8. It was necessary only to provide alkali for pH control.

(ii) CONTROL OF pH; Deviation from the set point caused the controller to start a peristaltic pump (3 or 4, Fig.13), which transferred alkali from the resevoir (pHR) to the fermenter. Once the set point was regained the pump was deactivated.

The form of control used was ON/OFF (see section 35A, Part B) and to provide a smoother response, a timer interrupted the power supply to the pumps. The timer was part of the Chemap F.Z.7 control panel (Fig. 17) and had variable on/off periodicity. (0.5 - 5 secs., or 5 - 50 secs.).

A/

a manual over-ride switch allowed the pH controller to act directly on the pump. The timer allowed the rate of delivery of the alkali to be adjusted to suit process load changes. The variable off time allowed for short and long lag times between addition of the alkali and registration of the pH change in the fermenters.

The pump used to deliver the alkali to the fermenters was a fixed speed peristaltic pump (Watson Marlow) and the pumping rate could be varied by varying the internal diameter of the silicone tubing used to conduct the alkali. Thus if the timer periodicity could not be adjusted sufficiently to give smooth control, the section of tubing at the pumphead could be changed aseptically to achieve good control. Within limits the same effect could have been achieved by use of a variable speed pump but it was decided that such an expense was not warranted since the F.Z.7 had already been provided.

Alkali was stored in a glass resevoir (pH.R. Fig.13) and supplied both fermenters through 2 separate pumps. The resevoir was recharged aseptically by means of a remote peristaltic pump (7) which transferred fresh alkali through (Z) to the resevoir (pH.R.).

It can be seen that there were three ways to adjust the pH control loop response as process load changes occurred.

I) Adjustment of timer periodicity.

II) Replacement of delivery tube with one of a different size.

III) Adjustment of the alkali molarity.

The first method was the easiest to perform. To change the molarity of the control alkali a measured quantity of sterile water was pumped in/

in to the resevoir to dilute the alkali. Concentrated alkali was added to increase the molarity.

(C) FERMENTATION TEMPERATURE:

(i) MEASUREMENT: A mercury-in-glass thermometer (range 0-130°C) immersed in glycerol contained in a chemap thermometer pocket was used to check sterilising temperature and to calibrate the system below.

(ii) CONTROL: Two matched platinum resistance thermometers, fitted to the fermenter head plates by PTFE adaptors, were linked to a 2-point resistance-bridge recorder. Each thermometer operated its own pair of control lock-in relays in the rear of the recorder via microswitches on the bridge-wire spindle. One relay of each pair opened a solenoid valve on the cold water line serving the appropriate fermenter; the other relay powered a water heater in the same water line (Fig.4). In all cases reported here, heat outputs from agitation and exothermic methane metabolism obviated the need for heaters. The ON/OFF control of temperature was improved by careful adjustment of the cold water flow rate via the hand valve beneath each fermenter platform.

(4) DESCRIPTION OF THE FERMENTERS:

I. GENERAL:

Chemap fermenters (model G.F. 007) were the only types which met all the requirements outlined in section (62) of Part B.

The G.F.007 consisted of a glass vessel of 7 litre capacity which fitted onto a stainless steel base plate. The drive shaft entered the fermenter through the base plate and extended upward through a carbon top seal and ended slightly above the head plate. The seal kept the hollow shaft in a central allignment and also maintained sterility after autoclaving/

claving. A diagram of the G.F.007 has been given in Fig.(18). All metal parts in contact with the fermenter contents were made of stainless steel and the 'O' rings were neoprene.

A network of tubing was fixed to the base plate and through which passed either water for the purposes of temperature control (see section 3c, Part C) or steam during in situ autoclaving (See Appendix I).

The eight entry ports on the head plate could be made multi-functional by means of branched adapters. Each was sealed by an interchangeable plug and plug holder containing a self-sealing rubber gasket (Fig.19). The plugs could be removed rapidly and a needle adapter (Fig.20) fitted equally rapidly by piercing the gasket. Thus, after sterilization, there was only a minimum risk of contamination when attaching medium-feed lines, for example.

The same design of holder accepted Chemap ceramic filter units. These filters had a male quick-release coupling attached so that dismantling of the fermenter was not impeded by the fixed gas recycle lines. Each fermenter had two filters, one on the gas inlet sparger and one on the outlet condenser. A diagram of the filters has been provided in Fig.(11).

The condensers used in the project described here were standard equipment and were essential to the project for several reasons:

I) The exit filter would not work when wet.

II) At an operational temperature of 40° the rate of evaporation from the fermenters would have been excessive.

III) As a corollary to point II, because the analysers operated with dry sample gas and because dry recycle lines were necessary from a safety aspect, condensers to dry the exit gas were essential.

The condensers, Fig (21) were fitted in a gas tight manner to an adapter/

adapter attached to the fermenter above the end of the shaft. Gas leaving the fermenters via the shaft passed through the condensers and exit filters and into the recycle lines.

The resistance to flow of coolant through the condensers was high and a powerful circulating pump was needed to achieve a sufficiently high rate of flow to give adequate condensation. At 40^{o} the temperature of the coolant required to give adequate heat transfer was found to be not greater than 4^{o} at a through-put rate of 1000 c.c/minute.

Cooling was provided by two cooling coils immersed in a mixture of 20% ethylene glycol/80% water. Circulation of the coolant through the condensers was by means of a centrifugal pump.

II: AGITATIONAL SYSTEM:

Chemap offered several agitational/aeration systems each designed for specific applications. The system used in the work reported here consisted of 3 impeller discs each possessing 4 blades at right angles to each other. The discs were sited on the shaft at regular intervals. Four baffles were provided, fixed to the tubing network described earlier (Section 4 (I)). Each baffle was $^{3}/_{4}$" wide and extended from near the base plate to the foam breaking device. The baffles were situated at equidistant positions along the circumference of the fermenter but not in contact with the vessel walls.

Around the impellers was a device resembling a cheese grater called an emulgator (See Fig.18). The emulgator was attached to the tubing network in such a way that the smaller holes were opposite the impeller blades. Gas entering the vessel via the sparge tube passed up the inside of the emulgator. Agitation by the impellers caused gas/liquid to be sucked into the emulgator through the larger holes and out through the smaller/

smaller ones in a uniform emulsion. The suction effect on the gas in the head space was so great that even if there is no bottom sparging a uniform emulsion can be produced.

With many conventional agitational systems much gas flow is wasted due to entrainment of the gas along the shaft. The gas then escapes without the agitational system acting on it. Chemap claimed that up to 5 volumes of gas/volume of liquid/minute could be employed without large gas bubbles rising to the surface. The bubble size in the emulsion could be varied depending on the size of the pores in the emulgator from $1\text{-}10_{\mu}$.

Mass transfer rates for the agitational system described above were determined using the sulphite oxidation method of Cooper et al (1944). (Section 1, Part D).

Power to drive the impeller shaft was provided by a standard 3-phase induction motor of $^{3}/_{4}$ horse power. Stepless speed control cannot easily be achieved with A.C. frequency motors and the standard equipment supplied by Chemap used a stepless D.C. speed controller (UNITROL) on an induction motor. Since such an arrangement could be used under the conditions of explosion hazard in the work reported here Chemap provided a stepless hydraulic pump motor or variator. The variator was essentially a variable-stroke radial-piston type of pump and a fixed stroke radial-piston hydrometer linked by a fluid which circulated in a distributor shaft on which the two rotated. Speed control was achieved by alteration of the length of piston stroke via a hand wheel on the variator.

III. CONTROL OF LIQUID VOLUME IN CONTINUOUS CULTURE:

At low agitation rates a conventional weir overflow device was used.

At/

At high agitation and gas flow rates the whole fermenter was essentially full of foam. Weir devices or probes measuring liquid level (e.g. capacitance probes) were therefore inappropriate.

If the density of this foam could be assessed a control loop for liquid volume could be designed. There were two possibilities:

(i) use of a differential pressure sensor which essentially measures the weight of liquid present in the fermenter

(ii) measurement of torque on the agitation shaft, as affected by foam density.

The former method was ruled out because of the small weight of liquid in the present fermenters. The latter method further required that the torque measurement be made by an intrinsically safe instrument for operation in hazardous areas.

Originally the equipment supplied by Chemap was supposed to measure the torque generated by the hydraulic variator situated between the motor and the agitator shaft. The torque measurement was indicated on an oil pressure gauge. Changes in torque were related to changes in the ratio of liquid to gas in the fermenter, i.e. as medium was delivered into the vessel the density of the foam increased. As the density increased the power needed to drive the shaft at the set speed increased also. The power drawn was related to the torque transmitted and thus formed the basis of volume control.

Unfortunately the manufacturer's claims relating to torque measurement were not substantiated and so a new system of volume control was developed. This was based on the electrical power drawn by the fixed speed motor driving the variator. The principle of the power meter and important ancillary facilities incorporated into the final design as a result of considerable development work, will now be described.

Power Meter

At a fixed mains voltage, power drawn by the motor can be related directly to the current flowing in the motor. By tapping into one phase of the 3-phase supply to the motor, the current flowing in that phase was passed through a toroidal current transformer (Fig.22). The small induced current in the secondary windings of the transformer was amplified to a voltage output and compared with a fixed reference voltage supply, the difference being measured on a voltmeter and also relayed to a potentiometric recorder. By altering the reference voltage the voltmeter needle could be set for any desired base-line power consumption in the fermenter. Through a variable amplifier gain switch the sensitivity of the power meter could be increased to monitor very small current changes in the fermenter motor. A switched range facility allowed the full sensitivity to be obtained at two base-line power consumptions, the latter varying according to the agitation conditions prevailing in the fermenter.

Control accessory to Power Meter

A photocell was used to sense movements of the voltmeter needle corresponding to rise and fall of foam density in the fermenter. Increases in power consumption (increased foam density) triggered the photocell relay which effected two things. The relay energised effluent pumps 1 and 2 (Fig.3), so removing a proportion of the fermenter contents. Concurrent with this, the input pumps 5 and 6 were de-energised, thereby speeding up the return to set point. At this point, effluent pumps stopped and input pumps started again. A necessary refinement was the interposing of a timer-relay betweeen photocell relay and pumps. The timer relay was adjustable from 0.5 - 10 seconds and meant that minor oscillations of the power meter needle were 'ignored', i.e. did not activate the appropriate pumps. Only when a 'trend' in power consumption was evident did the control circuit operate, an event occurring every 20-

30/

30 seconds. Without this intermediate timer, the pumps would not survive the frequent ON/OFF switching.

The effluent pumps were fitted with a remote manual over-ride switch so that continuous input and removal of liquid was possible. The rate of both delivery and removal medium was controlled remotely by means of potentiometers sited on the FZ7 control panel. (Fig.17).

(IV) MECHANICAL FOAM BREAKAGE:

Each fermenter was equipped with the means to break foam generated during the course of fermentation. The foam breaker consisted of 3 cones situated on the central agitation shaft above the emulgator. The cones were arranged so that the hollow bases faced downwards. Each of the cones fitted neatly over the one below. The lowest cone was the smallest and possessed no ridges on the inside of cup; the remaining two were identical in size and had a series of ridges along the inner surface.

The foam breaker rotated with the central shaft. The foam generated in agitation of the fermenter contents rose up and hit the inner surface of the cones and air and liquid separated by centrifugal action. The ridges channelled the liquid portion back into the fermenter; the gas escaped into the condenser.

(5) CONSTRUCTIONAL DETAILS OF OXYGEN ELECTRODE:

The electrode consisted of a cylindrical glass shell of which the lower section was expanded to accept the cathode and anode. Fig.(23) shows the electrode in longitudinal section. The upper end was fitted with a P.T.F.E. adaptor machined to mate with the Chemap blind plug holder on the fermenter head plate. The central cathode was a silver rod extending/

extending into a disc at the extreme lower end immediately prior to the membrane. Around the cathode rod an insulating sheath of silicone rubber was fitted. The anode was an aluminium welding rod coiled up to increase its surface area. This surrounded the cathode. The expanded lower section received the electrolyte - 95%digol/5% saturated potassium chloride - which could be autoclaved at 121° without boiling off. A Chemap gasket was fitted to ensure a gas tight connection to the fermenter head plate.

The electrode membrane was an unusual one in that it was composed of a silicone matrix into which a nylon reinforcing mesh was embedded. Such a design gave strength plus high permeability. The membrane was cut into 4" x 1/2" strips and a 2" square. The square was applied to the open end of the electrode over the silver dish cathode. The surplus material of the square was wrapped around the electrode stem and stuck to the stem with a special silicone rubber dissolved in cyclohexane. The strips were wrapped around the stem so that the membrane was leak proof. 'O' rings may be added to further enhance a proper seal.

The terminals of the anode and cathode projected from the PTFE adaptor and were fitted with a terminal strip carrying leads to a multi-point recorder (41).

The electrode, electrolyte and membrane were all autoclavable. During autoclaving pressure equilibration was facilitated by means of a syring needle sealed into the P.T.F.E. plug by silicone rubber. After autoclaving, the needle was sealed with a cap made from a blocked syringe needle base.

It can be seen that gas in the fermenter could only escape through the electrode fitment via the gasket in the fermenter port. Should the electrode/

electrode membrane have ruptured, then gas would have left the fermenter through the vent or through the electrode seals in the P.T.F.E. adaptor terminal area. To preclude this the ventcap was always closed off and a layer of silicaset applied to the terminal area in such a way as to constitute a gas tight seal.

(6) LIQUID MEDIUM SUPPLY:

(I) BULK PREPARATION:

The recipe for the salts medium used in this project has been given in table (18). Industrial grade chemicals were used throughout the project and were supplied by I.C.I. Billingham.

With the exception of Zinc sulphate, all the trace elements (B), Table (15), were present as impurities in the other chemicals.

Medium was made up in 60 litre lots as follows. Chemicals listed in Part (A) of Table (15) were dissolved at 60 x strength in about 500 mls. of warm water. Stirring increased the rate of solution; after 10 minutes 30.0 mgs. of Zinc sulphate were added to the solution. The mixture was then made up to 1 litre with warm water. This solution constituted the bulk concentrated medium prior to dilution to 60 litres.

Using Whatman No.1. filter paper, the 1 litre bulk solution was filtered twice to remove large particulate matter present after dissolution. This acted as a prefiltering stage, and increased the life of the sterilizing membrane filters in the medium filtration rig (Section 49), part B. The crudely filtered solution was then made up to 60 litres with tap water in a bulk storage vessel (Fig.24). The medium was mixed thoroughly by shaking the vessel and held ready for sterilization.

(II) MEDIUM STERILIZATION:

The advantages of filtration as a means of medium sterilisation to the work reported here have been outlined previously in section (48)

Part/

Part B. In essence two forms of filtration rig were designed, manual and automatic, although much of the equipment was common to both.

(i) MANUAL FILTRATION:

(a) CONSTRUCTION OF THE RIG; The filtration unit consisted of two P.V.C. 142 m.m. (A1, B1) filter holders fixed to a dexion frame (See Fig.(25). These units acted as pre filters only for, although fitted with membranes of 0.22 u pore size, they could not be autoclaved. The subsequent lines up to detachable swagelock fittings were permanent fixtures. The pre-filters could be replaced simply and rapidly during a filtration run without concern for asepsis.

Final sterilization of the prefilter effluent was achieved by means of two autoclavable 47 mm. Swinnex filtration units (A2, B2). The membranes used in thse units were again 0.22 um pore size. The Swinnex units were permanently attached to autoclaveable swagelock quick-release fittings matched to the swagelock fittings of the main rig (Q1, Q2). The quick-release arrangement allowed pressure connections to be made to the main rig as many times as was necessary without damage to the delicate plastic filter holders.

The Swinnex units, male quick-release coupling, autoclavable solenoids (see automatic filtration section (6C) below) and silicone transfer tubing were detachable from the dexion frame of the main rig. Prior to medium sterilization, this section was autoclaved separately and then re-attached to the main rig. Final connection of the sterilization unit was made by fitting the transfer lines (terminated in Q.V.F. cone connections) aseptically to the medium resevoirs (R1) and (R2). (Fig. 3).

Some difficulty was experienced in the matter of leak-proof connections/

connections of silicone rubber to glass or stainless steel mainly because the pressure drop across the different sections had been greatly underestimated. Initially nylon cable clips were tried but were not entirely successful, particularly after autoclaving. A second method employed a protective wrapping of surgical tape around the silicone rubber. Wire was tightened onto the tape and forced a good connection. Unfortunately, this tended to damage the silicone rubber tubing. Finally Uniclip tubing clips were used to give a leak-tight connection. To reduce damage and therefore prolong the life of the silicone tubing surgical tape was first applied. During autoclaving it was found advisable to loosen the clips slightly in such a way as to allow expansion but still to retain the tubing on its mounting.

The first rig constructed used ground glass joints on the transfer lines between the Swinnex units and the resevoirs to reduce the amount of untangling required after autoclaving, but these joints were easily broken and sterility lost. This idea was abandoned and the transfer lines were attached directly onto the stainless steel inserts of the solenoids.

The pressure drop was greatest between the 142 mm. prefilter outlets and the Swinnex units. Particular attention had to be paid to the tubing connections and couplings in this area and inspections of this section were performed on every day of continuous operation.

(b) FILTRATION PROCEDURE. (MANUAL) In the filtration rig Fig. (24) membrane filters and prefilters were inserted into each Swinnex A1, A2). It was found that prefilters increased the life of the membranes by effectively removing large particulate matter. In addition, the prefilter gave rear support to the membrane filter. This was found to be important when backflow of liquid occurred after rapid valve closure.

Support/

Support for the membranes was needed during bubble point testing (See below)

The autoclavable section of the rig (Q1, Q2 to A, B) was sterilized and reattached to the main rig. Aseptic connection of the transfer lines to the sterile medium vessels (R1, R2) was made at (A) and (B) and medium introduced into the pressure vessel (SP).

Hand valves (1), (2) and (3) were closed and the 20 litre vessel (SP) pressurised to 20 p.s.i with compressed air via a three way solenoid valve (T). Valves (1) and (2) were then opened slowly, allowing medium to be passed to the P.V.C. filter units. Vent valves (E1 and E2) on the filter units were opened slightly to allow trapped air to escape. Valve (3) was then opened and medium passed through the Swinnex filter units (A2,B2), where sterilization occurred, and into the medium resevoirs. When the resevoirs were full valve (3) was closed. As soon as the vessels became empty the above proceedure was repeated to refill the resevoirs. Occasionally an air lock developed in the swinnex units and this was released by means of vent valves (F1, F2) which were specially fitted for the purpose. Finally, valve (T) was de-energised, thereby releasing the pressure in vessel (SP).

(c) AUTOMATIC FILTRATION:

The flow sheet of the automatic filtration rig has been set out in fig. (24). Much of the design of the manually controlled filtration rig constituted the nucleus of the automatic rig. To allow automatic filtration, a relay circuit was designed (Fig.26) so that the only manual component in the operation was the filling of a 60 litre bulk storage vessel (Bu), with the unsterile medium.

The/

The heart of the automatic filtration process were the level probes (K1 to K7) which detected the level of liquid in the various vessels and transmitted the information to the relay box controlling the filtration cycle. Each probe consisted of a heated thermistor held in a glass or stainless steel dip-rod. As liquid in a vessel touched the hot thermistor a temperature change occurred in the thermistor, whose resistance fell. The current passed by the thermistor depended on its electrical resistance which was proportional to the temperature of the liquid. The warmer the liquid the greater the current in the thermistor. At 30-40° C the current was in the region of 40-50 milliamps. and at 15-25°C the current was in the region of 25-35 milliamps. The current also depended on the resistance value of resistors R1 and R2 (see Fig.26). Typical current figures at 20°C and with R1 = 10K ohms and R2 = 200 ohms were thermistor hot: 60-70 milliamps, thermistor cold: 25-50 milliamps.

FILL CYCLE:

With the bulk storage vessel charged with 60 litres of unsterile medium, the filling cycle was as follows;

START UP CONDITION

1) Low level probe (K3) deactivated 3-way solenoid valve (T) and thus cut off the compressed air supply to the empty vessel (SP) (which was empty at start up). (S.V.1) solenoid was energised and the delivery pump (P) switched on, by low pressure switch (Y). Medium entered the pressure vessel (SP-) until the 20 litre mark was reached, at which point level probe (K2) stopped (P) and energised 3-way valve (T) so that compressed air commenced to pressurise (SP).

2) At the 20 p.s.i. point (displayed on G) the high pressure switch (X-) caused (S.V.2) to open and medium was forced along the pipework of/

of the main rig as far as handvalves (1) and (2). At this stage the hand valves were opened carefully so that medium passed into the filter units (A1) and (B1). Air locks were released via vents (E1, E2) and medium allowed to pass into the sterilizing filter units (A2, B2) by valve (3).

3) The medium resevoirs (R1, R2) were empty and to prevent probes (K6, K7) from opening (S.V.3-6), two over-ride switches were installed. When the contacts of these switches were opened the relay contacts of (K6, K7) were isolated and inoperative. Vessel (R1) was first filled. The over-ride switch (SW1-) was closed so that the signal from (K6) opened valves (S.V.3, S,V.6). Medium then entered vessel (R1) until K3 in SP read 'empty vessel' condition. The high level probe in this vessel (K4) closed (S.V.3) to prevent overfilling of (R1). Over-ride switch (SW2) was thrown so that low level probe (K7) opened (S.V.4, S.V.5) and delivery of medium to the fermenters occurred. (See liquid line flow sheet Fig.13).

4) Pressure vessel (SP) was now empty and (K3) opened 3-way valve (T) so that the pressure in (SP) was released to the atmosphere. Eventually the low pressure switch (Y) started the cycle once more. During the second filtration cycle, medium passed into the transfer lines to fill vessel (R2) at which time high level probe (K5) closed valve (S.V.4) to avoid accidental overfilling of (R2).

5) During the operations 3 and 4 above, medium was being delivered to the fermenters and when vessel (R1) became empty level probe (K6) opened (S.V.3) and closed (S.V.5). At the same time valve (S.V.6) was opened and vessel (R2) began to feed the fermenters. As each vessel emptied the above cycle was repeated, thus ensuring a continuous supply of sterile medium. The logic sequence of the relays in the control unit for the filtration system has been set out in Appendix III.

(7) /

(7) MEASUREMENT OF FLOW RATES: GASES.

(A) ETHANE PURGE RATE. A system to measure the rate of ethane purge was designed. Gas from the main gas recycle line was periodically vented under the action of the ethane control loop (See section3, Part C). When the mixed culture was able to consume both the methane and the ethane present in north sea gas (see Table 16), the purge rate fell to a low value depending on relative consumption rates of methane and ethane.

In essence the rate of ethane purge flow was measured by a wet-test rotary gas meter in conjunction with a photo-cell and pulse integrator. As gas passed into the gas meter (situated inside explosion cubicle 1) the pointer shaft rotated but in place of the pointer arm, an extension shaft was fitted, carrying an 8" diameter disc perforated with 1 cm. holes in 6 concentric circles (Fig.27). On one side of the disc a light soource projected a beam of light onto a photo-cell on the opposite side of the disc; the beam was interuppted by the revolving disc. The greater the rate of gas purge, the more often the perforations passed in front of the photo-cell. The output of the photo-cell was transmitted to a remote pulse counter (which indicated the total pulses) and to a pulse integrator with a recorder output. The pulse integrator was designed so that the recorder output changed little with short term fluctuations over a 5 second period (i.e. it did not respond greatly to pulse peaks) but trends upwards or downwards over several minutes produced the appropriate recorder response.

It was important that the rate of rotation of the gas meter gave approximately the correct range of pulse rates, i.e. 1-2 per second maximum, 1 per three of four seconds minimum. To ensure this the position of the photo-cell could be adjusted in relation to the perforated disc so that fewer or more perforations per rotation passed in front/

front of the cell. Thus, the requisite number of pulses per second could be obtained for a given range of gas flow rates.

The time constants for insensitivity to pulse peaks and small fluctuations of gas flow could be adjusted by minor alterations to the integrator circuitry. If necessary variable resistors could be inserted to allow adjustable time constants.

The circuitry for the pulse integrator and relay have been presented in Fig. (14). Some points regarding the operation of the instruments follow:

Every time that the relay contacts 'made' (Fig.14) current flowed through the counter and partially charged the $25_{u}F$ condenser. Similarly current could flow through T3 (Fig.14) and develop a potential across the 30K + 130 ohm resistors. The potential was tapped off at the 130 ohm point to the recorder, and the 2800 $_{u}F$ condenser partially charged. This condenser discharged slowly according to the product of the resistance and capacitance;

$$30 \text{ K ohm} \times 2800_{u}F = 84 \text{ seconds.}$$

The photo cell lamp was under-run to prolong its life (Fig.15) and a sensitivity control had been incorporated into the transistor circuit so that the ambient light did not activate the photocell.

It was designed so that if necessary, the relay could carry 5 amps. at 250 volts. Terminals (A) and (B) were the 12 volt A.C. supply to the light scource.

(B) GAS FLOW ESCAPING THROUGH THE LUTE:

A simple method of measuring the rate of flow of gas escaping from the system via the lute was designed. The backbone of the method was a rotary gas meter through which discharged gas was passed. Two 1" bar magnets/

magnets were attached to the meter pinter, one on each side of the centre point. A reed switch was fixed in position close to the pointer (Fig.28). As the pointer rotated the magnets caused the reed switch to make and 'break' a contact in circuit with a digital pulse counter, which displayed the sum of the number of 'makes' received from the reed switch.

The volume of escaped gas was calculated as follows:

One pointer rotation = 0.25 litres of gas

= 2 impulses

$$\text{Total volume of gas (l)} = \frac{\text{Total No. impulses}}{8}$$

(C) MEASUREMENT OF GAS FLOWS INTO ANALYSERS AND FERMENTERS

(1) Analysers

Each of the analysers was provided with an adjustable glass rotameter measuring flows in the range 0-140cc/min. It was important that the rate of flow into the analysers was constant and reproducible especially in the case of the infra-red analysers. This was true both for normal running conditions and for calibration. It was noted that slight fluctuations in the indicated flow caused alterations to the calibration of these instruments.

Each of the flow meters was calibrated with a specific gas mixture (50% oxygen, 50% methane at 40°C) but the composition of the fermentation gas was of quite different composition which varied from time to time. However, as long as the same flow setting was used each time the instruments were calibrated, the results of fermenter gas analyses were satisfactory. (See calibration of the analysers, section 3, Part 1).

(2) Fermenters:

An adjustable glass rotameter was used to control and measure the rate of gas flow into each fermenter. They were calibrated for/

for operation at 40°C with a gas mixture of 50% methane and 50% oxygen, a compromise between the actual system gas composition (varying) and an extreme value of for example 100% oxygen. The accuracy of these rotameters was not great but their function was to give an indication of the flows into the fermenter. An accuracy of 5% was considered acceptable.

(D) MEASUREMENT OF METHANE AND OXYGEN FLOWS INTO THE MAIN GAS RECYCLE LINES:

Measurement of gas flows into the system under the direction of the control loops governing the gas composition of the circulating gas (See section 3 Part C) was accomplished by means of Fischer-Porter differential pressure cells converted to read flow by means of a capillary tube arrangement and a shunt tube. (Fig.(4) items 7 and 14).

These instruments had certain attractive features which led to their incorporation in the design described in this thesis;

1) They gave a pneumatic signal which was transmitted to a recorder (4) in Fig.(4) so that a continuous trace of gas consumption was available.

2) Adjustable damping could be used to exclude flow pulsations.

3) There was a positive overrange protection to 1500 p.s.i.

4) The through bolt construction allowed simple removal of the transmitter from its mounting.

5) The flow capillaries could be removed and replaced without disconnecting the instrument from the flanges.

6) The measuring elements were protected by sealing diaphragms.

7) The shunt tube assemply permitted quick changes of range whilst the instrument was in operation.

The flow transmitters were calibrated at I.C.I. Billingham and the calibration charts given in fig.(29) and (30). The working pressure was/

was 20 p.s.i. at 20°C. The flow rates used were:

Methane: 15 litres per hour maximum and 5 litres per hour minimum

Oxygen: 30 " " " " " 5 " " " "

The capillaries were degreased before use for safe oxygen service. (See section 67, Part B).

(E) MEASUREMENT OF CARBON DIOXIDE FLOW INTO THE MAIN RECYCLE LINES:

Carbon dioxide was measured by means of a glass rotameter calibrated for operation at 20°C at a pressure of 20 p.s.i. Carbon dioxide in the recycle lines was not automatically controlled but was added to the system under manual control to the desired level. (See start up procedure, section 1 of Part E). Flow was regulated by means of a flostat (18 in Feg.4).

(8) SAFETY ASPECTS OF THE METHANE BIOMASS PLANT:

The design described in section (2) of Part C conformed to the recommendations set out in section (67) Part B, and in addition possessed certain fail-safe devices which are described below; Fig.(4) shows the safety features of the plant.

Normally system pressure was automatically controlled but in the event of instrument malfunction, leaks, inoperative valves or any situation resulting in pressure increases or decreases fail-safe devices reduced the danger level;

I. WATER LUTE: An 18" water lute (V) in line on the low pressure side of the system.

This was open to the atmosphere and rapid let down of pressure occurred by this means.

II. PRESSURE SWITCHES: Pressure transducer (5) detected pressure changes and relayed the information both to a pneumatic multipoint recorder/

recorder and to pressure switches, one for high pressure and one for low pressure. If the system pressure either increased or decreased beyond preset points an electrical circuit was completed (via relays) to two solenoids (60) and (61). The solenoids were installed on pneumatic control lines to the oxygen and North sea gas valves (7) and (14) and could interrupt the control signal so that the valves closed and prevented any further entry of oxygen or North sea gas into the system.

III. PURGE SYSTEM: The gas analysers were intrinsically safe but in case of leakage of system gas into the casings they were supplied with a compressed air purge. The air rapidly diluted any dangerous gases present in the casing to below the lower explosive limit.

There were 6 peristaltic pumps involved in medium delivery, pH control and Level control (Fig.(13) 1-6). All six pumps were enclosed in a perspex cabinet so that only the pump heads projected from the cabinet. (See Fig.31). The cabinet was divided into two sections each of which was fed by a compressed air purge. The pressure inside the cabinet was slightly higher than outside and therefore the air constantly issued out through the pump heads. Gas leaking either from the gas lines themselves or from a ruptured liquid delivery line could not enter the cabinet and reach the hazardous electrical pump motors. Flow meters on the purge lines to both the cabinet and the anlysers indicated that a supply of air was being maintained. Should the air supply have fallen (e.g. compressor became inoperative; purge transmission lines broke) a pressure switch detected the fall in purge pressure and forced the closure of O_2 and CH_4 valves (7) and (14), in the same manner as for system pressure changes. The pressure switch acted via a relay box (Fig.32)/

(Fig.32). The relay was designed so that action by the HIGH/LOW pressure switches had the following effects;

(A) High pressure/low pressure condition:

(i) Solenoid S2 opened (Fig.4) and allowed gas in the system to be vented.

(2) Oxygen valve (14) was shut off.

(3) North sea gas valve (7) was shut off.

(4) The medium and effluent delivery pumps were switched off.

(5) A high pressure or low pressure trip light came on.

(B) PurgeFailure:

(1) The gas analysers and pH control pumps were switched off.

(2) A purge failure light came on.

(3) Medium and effluent delivery pumps were switched off.

(IV) POWER LOSS: In the event of a generalised power failure a series of events took place;

(1) The gas circulation pump (27) stopped.

(2) Solenoids (60) and (61) closed. This was because they were energised open. Thus the gas supply was effectively shut off.

(3) Solenoid (R1) (energised closed) opened. Gas still in the analysers could therefore escape from the system via the lute (V).

(4) Gas in the main system was vented to atmosphere through the lute.

Naturally in a condition of power failure none of the electrical equipment could function but should the power have returned the system could self-start. The low pressure switches, activated by loss of gas from the system, prevented fresh gas entering the system until the operator restored the correct conditions of operation, whereupon a manual reset switch/

switch incorporated into the relay box (Fig.32) was depressed.

(V) PARTICULATE CONTAMINATION: Small particulate matter present in certain concentrations of oxygen could be spontaneously oxidised with a clear risk of explosion when methane was also present (section Part B).

Sintered stainless steel filters (Z1-3)(Fig.4) were installed in the system at crucial points. They prevented particles larger than the pore size of the sinter (35u) from entering the main gas system.

(VI) EXPLOSION RISKS: To reduce the effect of an explosion from whatever cause, stainless steel flame traps were placed in line throughout the system (P1-P12). These flame traps attenuated the combustion wave of an explosion because they had a very large surface area and prevented the wave extending beyond the trap. They are only partially successful at containing an explosion but do lessen the overall intensity of an explosion.

(VII) PRESSURE RELIEF: If none of the above fail-safe procedures were successful a pressure relief pad (W) fractured and allowed gas to escape to atmosphere, thus preventing an excessive build up of pressure.

(VIII) FERMENTER STRENGTH: Certain elementary precautions were taken in addition to the above design features.

1) The fermentation vessels were tested at I.C.I. Explosives Factory, Ardeer, and found to resist at least 6 explosions from stoichiometric mixtures of methane and oxygen. This constituted the most severe examination of the strength of the fermenters. As a further precaution, stainless steel hoods bolted to the fermenter head-plates give added protection against flying glass.

2)/

2) The glass ware in the whole system was limited to a minimum. The carbon bioxide scrubbers were made of thick glass (100 p.s.i. safe working pressure) and were taped to reduce the chance of glass fragments flying around in the event of an explosion. Similarly the effluent culture collection vessels were taped. A 20 litre vessel constituted the greatest danger since here was the largest volume of free gas in the system. Fortunately the likelihood of ignition in these vessels was minimum.

3) The fermenters, the collection vessels, CO2 scrubbers and a greater part of the recycle piping were situated in a specially constructed explosion cubicle (Cubicle 1). The gas cylinders, (except those containing North Sea gas), the analysers and Fischer-Porter flow meters were fixed in an identical unit (Cubicle 2). Any explosion occurring in these cubicles caused a window to blow out so that damage to equipment caused by pressure waves was reduced.

The cylinders of North Sea gas were situated in a position remote from the oxygen cylinders to prevent mixing of these gases in the event of leaks from the cylinders and delivery pipes. Entry to the cubicles was restricted to authorised personnel and entered only for routine inspections and adjustments of equipment.

Although the fermenters contained approximately 2 litres of gas mixture this was in the form of a homogenous foam in the ratio of 5 parts liquid to 2 parts gas, (See agitational system section, Part C). and was factually non-explosive.

PART (D) MATERIALS AND METHODS:

COMMISSIONING EXPERIMENTS ON THE EQUIPMENT AND INSTRUMENTATION

PAGES: (142) TO (161)

(1) MEASUREMENT OF THE EFFICIENCY OF AERATION OF THE FERMENTERS; MASS TRANSFER COEFFICIENTS:

Mass transfer coefficients were calculated for different gas flows and rates of agitation by a method based on the sulphite oxidation technique of Cooper Fernstrom and Miller (1944). The effect of the emulgator was tested during this experiment.

The fermenters were filled to the working volume of 5 litres with a solution of sodium sulphite (0.5M). A solution of cobaltous sulphate was added to a final concentration of 0.001M. A I ml. sample of the fermenter contents was removed and added to 10 ml. of freshly prepared 0.1N Iodine solution as quickly as possible. It was found that keeping the pipette tip close to the surface of the iodine solution reduced the degree of initial oxidation. 5mls. of 2N hydrochloric acid were added and thoroughly mixed as rapidly as possible. Excess iodine was then titrated against 0.IN sodium thiosulphate with a starch indicator. By this means the initial or basal oxidation of sulphite by oxygen already dissolved in the solution was determined.

The aeration agitation system was started and samples removed at 30 second intervals and titrated in the same way as above. The equations relating to the method were:

$$O_2 + 2Na_2SO_3 = 2\,Na_2SO_4 \qquad (12)$$

$$Na_2SO_3 + I_2 + H_2O = Na_2SO_4 + 2HI \qquad (13)$$

$$I_2 + 2Na_2S_2O_3 = Na_2S_4O_6 + 2NaI \qquad (14)$$

It is evident that one mole of oxygen absorbed is equivalent to 2 equivalents of I_2 or 4 equivalents of $Na_2S_2O_3$. Thus Iml. of 0.I NI_2 + I ml. of 0.I $Na_2S_2O_3$ = I/40 millimoles of O_2.

Volumes/

Volumes of thiosulphate were plotted against time, and the slopes of the graphs were a measure of the rate of oxidation of sulphite under the imposed conditions. Multiplication of the slopes by 1/40 gave the rate of oxidation in terms of mM Oxygen/minute. Multiplication by 60/40 gave the result in terms of mM oxygen/hour.

It was assumed that oxygen transferred into solution reacted as fast as it was dissolved so that the concentration of dissolved oxygen was zero. It is known that the rate of transfer of oxygen (or absorption) is related to the mass transfer coefficient and the concentration driving force as follows; (See section 19 part B).

$$\text{Rate of } O_2 \text{ absorption} = K_L A\,(C_G - C_L) \qquad (15)$$

$$(mM/L/Hr.) \qquad (mM/L)$$

The saturation level of oxygen in 0.5 Molar sodium sulphate is 0.2 mM O_2/Litre. Thus the mass transfer coefficient

$K_L A$ = Rate of O_2 absorbtion/0.2 or

$K_L A$ = 5 X Rate of O_2 absorbtion

The $K_L A$ values were expressed in terms of mMO_2/Litre/Hour by multiplying the rate of absorption per hour by 1000, since the titres obtained were for 1 ml. samples.

(2) RESULTS OF MASS TRANSFER EXPERIMENTS:

The results of the examination of the gas transfer capability of the fermenters at different air velocities and agitation rates has been presented in table (19). Each titration run was completed 3 times and the average value taken as the titre. The rates of mass transfer increased both with increased air flow and with rate of agitation. The emulgator/

emulgator did appear to increase mass transfer rates but the greatest increase was at 3000 r.p.m. and 10 l/hr. This was calculated to 2460 without the emulgator and 2580 with it - a difference of 120. In one case there was a drop in the mass transfer rate with the emulgator in place (5.0l/hr. at 1000 r.p.m.) This may have been a reflection of the method itself rather than an effect of the emulgator.

(3) CALIBRATION OF GAS ANALYSERS:

The four gas analysers were calibrated in a simple on-line manner which did not require any plant shut down. Calibration was complete in less than 4 minutes. For calibration of the Servomex O_2 analyser, a source of nitrogen and air was needed; for the infra-red analysers nitrogen and a source of mixed ethane, methane and carbon dioxide were required. I.C.I. Billingham, provided a cylinder of gas for use in analyser calibration of the following composition;

METHANE	:	60.5 % V/V
ETHANE	:	26.0 % V/V
CARBON DIOXIDE	:	13.5 % V/V

In all cases except the calibration of the servomex, it was possible to calibrate by means of regulating one hand valve and two solenoids. The Servomex required a supply of compressed air for calibration.

(1) ZEROING:

(i) Hand valve (C) and solenoid (R1) were closed. This effectively isolated the analysers from the rest of the recirculatory system, and was done even when there was no gas mixture circulating in the plant, thereby reducing the amount of nitrogen finding its way into the gas lines.

(ii)

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(ii) Solenoid (S2) and solenoid (N2) were opened so that nitrogen passed from the nitrogen cylinder through the analysers and out to atmosphere. After about 45 seconds the zero deflection of the analyser pointers was complete. Accurate zero adjustment was made with the zero potentiometer provided on each instrument. It was sometimes necessary to adjust the flow rates of nitrogen to each analyser and this was achieved by means of rotameters (F1-F4). It was found that a flow rate of 140 c.c./min for the infra red analysers and of 100 cc/min for the servomex gave the most rapid and stable results.

(2) SPANNING:

(i) Solenoid (N2) was closed.

(ii) Solenoid (Mx) was opened. This allowed the special gas mixture to pass into infra-red analysers and out to atmosphere. The flow was adjusted to 140 cc/min with (F2-F4).

(iii) The sample connection tube of the servomex was transferred to the span connection. This directed compressed air from the analyser purge supply into the 02 analyser. Span adjustment was made with the span controls on the analysers.

The above procedure was repeated until stable calibration was achieved.

After calibration the analysers were placed on line as follows:

(i) The servomex span connection was transferred to the sample connection.

(ii) Solenoids (N2) and (Mx) were closed.

(iii) Hand valve (C) was opened to allow gas from the main system to flush out the analysers for about 20 seconds.

(iv) Solenoid (R2) was opened and (R1) opened at the same time.

(4) ANALYSER CALIBRATION STABILITY AND DRIFT:

In order to estimate how often to calibrate the analysers in a routine/

routine operational check, it was necessary to gain some information on the stability of calibration of the analysers.

A gas of fixed composition was introduced into the fermentation system according to the procedure given in section (1) of Part E, and allowed to circulate for several days. The fermenters were sterile and therefore no gas consumption was expected, and therefore any change in the measured composition of the gas (see below) was due to drift in the analyser circuits.

O_2 :	14.5%
CO_2:	5.0%
CH_4:	75.5%
C_2H_6:	5.0%

The agitational system was adjusted to 2000 r.p.m. and after about an hour the gas composition stabilised. An initial change occurred as gas in solution in the water in the fermenters equilibrated with the circulating gas. The circulating gas composition was continuously recorded, O_2 on recorder (13) and the output of the infra-red analysers on recorder (22).

(5) RESULTS OF DRIFT MEASUREMENT OVER 72 HOURS:

Table (20) shows the percentage drift at various stages in a 72 hour period.

(6) CALIBRATION AND PERFORMANCE OF DISSOLVED OXYGEN ELECTRODES:

CALIBRATION: Calibration of electrodes was performed prior to each fermentation run at 40°C and pH 6.8 (controlled).

(i) Zero Adjustment: This was accomplished in one of two ways. When the electrodes were removed from the fermenters at the end of a fermentation run for adjustments and maintenance, the electrodes were immersed/

immersed in a 0.5 Molar solution of sodium sulphite and the electrode terminals attached to recorder (41). The sulphite solution absorbed any free oxygen in the vicinity of the electrodes and enabled the 'zero oxygen' output of the electrodes to be determined. The current output of the electrodes was also measured, using an avometer. It was noted that a constant output was not achieved for about two hours, the current dropping steadily over this period.

The recorder trace was adjusted on the recorder scale by means of a potentiometer on the side of recorder (41).

In a second method of zeroing, used in routine calibration checks at the beginning and end of a fermentation run, or during the run, the 'gassing out' method described by
The fermenters, containing fresh medium, were flushed through with oxygen free nitrogen, via solenoid valve (N2) until a stable and constant electrode output was obtained on the recorder, when the current was measured by the avometer. The rate of drop in current to the basal level was increased by agitation at about 2000 r.p.m. and measurements completed within 20 minutes.

(ii) Spanning: For calibration outside the fermenters the electrodes were immersed in air-saturated water and the outputs measured as for zero adjustments. For in situ calibration of electrodes the nitrogen supply was shut off and a flow of compressed air allowed to pass through the fermenters. In the fermentations proper it was not proposed to use an oxygen concentration of greater than 14.5% and therefore air was regarded as suitable for spanning.

(7) MEASUREMENT OF ELECTRODE RESPONSE TIMES:

The recorded change from zero oxygen to that for air saturated water and/

and vice versa was used to calculate the electrode response times (Fig.33). It was evident that each electrode had individual characteristics in terms of response times, which were

(8) MEASUREMENT OF TEMPERATURE COEFFICIENT OF ELECTRODES:

Using air saturated water a stable output from each electrode was established at 40°C and pH 6.8. This was 42 uA. for electrode (2) and 37 uA. for electrode (1). The temperature was changed to 50° and the output measured over a period of 5 minutes.

The temperature was readjusted to 40° and the output measured once more. Stable outputs were obtained in each case after about 1.5 minutes. The temperature was dropped to 30° and similar readings taken (Fig.34a).

The temperature effect was noticeable in actual fermentations, particularly if the temperature control of the fermenter was not tuned correctly. (Fig. 34b). Accurate measurement of dissolved oxygen tension required either some form of temperature control or a temperature compensation circuit. (See section 15 of part B.).

Although each electrode had its own characteristics, both electrodes exhibited extremely stable outputs over a long term provided that operating conditions of circulating gas composition pH, gas consumption rates, and temperature remained constant. It was noted that the output of both electrodes did not vary more than 0.4 u Amps throughout a 200 hour run. The stability of the electrodes at zero O_2 levels was good over a period of several weeks, Fig.(35). The stability of the electrodes at 14.5% oxygen (V/V) in the circulating gas was also good (Fig.36).

(9) CALIBRATION OF THE DIFFERENTIAL PRESSURE FLOW TRANSMITTERS:

Both the oxygen and north sea gas flow transmitters were calibrated at

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at I.C.I. Billingham, Fig (30), (29). In fact the capillary shunt tubes supplied by Fischer-Porter were different sizes to those stated by the manufacturers and calibration was not satisfactory. The capillary sizes were altered and the 'h' values and the calibration curves determined for the modified instruments (Figs. 29, 30).

It was noted that the relationship between flow and pressure drop did not follow a square root law but more of the order

$$Q = h^{0.8}.$$

This was because the Reynolds numbers were down below 2000, and the flow was transitional/laminar. The capillary size for north sea gas service was finally 0.10" and the 'h' value 32" water gauge. For oxygen service, the flow/pressure drop relationship was:

$$Q = h^{0.65}.$$

The Reynolds numbers oxygen were higher than for the north sea gas instrument. The adjusted capillary size was 0.014" and the 'h' value was 43" water gauge.

In the above calibration conditions at 20°C the following flows were obtained;

North sea gas :	Maximum flow	=	15 litres/hr.
	Minimum flow	=	5 litres/hr.
Oxygen :	Maximum flow	=	30 litres/hr.
	Minimum flow	=	10 litres/hr.

(10) CALIBRATION OF TAYLOR 701 and 721 TRANSDUCERS:

The 701 instrument was calibrated with 0 - 5 mAmp. signals and gave a correct 3-15 p.s.i. output. Similarly the 721 instrument gave a correct 3-15 p.s.i. output in response to a 0 - 10mV input. Fig.(37) and Table (21).

(11)/

(11) CALIBRATION OF THE LIQUID LEVEL CONTROL SYSTEM:

It was necessary to test the torque-meter method for the control of liquid level in the fermenters especially in relation to sensitivity and stability. Each fermenter was tested at different rates of agitation by removing fixed amounts of the fermenter contents and measuring the change in power consumption. Fig (38) and (39).

Stability was measured by merely leaving the fermenters running at a fixed rate of agitation with the working volume of 5 litres present. No volume changes occurred so that changes in the power consumed represented the inhererent drift of the system. Both of the above tests were performed at 40°C and pH 6.8 with nitrogenous liquid media. No cells were present. Only an insignificant amount of drift occurred over a 200 hr. run (Fig.40). The sensitivity of the control loop was estimated as follows. The power consumption was stabilised after the motor was switched on for there was an initial decrease in power consumption as the oil in the hydraulic variator warmed up and became less viscous. A load change was achieved by switching on the medium in pumps so that liquid was delivered continuously at a constant rate. Once the current drawn passed the set point on the power meter, the effluent pumps were allowed to remove liquid under the control of the photocell and relays.

For this experiment a 10 grams/litre suspension of dead Brewer's yeast was used in the nitrogenous medium at 40°C and at pH 6.8. Over a period of 120 hours the volume delivered was compared with the volume removed under the action of the photo-cell control device. (See section of part B.). Observations on the volume change were made every hour.

The/results of the tests on the efficiency of the level control system have been presented in Fig.(41). A maximum volume deviation of 400 ml. was measured (± 200 ml). A control efficiency of 12.5% of the/

the total volume was considered acceptable.

(12) ESTIMATION OF THE PERCENTAGE EFFICIENCY OF THE CHEMAP CERAMIC FILTERS:

An indication of the percentage retentivity of the ceramic filters used in the inlet and exit ports of each fermenter was obtained at gas slow rates of 2.5, 5.0, 7.5 and 10 $l.min^{-1}$.

An aerosol was generated in a 500 ml flask containing 100 ml of a nutrient broth culture of Serratia marcescens by bubbling air through the broth. The air at the test gas velocity passed through a condenser, to prevent the filter from becoming wet when gas flow would be impeded.

A sterile millipore membrane in a 47 m.m. holder was placed in series before the ceramic filter and the aerosol allowed to pass through the membrane for a period of 10 minutes. The gas flow was shut off and the membrane was then removed from the holder and aseptically transferred to a nutrient agar plate. The membrane was incubated at 37°C to give an estimation of the number of organisms present in the aerosol. The gas flow was resumed and the number of organisms passing through the ceramic filter was ascertained by passing the gas through a second membrane filter in line after the ceramic filter and incubating in the same manner as the first filter. Individual colonies could be differentiated after 24 hours incubation with a binocular microscope (Table 22). Counts made after 48 hr incubation were often not possible because of coalescent growth of the colonies.

(13) ESTIMATION OF THE DIRT HANDLING CAPACITY OF THE CHEMAP FILTERS:

An indication of the maximum time before filter blockage occurred under conditions which reflected the actual working conditions as closely/

closely as possible was obtained by passing air (10 litres/minute) through the fermenters containing 5 litres of water. A suspension of heat killed Baker's yeast ($10g.l^{-1}$) was added to the fermenters to charge the aerosol with cells.

The gas and cells were passed through the filters for 72 hr. and the degree of blockage of the filter was estimated by measuring the drop in air flow through the fermenters. Readings were taken every 6 hours. Tests were done with and without coolant flowing through the effluent gas condenser. It was clear (Table 23) that an efficient condensation of moisture was essential to a prolonged working life of outlet filters. No drop in flow through the filters was recorded over 72 hr. when the condenser was in place. The absence of the condenser caused a rapid and progressive drop in flow as the filter became increasingly blocked. Thus the ceramic filters could be used confidently for long periods in fermentations with high cell concentrations.

(14) PRELIMINARY TESTING OF THE CONTROL LOOPS:

(i) CONTROL OF GAS COMPOSITION: A mixture of oxygen and span gas was fed into the system by manual regulation of the oxygen controller (11) and by admitting span gas via solenoid (Mx). The final gas composition was; Methane 75.5%; Ethane, 5%; Oxygen 14.5%; carbon dioxide 5% (V/V), and a pressure of 7.5" water gauge.

The controllers were set to AUTO with appropriate set points for each. A small leak was induced in the system. This caused the pressure to drop with consequent input of north sea gas through control action. As more north sea gas entered the system the oxygen content tended to fall so that more oxygen was fed into the system to re-establish the set/

set concentration of oxygen. Figs. (43) and (44) show the control achieved in the composition of oxygen and in system pressure respectively. Control of O_2 gas phase concentration was excellent. Virtually straight line control was achieved with a maximum deviation from the set point of less than 0.1%.

System pressure control was reasonably effective but although the general trend of control produced a straight line, the pressure oscillated rapidly by about 0.5% of maximum (0.75" water gauge). This was considered good enough since the object of pressure control was to control the concentration of Methane in the gas phase. The oscillation in the pressure did not seem to upset the control of methane in the gas phase significantly (Fig.46).

The ethane control loop was tested by dropping the set point to below the indicated concentration. This opened the purge control valve (26) and gas vented until the new control concentration was reached. Excellent control was obtained. The control of Ethane was remarkably good, with almost no deviation from the set point.

By admitting carbon dioxide to the system (regulated to 1 litre/hour by flostat (18)), the set point was passed and control initiated through the scrubbers. (Fig.(46) represents the degree of control of the CO_2 composition). Although some oscillation about the set point, the magnitude of the deviation (0.5%) was not considered to be great enough to warrant redesign of the loop.

The above procedure gave some insight into how the gas composition control behaved for a given load change but complete testing was only possible in fermentation conditions.

(ii)

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(ii) TEMPERATURE AND pH CONTROL: Temperature control was easily tested by setting the rate of agitation to 3000 r.p.m. The heat generated from agitation normally caused the temperature to rise to above 55°C. Control was held at 40°C.

Control of pH was tested during a run in which medium at pH 2.6 was present in the fermenters. The pH set point was adjusted to 6.8; and alkali was delivered to the fermenters until the set point was reached. It was found that provided the rate of agitation was in the region of 1500 r.p.m. or above (efficient mixing) then very close control of pH and temperature was possible. Charts representing the efficiency of pH and temperature control have been set out in Fig. (47) and (48). The degree of control achieved over both pH and temperature was excellent, with almost no deviation from the set points. It is unlikely that this degree of control could have been improved upon even with more sophisticated control loops.

(15) LONG TERM PERFORMANCE OF THE CONTROL LOOPS AND ELECTRODES:

It was observed that over a period of 12-18 months the performance of certain components of each control loop deteriorated, necessitating shut down and fault correction.

(1) ETHANE CONTROL: After 18 months operation oil-contamination of the University compressed air supply completely prevented the ethane recorder/controller from functioning. It was necessary to remove the controller from line and thoroughly clean it. Similarly the minimum pneumatic control valve needed a complete clean before correct functioning was obtained.

A/

A further complication was that the ethane analyser gradually became insensitive to changes in the ethane concentration. This was due to progressive ageing of components, particularly the condensers in the amplifier, and a new amplifier was fitted.

(2) OXYGEN CONTROL: The servomex analyser gave trouble free performance over a three year period but the associated control valve was affected by the oily air supply, and had to be overhauled after 18 months operation.

Although not connected with a control function, it was gratifying that the stability and response times of both oxygen electrodes showed only insignificant change in performance over a 2 year period. The output of electrode (2) dropped from 42 uAmps to 39 uAmps over this period, corresponding to 14.5% oxygen in the gas phase.

(3) pH CONTROL: The output and stability of calibration of both pH electrodes remained constant after several autoclavings over 2 years.

Control was interuppted after about 9 months when the pH indicator/controller governing fermenter (1) stopped working completely. The instrument had to be replaced and no reason for the stoppage was discovered. Apart from this upset, control did not deteriorate significantly, throughout a 2 year period.

(4) TEMPERATURE CONTROL: Very little trouble with either temperature measurement or control was experienced but it was found that the $^{1}/_{4}$" solenoid valves allowing cold water into the fermenter were too small for adequate control at rates of agitation above 2000 r.p.m. These solenoids were replaced with $^{3}/_{8}$" models and correct control was achieved even at 3000 r.p.m. At one stage both of the lock-in relays in the resistance recorder/controller were replaced - apparently they had been temporarily overloaded.

(5)/

(5) CARBON DIOXIDE CONCENTRATION CONTROL: Adequate control was maintained throughout a 2 year period except that the amplifier in the carbon dioxide analyser aged in the same way as that in the ethane analyser. This was replaced after a period of 18 months. At the same time it also became necessary to replace the motor driving the chopper arm in the measuring cell of this instrument.

(6) PRESSURE CONTROL: Several mishaps occurred to the pressure control loop during 2 years of operation. The oil contamination in the compressed air supply prevented correct control action. In this case both the recorder and the control station were rendered inoperative and together with the minim control valve were removed from line and cleaned.

The pressure controller never fully recovered from its contact with oil and leaked when the control lever was in the 'seal' position. Fortunately this did not affect automatic control.

Oil contamination of the pressure regulator (18) caused the low pressure readings to fluctuate, severely reducing the ability of the controller to maintain the pressure at the set point. (See Fig.49). After thorough cleaning, full sensitivity and performance were restored (Fig.44). The effect of oil contamination of the control valve (fig.50) severely reduced the efficiency of the pressure control loops. One of the major limiting factors in the effectiveness of the pressure control loop was the persistant leak of gas from the system. It was not possible to isolate and cure every small leak in such a complex system. The rate of leakage was estimated by circulating gas in the system at a controlled low pressure (7.5" water gauge). Only the pressure control was allowed to function so that any leakage was made up by an inflow of north sea gas into the system. The leak rate at the time/

time of commisioning was negligible (Fig.51) but a steady increase in the leakage rate to a maximum of 1.2 l/hr. was noted after 6 months operation (Fig.60).

The nature of the fermenter agitation system caused the recorded low pressure value rapidly to fluctuate by plus/minus 1" water gauge due to the fluctuation of the gas head space volume in fermenter (2) as foam was generated and destroyed. This fluctuation was superimposed onto the controlled value in such a way that a straight line form of control could not be attained. Fig (44).

(7) FERMENTER LEVEL CONTROL: The greatest deterioration in control effectiveness occurred in the control of the volume of liquid in the fermenters. This was attributed to the mal-function of the hydraulic variator and the electric motors of each fermenter.

The initial success of the control loops (Fig.41) lasted for a period of only 3 months after which time it became necessary to replace both of the hydraulic variators (Fig.52). Subsequent to the replacement of the hydraulic variators the control of the liquid volume was improved in fermenter (2) but it was not possible to rectify the control defect in fermenter (1) entirely. Fig. (53).

The maximum deviation from the set point observed for fermenter (2) volume was 45% - an obviously unacceptable figure. Fermenter (1) gave slightly better control, Fig (52), where the maximum deviation recorded was 15%. The replacement of the hydraulic variators enabled control of volume in fermenter (2) to within 13%(of volume) which was acceptable. The control achieved with fermenter (1) remained with a maximum deviation of 15%.

It/

It was noticed that at irregular and unpredictable times, voltage changes occurred in the power supply to the fermenters and instrumentation. These voltage surges affect current drawn by the motors; in turn this affected the control of liquid volume in the fermenters (Fig. 54). The effect was more severe in fermenter (1) and was superimposed on the already erratic control of level in fermenter (1). This was sufficient to completely prevent control of volume . It was still possible to control volume in fermenter (2) by this method. Why one control loop was satisfactory and the other faulty was beyond solution by a number of experts. Both torque meters were themselves working correctly. Possibly the hydraulic variator of fermenter (2) was developing a fault, as had its predecessors, which affected friction within the drive system; increased friction would draw more current through the motor independent of fermenter liquid volume.

(16) TUNING OF THE CONTROL LOOPS:

The methodology for tuning control loops has already been discussed in relation to the practical aspects of the design of the biomass plant in Part C. In steady state conditions of continuous culture where a constant load change in each of the measured parameters occurred, the control loops were tuned to give the smoothest control. Only approximate settings of the valves, proportional band, reset rate, and timers of each control loop could be derived for start-up conditions where batch culture conditions prevailed. Since the load changes were not constant during the first few days of isolation of the culture, the control settings were not constant either. (See section 35B of Part B).

It must be emphasised that incorrectly tuned control loops could not give proper control. This was observed for some of the control loops during start-up. The result of too high a reset rate on the control of pressure/

pressure caused the pressure to fluctuate with a maximum oscillation of 2-3" water gauge. (Fig.55). Similarly, too low a proportional band width caused the pressure to fluctuate less rapidly than for too high a reset rate but with the same maximum deviation - 3" water gauge. (Fig.56).

A regular cycling was observed in the gasohase oxygen concentration when the proportional band was less than optimum. The cycle had a 35 minute periodicity although the deviation in concentration was less than 2%.

To some extent the control of fermenter temperature depended on the aperture of the cooling water and valves. Thus poor control resulted if the aperture of these valves was not correctly adjusted. Fig.(58) shows the effect of too large an aperture which caused the temperature of fermenter (1) to oscillate through 2 degrees and fermenter (2) by about 1 degree, but over an extended period of almost two hours.

(17) PRELIMINARY TESTS ON THE MEDIUM FILTRATION PROCESS:

The filtration rig described in section (6) of Part C was examined to determine the maximum flow rates of sterile medium obtainable. The rig was tested with all membrane filters and prefilters in position and with only the 47 m.m. sterilising membranes plus prefilters in place. The flow rate of medium through the 142 m.m. filters was also determined. (Table24).

It can be seen that the rate limiting section of the rig resided in the two 47 mm filters. The minimum flow rate obtained was 256 ml./minute which meant that the 20 litre medium resevoirs took 78 minutes to fill. Allowing an 8 minute contingency margin, the maximum dilution rate possible was calculated as follows:

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Total fermenter volume = 10 litres.

Volume in medium resevoir = 20 litres

Maximum rate of emptying the resevoir = 70 minutes/20 litres or 17.2 L/hr.

Therefore the maximum dilution rate obtainable for both fermenters was 1.72^{hr-1}.

The dilution rate calculated above was composite for both fermenters but each fermenter could be supplied with different rates of medium flow provided that the total did not exceed 1.72^{hr-1}.

Measurement of medium flow rates was done using both crudely filtered medium (see section 6 of Part C) and untreated freshly made up medium (Table 24). It was evident that even the rough initial filtering was instrumental in removing particulate matter from the medium. Without such treatment the flow rate through the filtration rig rapidly dropped off as the filters became blocked. Although the freshly prepared medium was not visibly loaded with particles the need for prefiltration when using membrane filters was highlighted.

The automatic filtration scheme (Section 6, part C) was examined. It was found to function adequately provided that the ambient temperature did not rise above 25°C. When this occurred, the thermistor probes did not respond to changes in temperature from the medium and rendered the apparatus non-functional. In practice it was extremely difficult to keep the ambient temperature below 25°C and the rig was redesigned with thermistors of different characteristics. The results of the tests on this rig will be reported elsewhere.

(18)/

(18) THE METHOD FOR BUBBLE POINT TESTING THE MEMBRANE FILTERS:

The rig was designed so that in line bubble point testing could be performed before, during or at the end of the filtration cycle (Fig.24).

(i) MANUAL RIG: Hand valve (3) was closed and compressed air from (5) allowed to force residual liquid from the line after (3) through the filters (F1, F2). The air pressure was increased until air bubbles were seen to bubble through the filters into the transfer lines (visible in the silicone rubber lines). The pressure at which a continuous stream of bubbles began to emerge was recorded as the bubble point. For intact membranes this was normally 55 p.s.i. (millipore membranes) and when the membrane was ruptured even to a slight degree, the bubble point was less than 2 p.s.i.

Once the test was completed valve (5) was closed and (3) reopened. Medium then passed through the filters in the normal way. Occasional air locks developed in the filters after bubble point testing and these were released via vents (A2) and (B2).

(ii) AUTOMATIC RIG: The bubble point test was conducted in the same way as for the manual rig except that solenoid (SV3) or (SV4) were opened by means of an over-ride switch (Fig.26). This was necessary to allow free passage of liquid or medium at the bubble point.

PART (E) MATERIALS AND METHODS:

COMMISSIONING EXPERIMENTS

AND RESULTS;

GROWTH EXPERIMENT:

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(1) START-UP PROCEDURE:

The complexity of the fermentation rig necessitated a strict adherence to the correct procedures for start-up and shut-down to avoid blow back of culture fluid into filters and pipework. The correct procedure for start-up has been presented below as a sequence of operational steps.

(1) Calibrate the gas analysers. (See Section 3 of Part D).

(2) Autoclave the fermenters (See Appendix 1)

(3) Fill the fermenters with sterile medium. (See Section 6,Part C).

(4) Aseptically connect all liquid lines to their appropriate ports on each fermenter. (See plan of port designations for each fermenter in Fig.63).

(5) Switch on air compressor and supply; (i) 20 p.s.i to instrument panel, (ii) 15 p.s.i. to purge lines in the gas analyser cases, (iii) 20 p.s.i. to differential pressure cell (5)(See fig. 4), (iv) 10 litres air/minute to the peristaltic pump cabinet. (v) 20 p.s.i. to pH electrodes.

(6) Switch on freeze units (1) and (2) and the coolant circulation pump, ensure sufficient coolant circulation through the condensers. This should not be less than 1 litre/minute to each condenser.

(7) Connect swagelock fittings on the fermenter filters to the main recycle piping.

(8) Fill lute to 15" water gauge.

(9) Check chart supply and ink levels in the recorders on the instrument panel.

(10) Correctly align the liquid lines in their appropriate shoes on the peristaltic pumps, giving regard to the direction of flow needed for each line. Connect the effluent lines to the collection vessel.

(11)

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(11) Attach the gas return line from the collection vessel to the main recycle line via the solenoid valve (R2).

(12) Fit the fermenter sampling lines to the peristaltic pump next to the F.Z.7 control units.

(13) Fill carbon dioxide scrubbers with carbasorb. This is done so that layers of glass wool (2" thick) alternate with the carbasorb (6" thick). Glass wool to form the initial and terminal plugs.

(14) Close off the top and bottom hand valves to one of the carbasorb columns.

(15) Set up temperature control loop as follows:

(i) Switch on recorder/controller (36) and power supply to the cooling water solenoids at the 13 amp. socket bank.

(ii) Open the cooling water hand valves under each fermenter table approximately $1/1/2$ turns.

(iii) Open mains water supply to the solenoids. Hand valve on the wall in cubicle 2.

(iv) Agitate fermenter contents at about 600 r.p.m. and allow temperature to reach $40^{\circ}C$ in both fermenters.

(v) Adjust response of the controller via the fermenter hand valves so that control occurs between the limits of $39.5-40.5^{\circ}C$ or better.

(16) Set up pH control loop as follows:

(i) Check alkali resevoir full. If not supply resevoir by means of peristaltic pump next to F.Z.7 control unit. The refill tube terminates in a blind plug which must be replaced when filling has been completed. Filling can be accomplished when the gas is circulating during full operation.

(ii)/

(ii) Connect pH electrode leads to the pH meter/controller (38) and set desired control point (6.85).

(iii) Adjust the pH recorder (41) so that the trace lies in the desired segment of recorder chart for each pH electrode output, by means of potentiometer on the side of recorder (41).

(iv) Selected F.Z.7 pH timer settings. At start up the ON time should be minimum and the OFF time maximum. Switch F.Z.7 control switch to vertical position. (timed doses)

(v) Ensure that the alkali delivery tubes to the fermenters are correctly aligned with no crimping constrictions to impede liquid passage.

(vi) Set pump switch on the F.Z.7 unit to ON position so that alkali is delivered to the fermenters at a low rate. The fermenters should be stirred to allow better mixing of fluids. At start-up it is necessary to short out the low pressure and high pressure fail safe relays to allow pH control. The shorting switches are on the relay box outer casing.

(17) Delivery of circulating gas mixture:

(i) Select mode of operation i.e. series or parallel gas flow, by means of appropriate hand valves on the perspex window of cubicle 1. (See section 2, Part C).

(ii) Close solenoids (S2 R1 R2) and open solenoid (S1).

(iii) Set pressure controller (6) to seal position and adjust set point needle to 50 mark.

(iv) Set upstream pressure of oxygen, north sea gas, and carbon dioxide to 20 p.s.i. Read on gauges (2,9,16).

(v) Short out fail safe relays (high and low pressure) with shorting switches on the relay box panel.

(vi)

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(vi) Switch on recorders (22) (4) (20B) (13) and transducer (20A).

(vii) Supply power to the ethane purge photocell and integrator/ pulse counter. Switch on counter for rotary gas meter (26A).

(viii) Set pressure controller (6) to MANUAL position to allow north sea gas to enter the system. Ultimately the north sea gas flushes any residual air in the system to atmosphere through (S1). When the analysers indicate no oxygen is present and that the concentration of ethane is 3.25 with methane at 94.0% switch (S1) closed.

(ix) Allow the pressure to build up. It may be necessary to adjust the set point of controller (6). Care must be taken to prevent the pressure from tripping the high pressure fail safe relay.

(x) Switch on the gas circulation pump (N) and adjust the flow rate of gas to the fermenters by rotameters (29,30).

(xi) Adjust the high pressure side to 10 p.s.i. with manometer (27).

(xii) At this point the gas should be circulating oxygen free at about 10 litres per minute to each fermenter. The pressure controller (6) should be set to the seal position after adjusting the set point needle to zero. Solenoid (S2) may now be opened so that the low pressure side reaches the lute pressure of 15" water gauge.

(xiii) The oxygen recorder/controller (13) is switched to the MANUAL position and the set point adjusted to allow the ingress of a small amount of oxygen to the system until the oxygen concentration reaches 15%.

(xiv) Carbon dioxide is allowed to enter the system by manual adjustment/

adjustment of the flostat (18) in cubicle 2. The flow rate is adjustable via the rotameter (17). The concentration of CO_2 is allowed to reach 5%. During this operation both oxygen and north sea gas are flushed out of the system. If the oxygen concentration is above 12% at this stage allow more north sea gas into the system by following step (viii). Mutual dilution occurs but by alternately bleeding in carbon dioxide, oxygen and north sea gas the following gas composition in the system is arrived at;

OXYGEN	14.5%	V/V
CARBON DIOXIDE	5%	V/V
NORTH SEA GAS	80.5%	V/V

Once the gas phase constitution is established as above, the gas composition controllers can be switched to 'AUTO' so that automatic make up of the gases can proceed. Thus recorder/controller 20B, (Ethane), 13 (Oxygen) 6 (methane, via pressure), are each adjusted so that the set points reflected the desired values of the gas composition. In the case of oxygen, ethane and carbon dioxide this is a simple concentration setting. For ethane it was decided to set the initial concconcentration at a controlling level of 5%. This can be adjusted to accommodate different culture kinetics. Methane since it is regulated via pressure control is adjusted so that control occurs at the low pressure value of 15" water gauge on controller (6).

(18) The fermenters are inoculated with the isolation material in the manner given in section (3) of Part E.

(19) Initial growth occurs in the batch mode until the culture becomes established. This can be observed through the gas consumption traces and pH delivery rates. Continuous culture is arranged by setting up the level control loop for each fermenter, thus;

(i)
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(i) Switch on the power measuring units and then fit the photo cells to the set points on the meters.

(ii) Turn fermenter agitation speed controls to required speed. For good level control this is in the region of not less than 2000 r.p.m. Allow the power drawn to stabilise - approximately 2 hours.

(iii) Connect the recorder output leads of the power measuring units to recorder (41). Adjust recorder trace position with the potentiometer on each measuring unit as required.

(iv). Set medium delivery pump speed to required setting by means of the potentiometers on the F.Z.7 units. The delivery of medium is controllable at the F.Z.7 units, but power to the pumps is controlled by two toggle switches situated on the side of the relay box.

(v) Set effluent pump speed by means of the potentiometers on the F.Z.7 units. Power to these pumps can be controlled either manually or automatically. For continuous culture operation set the selector switch to AUTO position.

(vi) Check the flow rate of medium to the fermenters by means of the calibrated tubes on the window of cubicle 1. (See section 19, Part D).

(vii) Ensure that the oxygen electrode leads have been attached to recorder (41), and adjust the trace position via potentiometers on the side of this recorder.

(viii) Replace the inoculation container with a sterile sampling jar.

When the above procedure has been completed the fermenters should be operating at the correct gas flows and dilution rates in the continuous mode.

(2) /

(2) SHUT-DOWN PROCEDURE:

For shut down the following sequence of operations must be adhered to, both for temporary and long term shut-down.

(1) For temporary shut-down the gas mixture can be retained in the system by closing solenoid valves (S1, S2, R2).

(2) Long term shut-down requires the removal of explosive gas from the system. This is achieved by opening solenoid (S1), closing (S2,R2). Nitrogen is then flushed through the system by opening solenoid (N2) for several minutes until the recorder trace bearing the output from the gas analysers indicates zero methane/oxygen concentration. Then close (N2).

(3) Isolate the fermenters from the gas stream by closing hand valves (F5, F6) and immediately opening valves (H1, H2, H3). This prevents back flow of the fermenter contents into the gas inlet filters and the pipework generally.

(4) Shut off the gas circulation pump (N).

(5) Turn the fermenter agitation speed control handle anti-clockwise

(6) Once the pressure on the high side has dropped from 10 p.s.i. to zero the fermenters can be dismantled if necessary or adjustments made to the system.

(7) Under conditions of long term shut-down the recorders, transducer, integrator/photocell and rotary gas meter counter should be shut off. Similarly, the air compresser supplying the pneumatic instruments (4,5,6, 3, 7, 14, 10, 12 of fig.4) purge line and pH electrodes can be shut down.

(8) The power to the cooling water solenoids and the CO_2 scrubber solenoid should be shut off for long term shut-down.

(9) If the pH electrodes are to be removed the pH sensitive glass bulb/

bulb should not be allowed to dry out, but should be immersed in dilute Hydrochloric acid until required. The O_2 electrode should be immersed in a solution of sodium sulphite with the terminals shorted out to prevent the potential building up through reaction with Oxygen.

(3) INOCULUM SCOURCE AND INOCULATION PROCEDURE:

Samples of soil from several sites were used as a scource of methane/north sea gas oxidising organisms in the first commissioning experiments. In fact each of the soil samples were mixed together for the commissioning data, the crude overall mixture was then prepared for inoculation in the following manner:

(1) About 50 grams of the mixed soil was added to a solution of the nitrogenous salts media (See table 18) adjusted to pH 6.85 and thoroughly homogenised.

(2) The rough, particulate matter was then filtered off by means of a Whatmans No.1. filter paper and discarded.

(3) The filtrate was added to an inoculation bottle and attached to the sampling lines at the peristaltic pump next to the F.Z.7 units.

(4) Approximately 100 mls. of inoculum were transferred to each fermenter under the action of the pump. A clamp was present on the sampling lines as an extra safeguard to prevent gas under pressure in the fermenters escaping along the sampling lines in the event that the lines became detached from the pump shoe. These clamps were reattached after inoculation was completed.

It was possible to inoculate either before the start-up procedure described in section (1) of Part E was completed, providing the conditions of growth were established as soon after inoculation as possible, or, after the system had been pressurised and Start-up completed. It was found/

found that the latter system was most convenient partly because contamination of the fermenter filters and recycle lines was precluded in the event of a 'blowback', and partly due to the fact that any faults arising from the Start-up situation were shown up before the inoculum was added. Had shut down been necessary and had the fermenters been inoculated before Start-up then the isolate would have been wasted.

(4) COMMISSIONING EXPERIMENT: PARAMETERS AND CONDITIONS USED:

Using the Start-up procedure given in section (1) part E, the fermentation system was established so that the following conditions prevailed:

1) pH 6.85

2) Temperature 40°C

3) Agitation speed 3000 r.p.m.

4) Gas flow to the fermenters 10 Litres/minute to each

5) Gas phase composition : (V/V)

Methane	75.5%
Ethane	5%
Oxygen	14.5%
Carbon dioxide	5%

6) System pressure; High 10 p.s.i.

Low 15" water gauge

(5) MEASUREMENT OF CELL DRY WEIGHTS AND CULTURE OPTICAL DENSITY:

(1) Dry weights: 5 ten millilitre samples of each fermenter culture were placed in clean dry and previously weighed centrifuge tubes. A control consisting of newly filtered liquid medium (See section 6, Part C) was centrifuged at the same time as the fermenter samples. Each sample was/

was centrifuged at 3000 r.p.m. for 10 minutes and then washed with distilled water. After a further 10 minutes at 3000 r.p.m. the water was discarded and the centrifuge tubes were transferred to a hot air oven and dried to constant dryness at 100°C. Constant dryness was achieved after about 24 hours. Each tube with its pellet of cells and the control were accurately weighed on a balance and once constant dryness had been achieved the average weighing of the 5 samples was subtracted from the control weighing to give the dry weights of the cellular material in the fermenters.

(2) Optical densities: For the measurements of culture optical density, a Klett-Summerson Colorimeter was employed. Freshly filtered medium was used as a blank and measurements were made with a green filter at 570A. For both dry weight and optical density measurements it was essential that the tubes and cuvettes be clean and dry before use. To ensure this, the glass ware associated with these tests was kept immersed in ethanol when not in use and rapidly and carefully dried with air immediately before use.

(6) MEASUREMENT OF GAS CONSUMPTION DURING GROWTH:

Recorder (4) displayed the amount of oxygen and north sea gas which entered the recycle lines in a given period. The total input of oxygen and north sea gas was a function of loss due to microbial consumption, leaks, ethane purge and pressure control (through the lute - if any). Thus:

Total input - leakage = lute loss - ethane purge - consumption.

for both oxygen and north sea gas.

The total gas lost from the system in the ethane purge and lute loss was with time registered on the intergator/pulse counter and reed switch counter/

counter respectively. Therefore the amount of oxygen and north sea gas lost through each of these routes was derived from the analyser traces (i.e. x c.c.s. of gas lost, 75.5% methane). Provided that control of the gas composition was good, i.e. a constant composition of each gas was maintained, then the consumption of oxygen and north sea gas by organisms could be estimated.

The culture was allowed to grow in batch culture conditions and during the first three days no apparent consumption of north sea gas or oxygen was observed. After the beginning of the fourth day a rise in the optical density was noted (Fig.64) which was accompanied by an input of north sea gas and oxygen. The traces of oxygen and north sea gas flow into the system were difficult to interpret because the flows were not constant but fluctuated rapidly under the influence of the oxygen and pressure control loops. An estimation of the gas flow was obtained by taking an average of the highest and lowest flow rates for each trace for each hour of growth (Fig.69). No loss of gas through the lute or ethane purge valve was observed.

Consumption of north sea gas and oxygen began abruptly on the fourth day and reached 0.2 l/hr. and 1.1 l/hr respectively, in about 7 hours. The rate of consumption progressed steadily for a further 8 hours (Fig.69) when the rate became more rapid. After about 23 hours the rate of consumption of north sea gas began to drop and at 25 hours the rate of oxygen consumption followed. The maximum rate of consumption recorded for north sea gas was 0.98 l/hr, and 6.3 l/hr. for oxygen.

(7) <u>GROWTH OF NORTH SEA GAS OXIDISING ORGANISMS DURING THE COMMISSIONING RUN</u>:

The/

The commisioning run was designed merely to establish that the fermentation plant could function under conditions of growth of methane/ethane oxidising micro-organisms. It was established that the control loops were able to maintain the measured parameters at values acceptably close to desired settings, (see Section 14, part D). The inoculum was dilute soil and therefore growth of organisms other than north sea gas oxidisers was possible. In such a case oxygen would have been consumed and replaced under control action. This in turn would have diluted the ethane, methane, in the circulating gas mixture. Figs. (65) and (66) indicate that this did not occur. It can be seen that the concentrations of these gases remained constant, up to time (X).

As a check to verify that north sea gas oxidisers were present in the culture both the oxygen and pressure control stations were switched to the manual position, with the set point at zero. The CO_2 flow solenoid supplying the scrubbers was shut off. Any methane and/or ethane oxidisers present consume these gases along with oxygen. Thus from the time at which the control stations were switched to MANUAL a drop in the concentration of methane, oxygen and possibly ethane would commence. Carbon dioxide would tend to rise in concentration due to the fact that neither scrubbing with carbasorb nor dilution by the oxygen and north sea gas occurred. Figs. (65) to (66) show clearly that both methane and ethane were consumed by the culture, and a corresponding drop in oxygen concentration was recorded (Fig.67) - from time (X). At the same time a pressure drop ensued due to the consumption of gases by micro-organisms Fig (68) from time (X).

Dry weight measurements and optical density readings were according to the method in section (5) part E. Dry weight measurements were taken every day and optical density readings were made every hour for the first day and every 5 hours thereafter. During the growth experiment the optical/

optical density of the culture did not change for the first 4 days but increased steadily after this time (Fig.64). Similarly the dry weight of the culture increased steadily after the fourth day of isolation. The increase in both dry weight and optical density slowed down after the 24th hour of growth and remained at a peak from the 27th hour.

The growth rate (u) of the culture was calculated from Fig.(64) and the relationship;

$$u = \frac{\log_e 2}{t_d} = \frac{0.693}{6.1} = .113 \ hr^{-1} \qquad (15)$$

(t_d is the doubling time in hrs.).

The yield of the culture (Y_{nsg}) was calculated from the relationship;

$$Y = \frac{X}{\text{nsg consumed}} = \frac{0.529}{0.8} \qquad (16)$$

$$Y = 66\%$$

(8) MICROBIOLOGICAL EXAMINATION OF THE CULTURE:

Microscopic examination of the culture revealed that there was a mixed culture present. At the start of the isolation run it was not possible to detect predominance relationships because the culture was so heterogeneous. Once growth of the north sea gas oxidising cultures began it was evident that at least three distinct types of organism predominated. It was difficult to determine the exact relationship between the three types by microscopic examination only but a gram negative rod was persistently present in the greatest numbers. A gram positive/

positive yeast was also present together with a gram positive rod - non chaining.

No further examination of the culture was undertaken at this stage as it was felt that the commissioning run had served its purpose - to establish that the fermentation plant was capable of isolating and growing a north sea gas oxidising culture at high gas transfer rates and at 40°C. (See section 26 part A). The results of the experiments in continuous culture will be reported elsewhere.

TABLE (19)

INFLUENCE OF THE EMULGATOR ON MASS TRANSFER COEFFICIENTS AND OXYGEN ABSORBTION RATES AT DIFFERENT GAS VELOCITIES AND AGITATION RATES.

AGITATION RATE (R.P.M.)	AIR FLOW RATE (l/hr)	WITHOUT EMULGATOR		WITH EMULGATOR	
		O_2 ABS. (mM $O_2 hr^{-1}$)	$K_L A$ ($hr.^{-1}$)	O_2 ABS. (mM $O_2 hr.$)	$K_L A$ ($hr.^{-1}$)
1000	2.5	105	525	121	605
	5.0	149	745	144	740
	7.5	216	1080	223	1115
	1.0	241	1205	256	1280
1500	2.5	162	810	166	830
	5.0	194	970	226	1130
	7.5	247	1235	258	1290
	1.0	273	1365	288	1440
2000	2.5	227	1135	232	1160
	5.0	246	1230	251	1255
	7.5	302	1510	314	1570
	1.0	372	1860	387	1935
2500	2.5	281	1405	288	1440
	5.0	309	1545	321	1605
	7.5	347	1735	394	1970
	1.0	399	1995	419	2095
3000	2.5	300	1500	312	1560
	5.0	318	1590	329	1640
	7.5	414	2070	422	2110
	5.0	492	2460	516	2580

(At 25°C, pH circa 6.0)

TABLE (20)

DRIFT OVER A 72 HOUR PERIOD IN THE GAS ANALYSER MEASUREMENTS USING A FIXED CIRCULATING GAS COMPOSITION:

TIME	GAS COMPOSITION MEASUREMENT (% TOTAL MIXTURE)			
(hrs)	O_2	CO_2	CH_4	C_2H_6
0	14.5	5.0	75.5	5.0
1	14.4	5.1	75.5	5.2
2	14.6	5.2	75.3	5.0
5	14.8	5.2	75.5	5.5
12	14.6	5.1	75.6	5.3
20	14.7	5.3	75.8	5.4
24	14.7	5.2	75.9	5.6
36	14.9	5.5	76 .2	5.9
48	15.4	6.0	76.8	5.9
72	16.8	6.8	77.1	6.3

TABLE (21)

CALIBRATION OF THE TAYLOR 721 AND 701 TRANSDUCERS:

(721):

INPUT (mV)	OUTPUT CURRENT (mA)	OUTPUT PRESSURE p.s.i.
0	0.99	3
1	1.386	4.2
2	1.789	5.4
3	2.193	6.6
4	2.596	7.8
5	3.0	9.03
6	3.404	10.24
7	3.806	11.44
8	4.207	12.64
9	4.613	13.84
10	5.013	15.02

(701):

INPUT (mA)	OUTPUT PRESSURE p.s.i.
0	3.01
1	5.21
2	7.52
3	9.63
4	12.03
5	15.02

TABLE (22)

PERCENTAGE RETENTIVITY OF THE CHEMAP CERAMIC FILTERS:
AT DIFFERENT GAS FLOW RATES:

GAS FLOW (hr/l)	No. ORGANISMS PRESENT IN AEROSOL	No. ORGANISMS PRESENT AFTER FILTRATION	PERCENTAGE RETENTIVITY
2.5	1,060	0	100
5.0	1,165	2	99.99
7.5	1,005	2	99.99
10.0	1,010	6	99.95

TABLE (23)

THE INFLUENCE OF THE FERMENTER CONDENSERS ON THE DIRT HANDLING CAPACITY OF THE CERAMIC FILTERS AT 10 LITRES/HOUR:

TIME (hr)	FLOW RATE OF AIR (Litres/hr) WITH CONDENSER	WITHOUT CONDENSER
0	10	10
6	10	10
12	10	9.5
18	10	8.9
24	10	8.1
30	10	7.2
36	10	6.4
42	10	4.8
48	10	3.4
54	10	2.8
60	10	2.3
66	1.0	1.2
72	1.0	0.6

TABLE (24)

FLOW RATES OF MEDIUM THROUGH THE FILTRATION RIG:

MEMBRANE COMBINATION	FLOW RATES (i) (ml./min) CLEAN		DIRTY	
	INITIAL	FINAL	INITIAL	FINAL
47 m.m. only	382	256	378	145
142 m.m. only	813	804	816	722
Both 47 m.m. and 142 m.m.	388	381	379	118

(i) At 20 p.s.i. differential pressure)

FIG. (1)

PROJECTED WORID POPULATION (U.N., 1960)

P = Pessimistic number
R = Reasonable "
O = Optimistic "

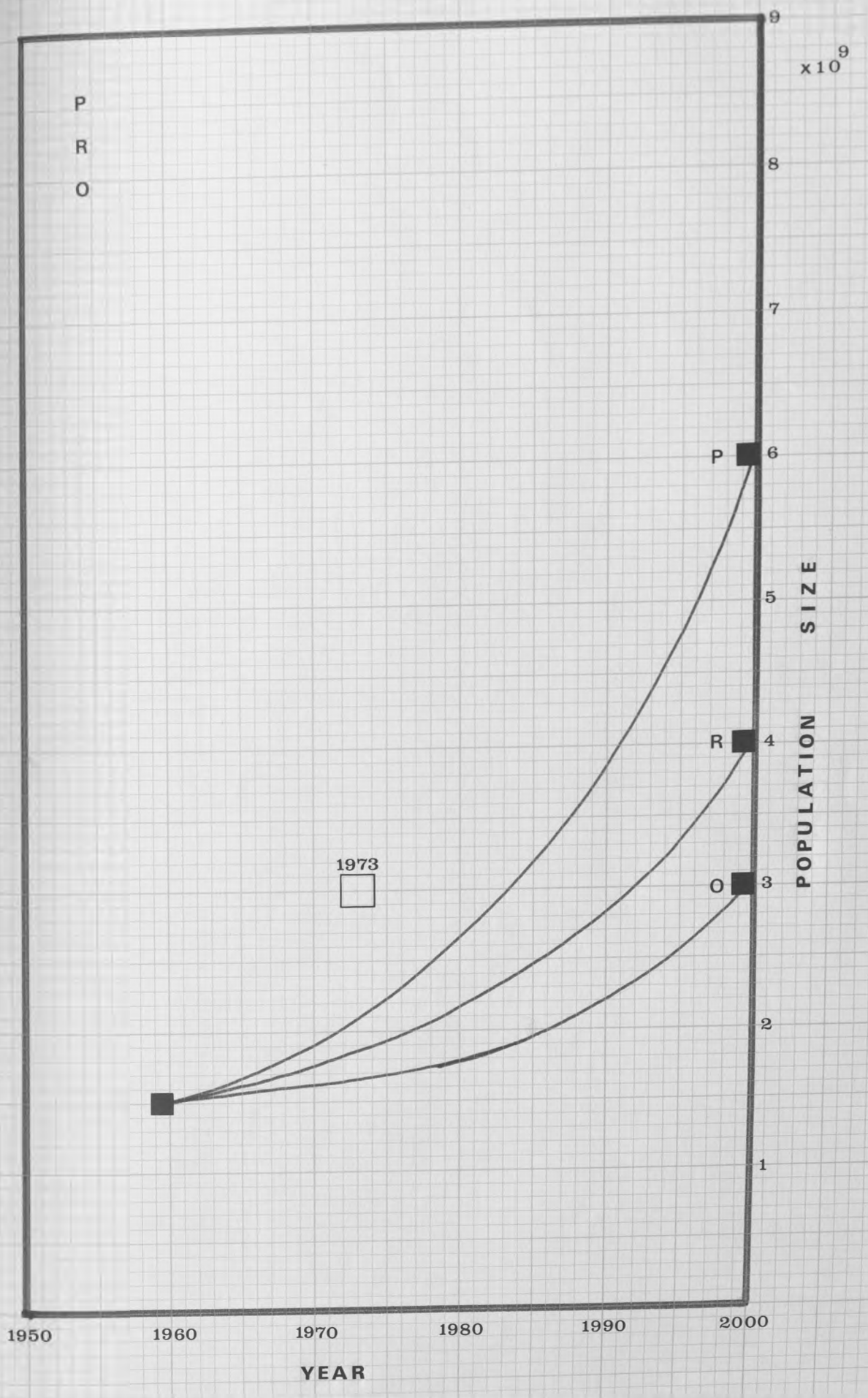

P
R
O
9
x10^9
8
7
6
5
4
3
2
1
P
R
O
1973
POPULATION SIZE
1950
1960
1970
1980
1990
2000
YEAR

FIG.(2) LAYOUT OF THE INSTRUMENT PANEL :

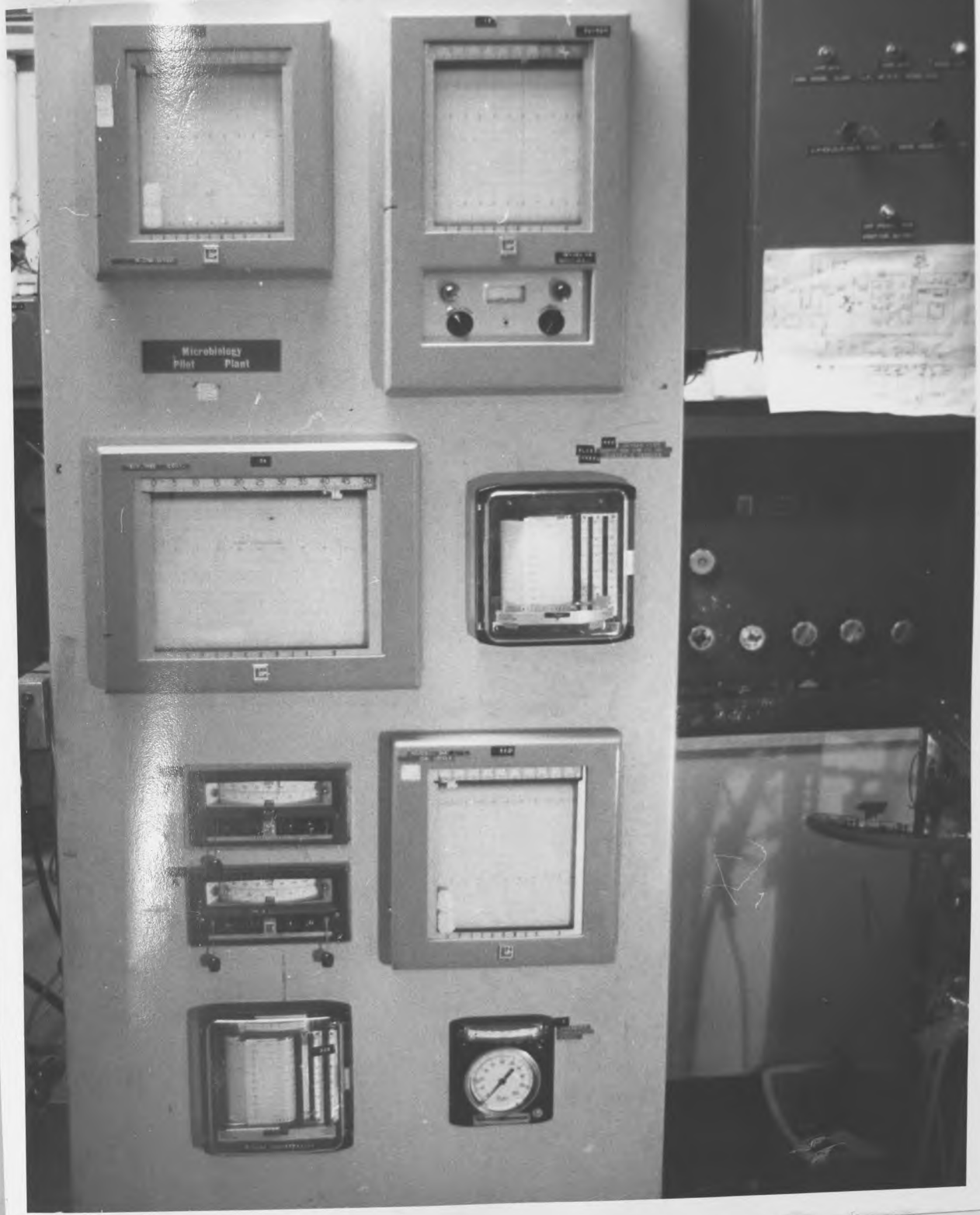
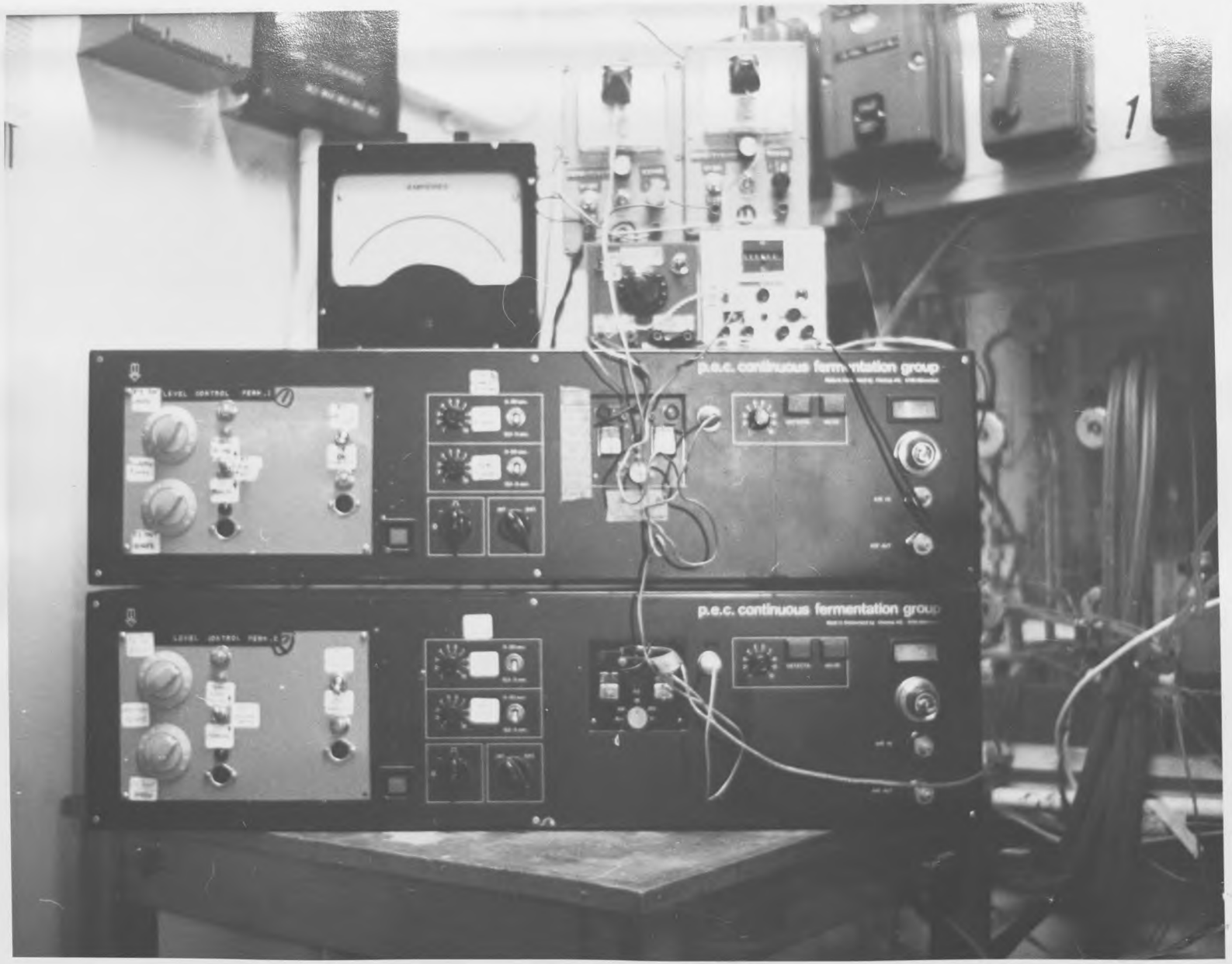

Microbiology
Pilot Plant

FIG. (3): POWER CONSUMPTION MEASUREMENT UNITS


p.e.c. continuous fermentation group
p.e.c. continuous fermentation group

Black

5 — Measuring instruments

20B — Recording "

13 — Control "

Blue

29 — Rotameter flow measuring

or

53

3 — Flow Transmitter, Differential Pressure type

27 (X) — Automatic Pressure adjustment for varying flow rates

26 — Rotary Gas meter fitted with photocell or Reed switch unit

RGM 52 — for remote pulse read-out of gas flow.

Pulse counter or Integrator

Peristaltic metering pump

N — Gas Recycle pump

NSG — Cylinders of gases

28 — Pressure gauge

H2 — Hand operated valve

FS — Solenoid " "

P — Sintered steel flame trap or filter

5A 2 — Non Return valve

Pneumatic proportional valve

MR Multipoint Recorder

TRC Temperature Recorder-Controller

---

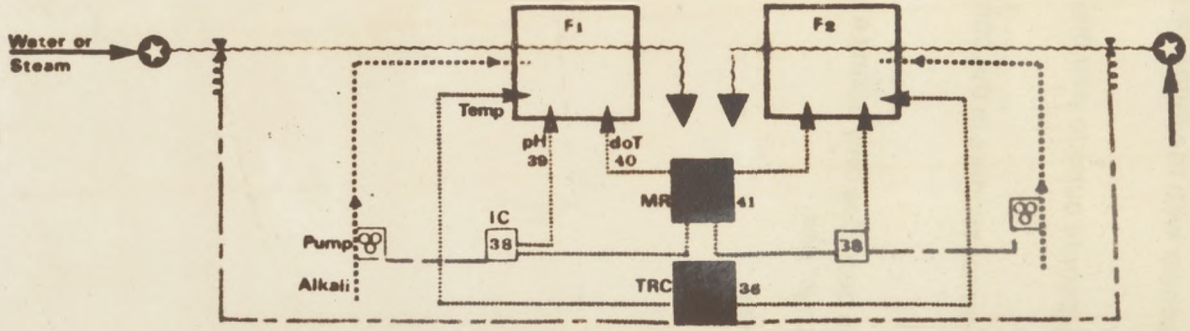
1 Basic fermenters with control loops for Temperature
pH
and monitoring of DCT

2 Supply of essential gases, serving F_1 and F_2 in series or in parallel and gas recirculation facility.

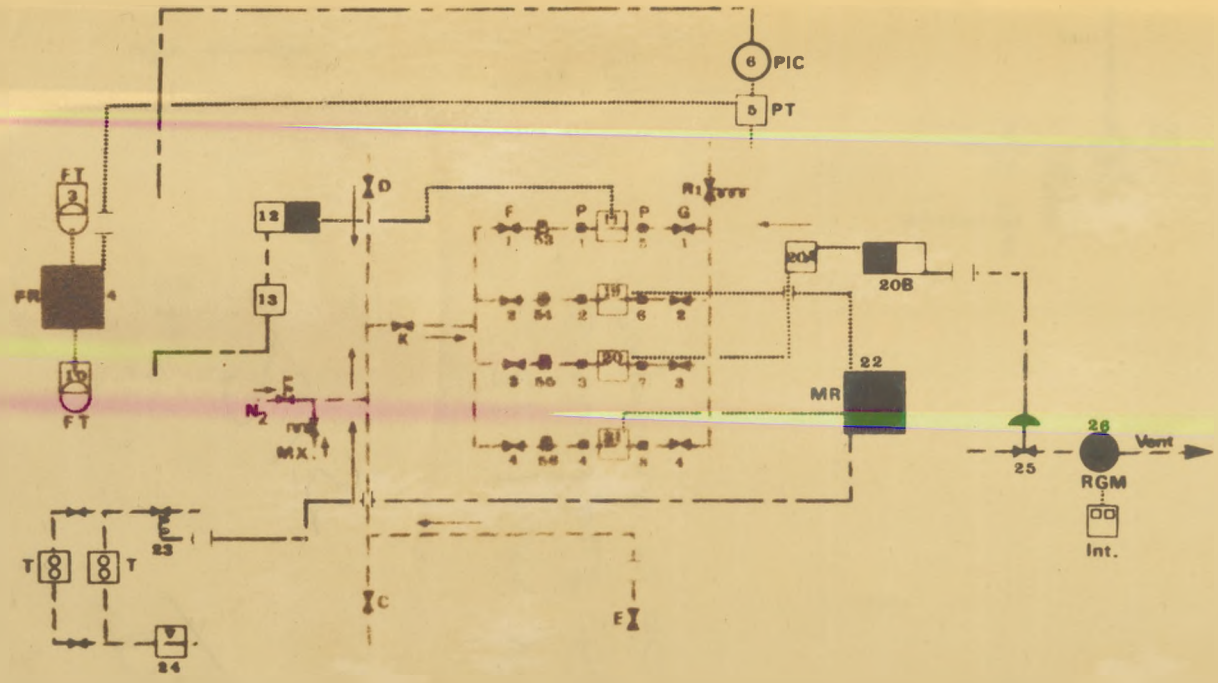
3 Analysis of: Gas flows for O_2 NSG
Gas concentrations CO_2 C_2H_6
and provision for 'span gas' calibratio of instruments

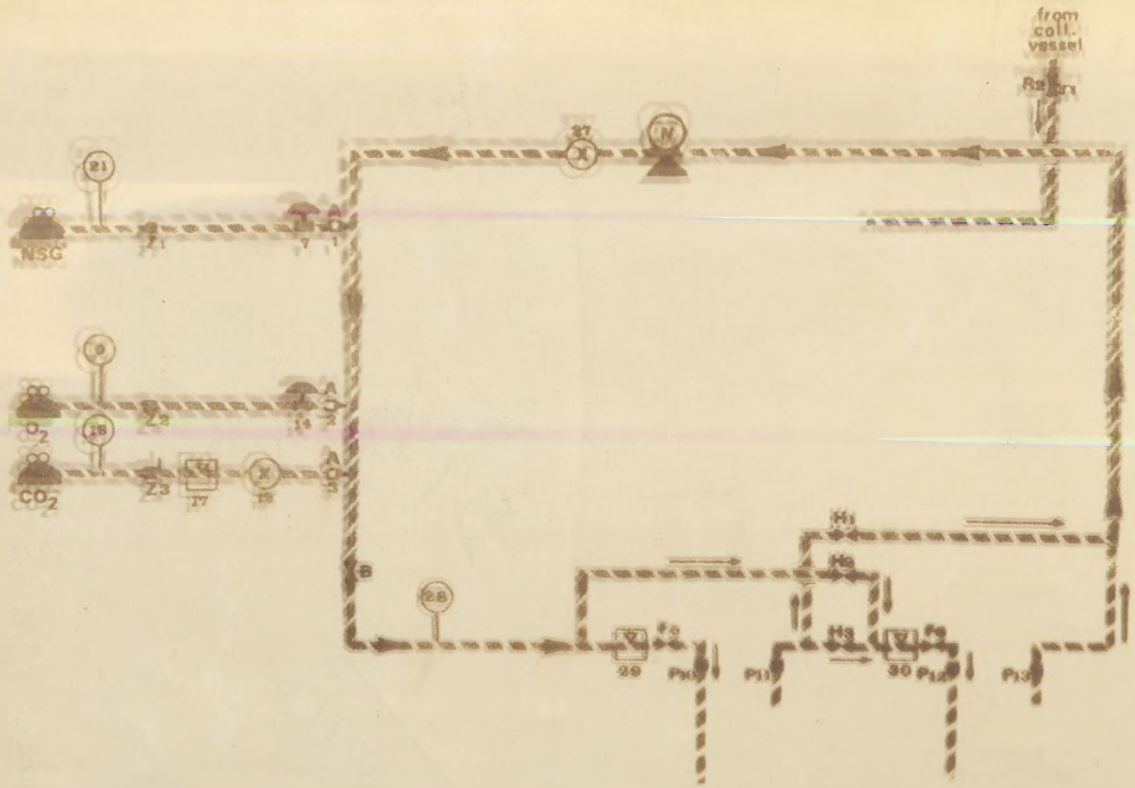
4 Full recording and control loops for system pressure, O_2 concentrati system purge, CO_2 removal.

5 Safety features.


Water or
Steam
F1
F2
Temp
pH
39
doT
40
MR
41
IC
38
Pump
Alkali
38
TRC
36

1

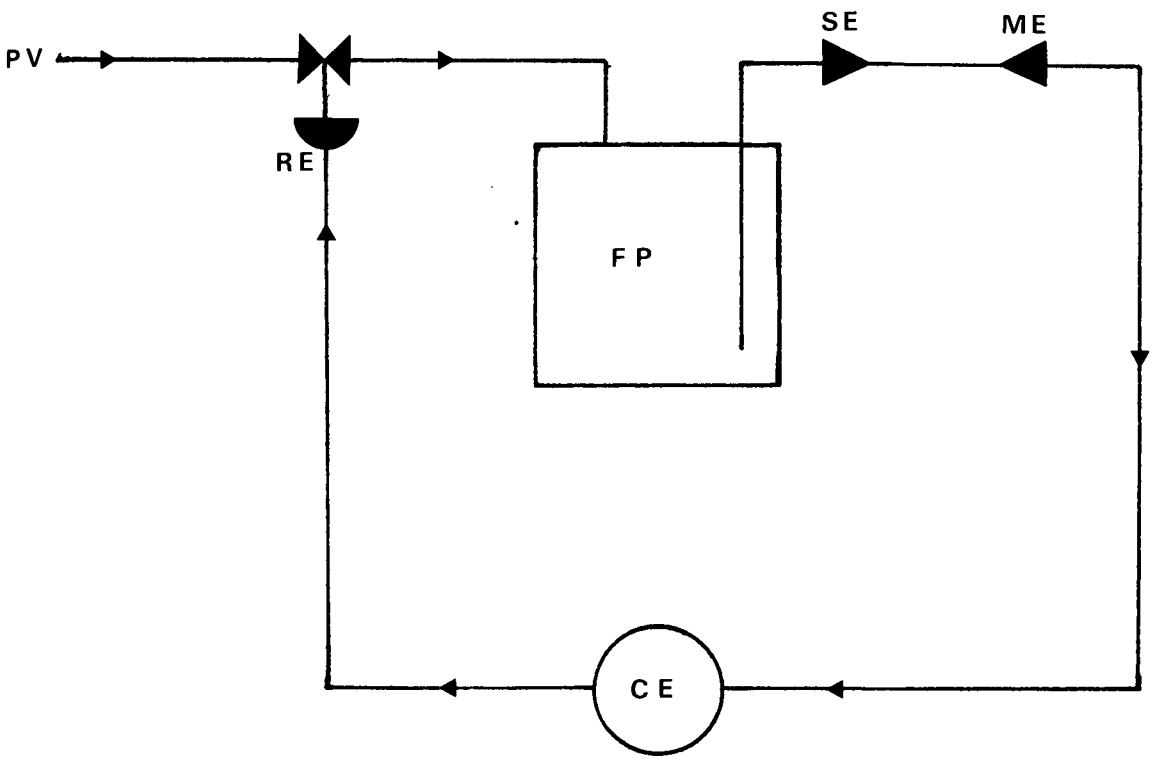

6 PIC
5 PT
FT
3
FR
4
FT
12
13
D
R1
F
P
P
G
20A
20B
22
MR
MX.
N_2
K
E
C
T
T
23
24
25
26
Vent
RGM
Int.


from coll. vessel
NSG
O_2
CO_2


to 50
to FS
50
Vent
S3
S2
RGM
S1
V

FIG. (5)

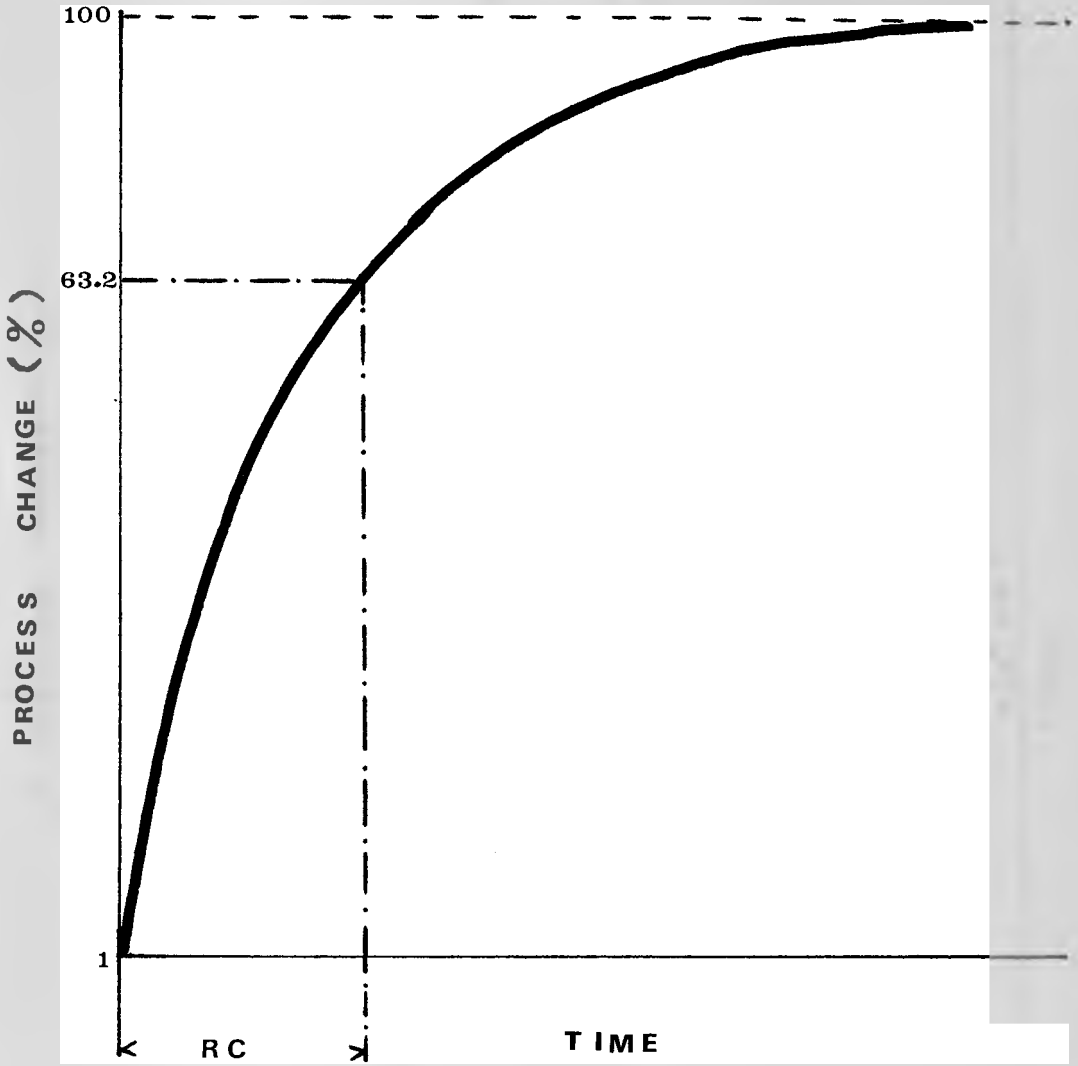
THE COMPONENTS OF A BASIC CONTROL LOOP



P V	Process Variable
F P	Fermentation Process
S E	Sensory Element
M E	Measuring Element
C E	Control Element
R E	Regulating Element

FIG.(6)

THE TIME CONSTANT FOR ANY CONTROL LOOP



R C Time constant

FIG.(7) THE EFFECT OF THE CONTROL LOOP TIME CONSTANT ON THE MEASURED VALUE OF A PROCESS PARAMETER

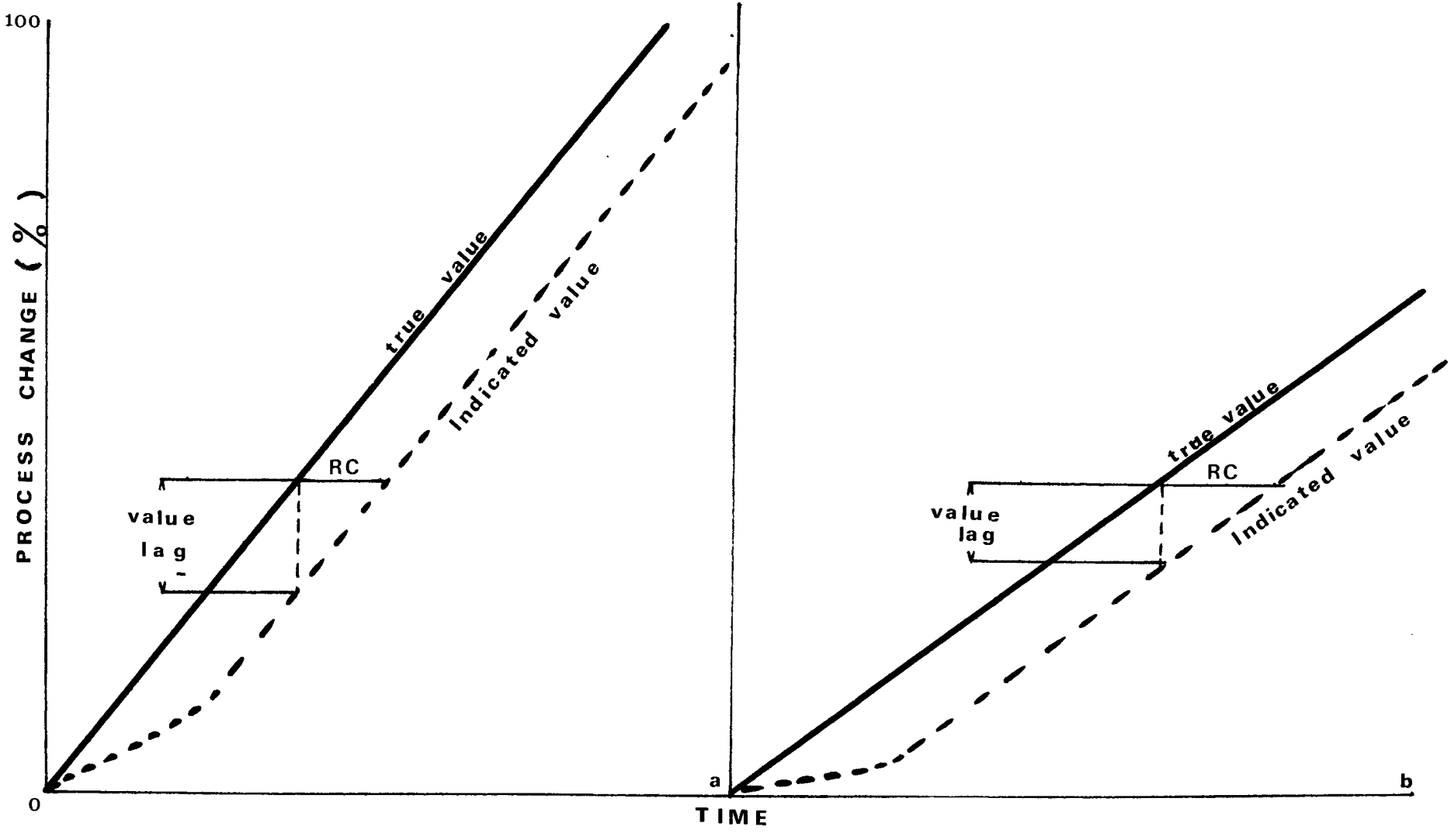


FIG. (8)

THE EFFECT OF PHASE LAGS ON THE OPERATION OF A CONTROL LOOP

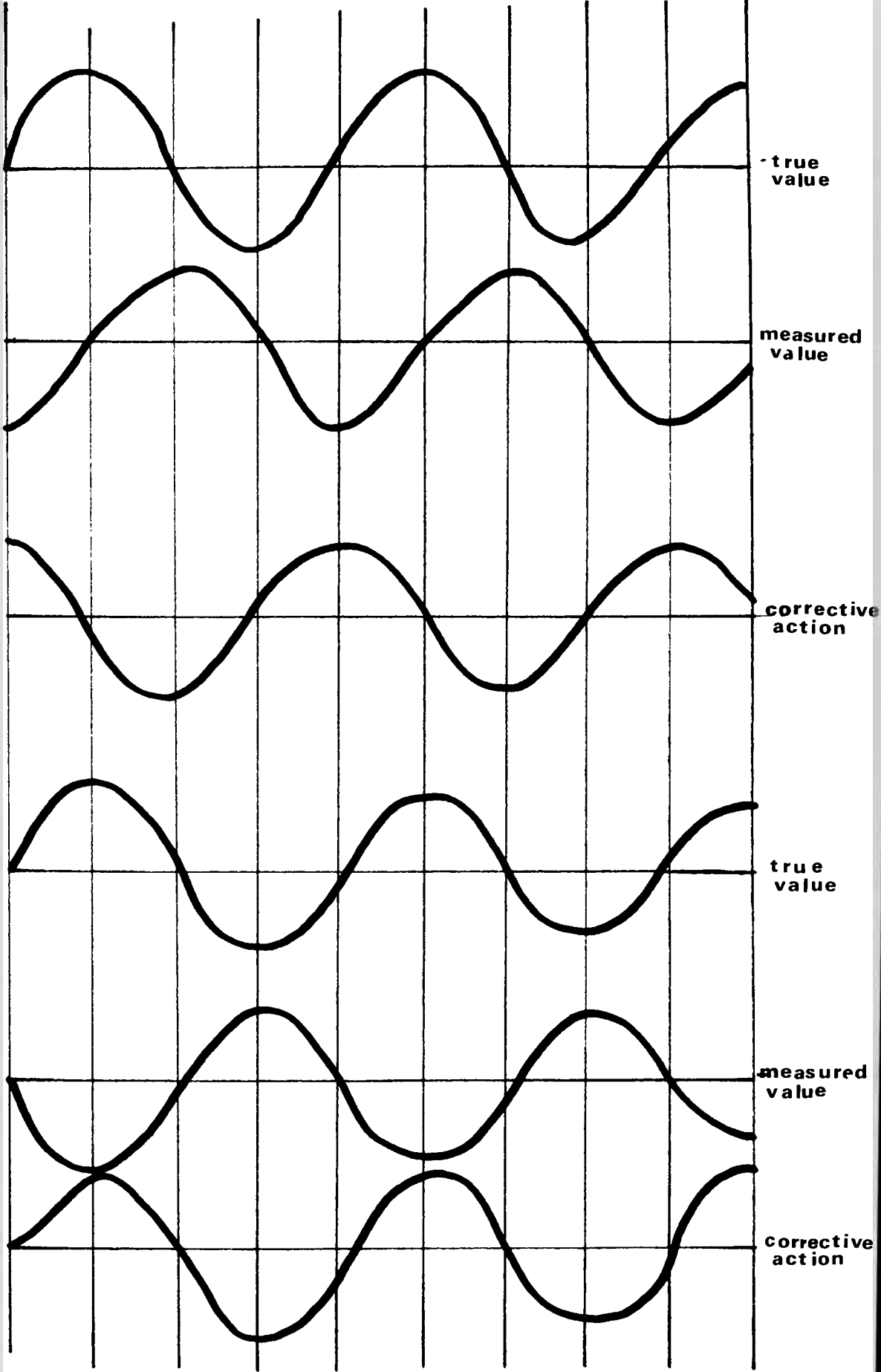

true value
measured value
corrective action
true value
measured value
corrective action

FIG.(9)

THE RELATION STEP OF FLOW RATE TO PARTICLE PENETRATION

IN DEPTH FILTERS

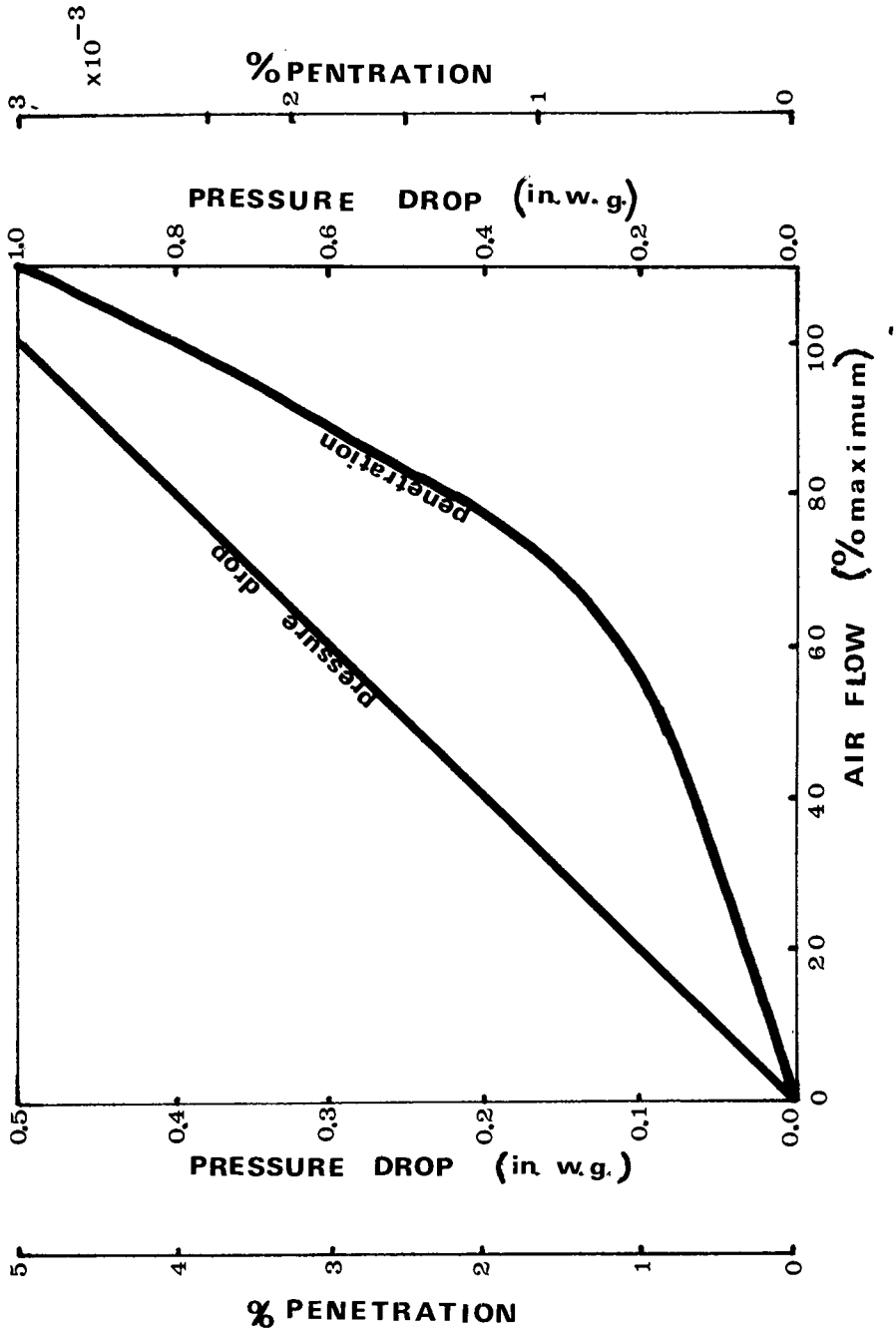


FIG.(10) THE RELATIONSHIP BETWEEN FILTER RETENTIVITY AND GAS FLOW RATE

(From HUMPHREY, 1960)

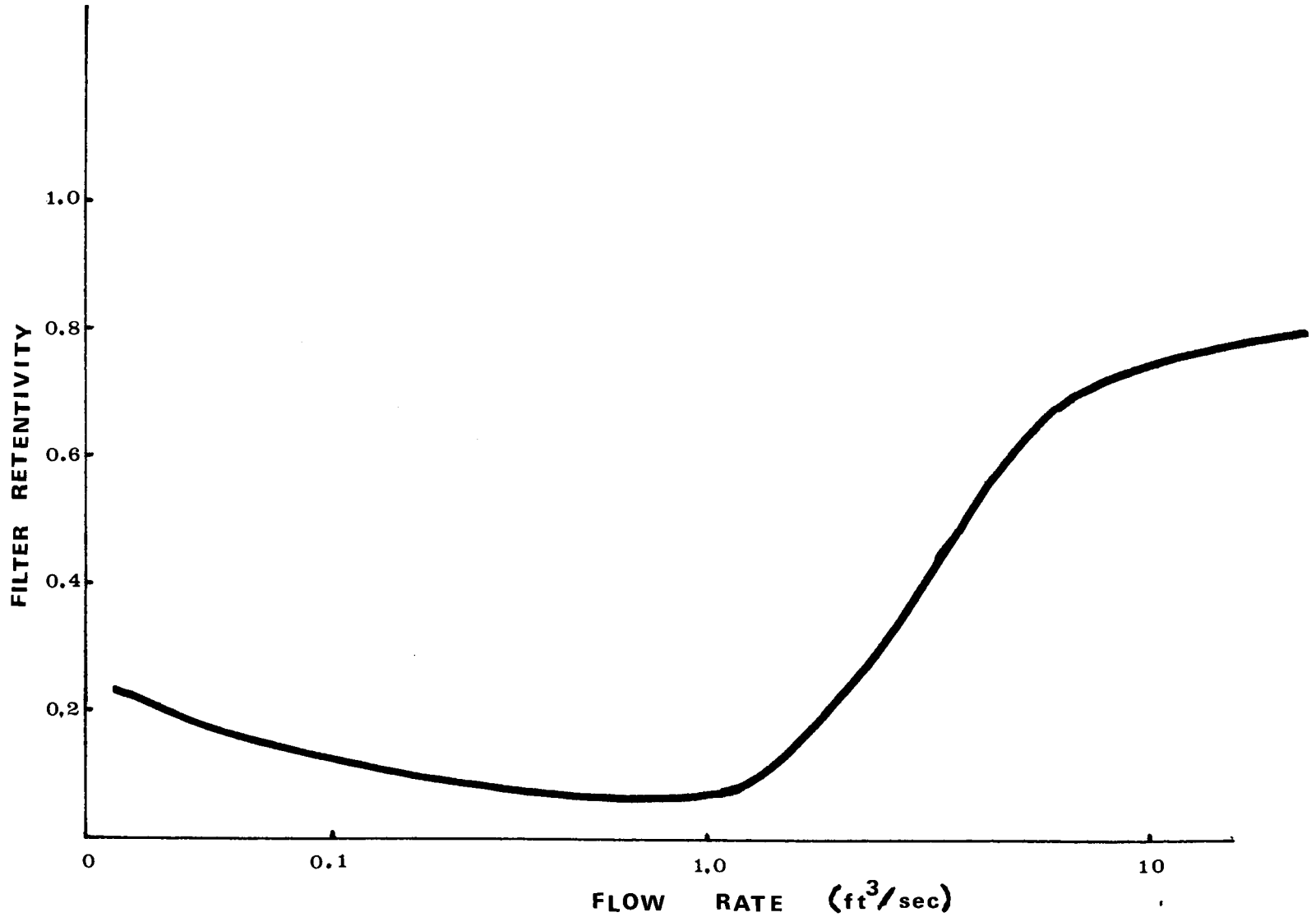


FIG.(11)

SCHEMATIC DIAGRAM OF CHEMAP CERAMIC FILTER

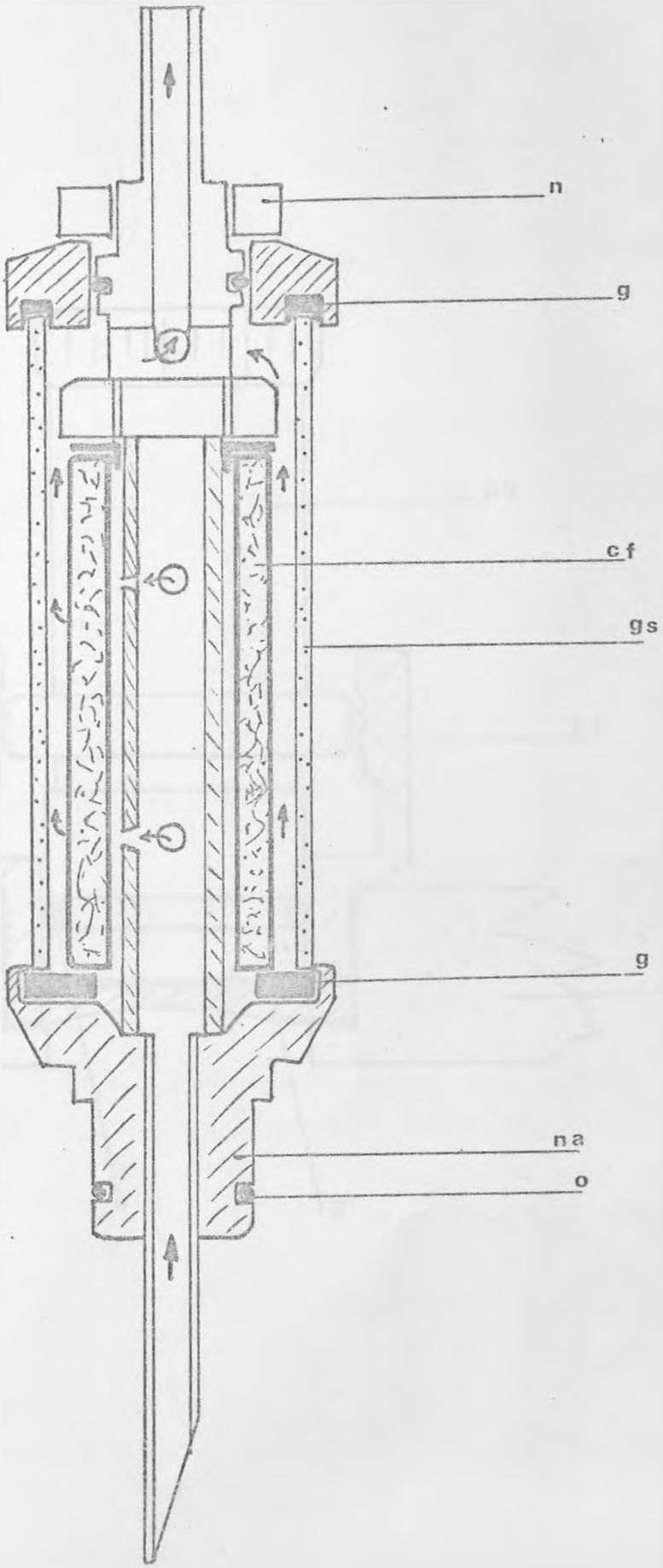
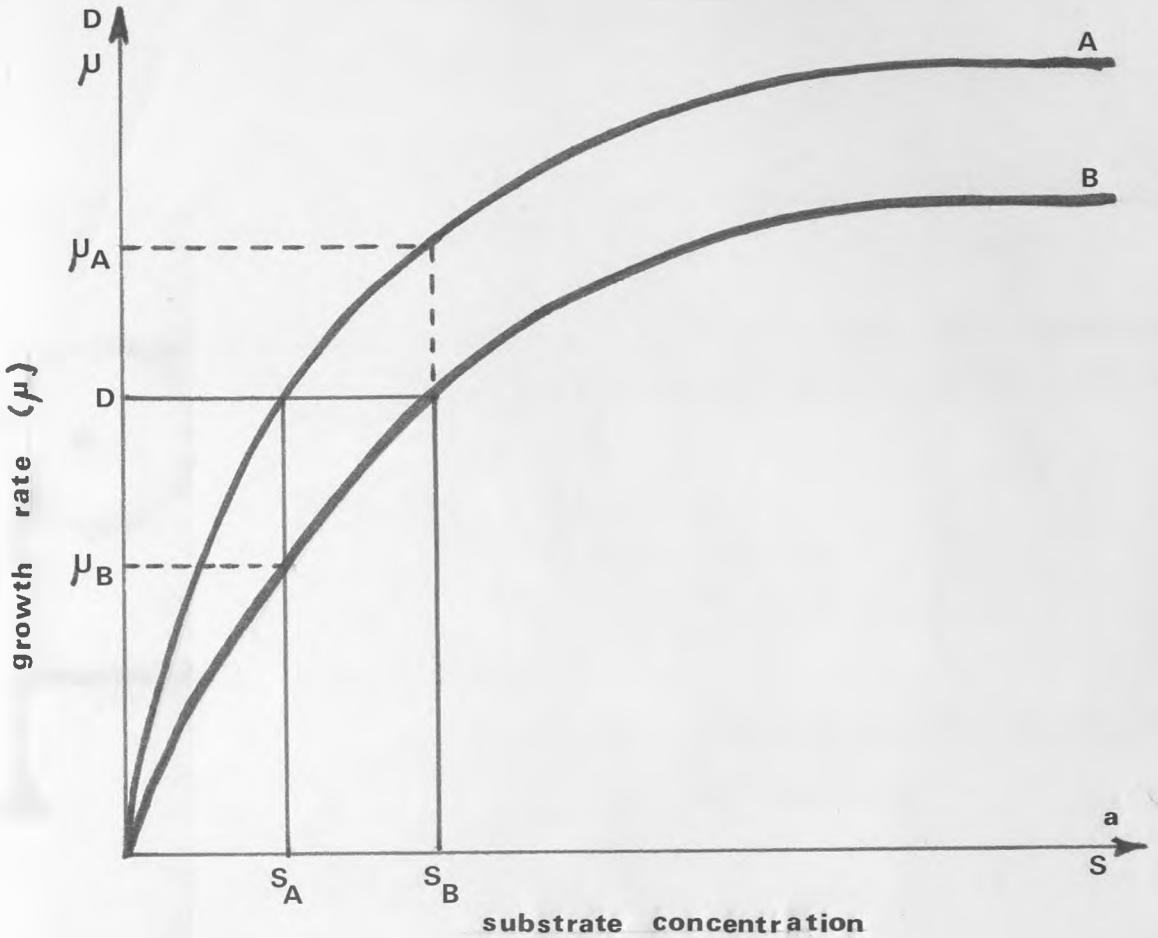
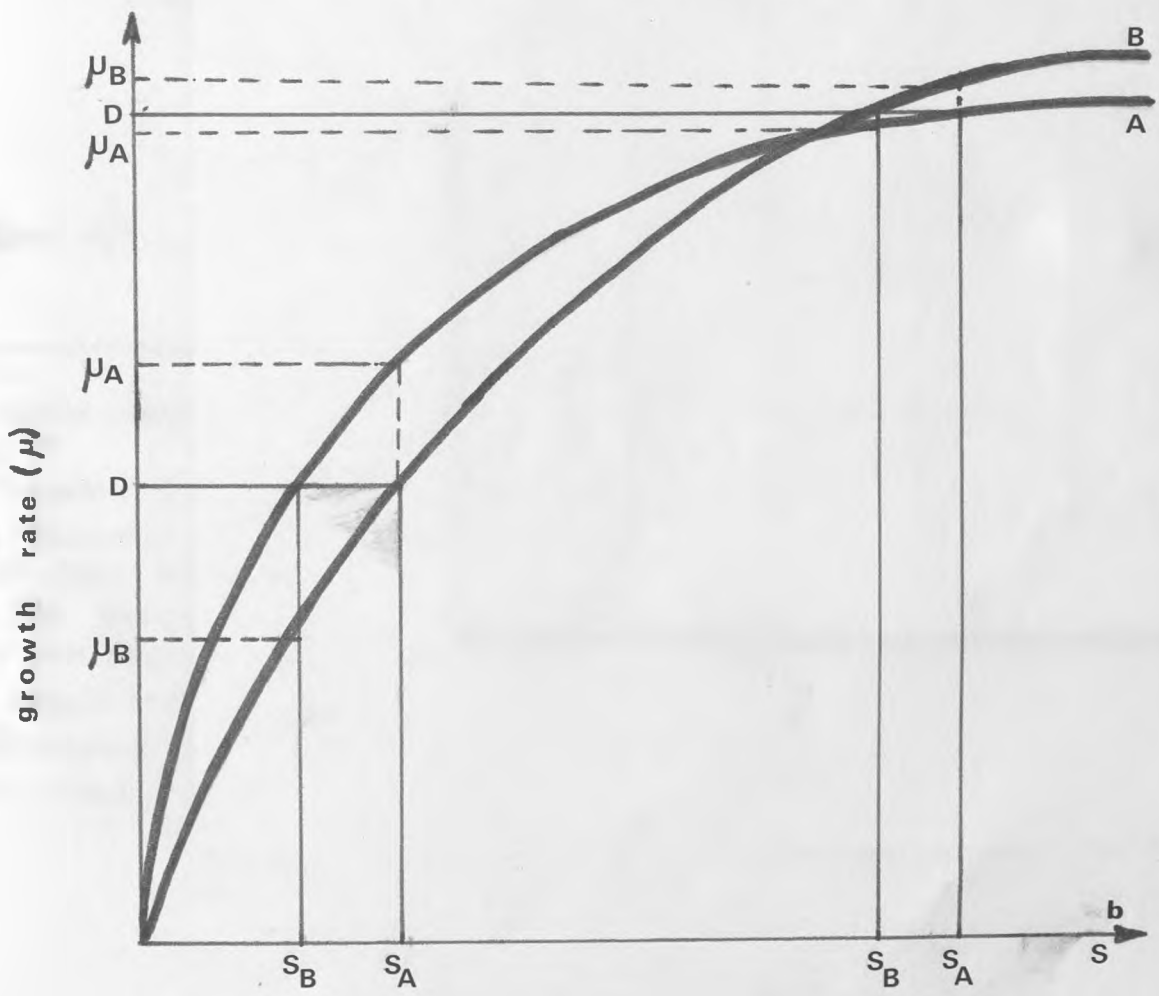
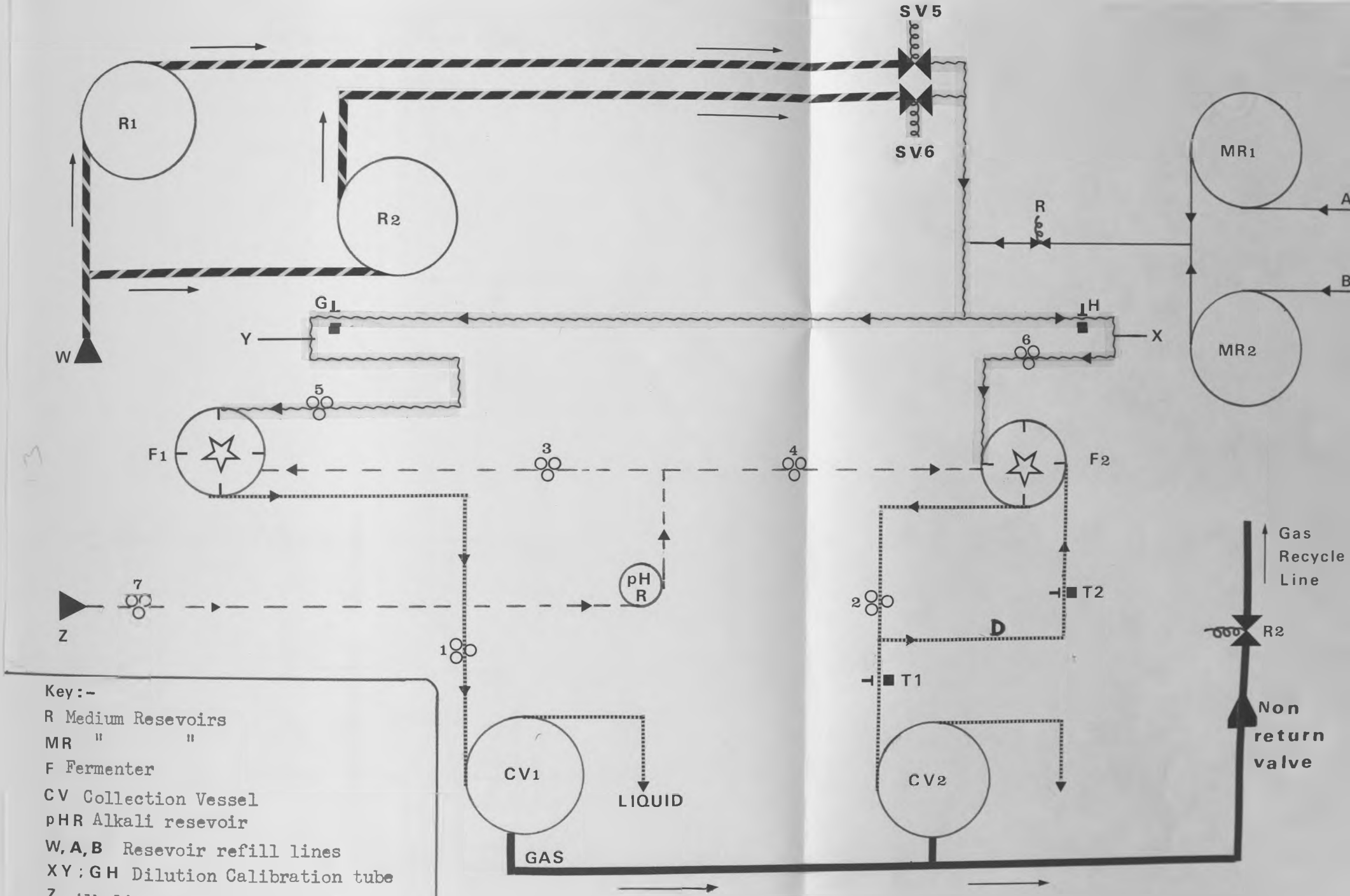


FIG .(12)

SELECTION OF MICROORGANISMS IN THE CHEMOSTAT (FROM TEMPEST 1970)


D
μ
A
B
μA
D
μB
growth rate (μ)
a
S
SA
SB
substrate concentration


B
μB
D
A
μA
μA
D
μB
growth rate (μ)
b
S
SB
SA
SB
SA
substrate concentration



Key :-

R Medium Resevoirs

MR " "

F Fermenter

CV Collection Vessel

pHR Alkali resevoir

W, A, B Resevoir refill lines

XY ; GH Dilution Calibration tube

Z Alkali resevoir refill line

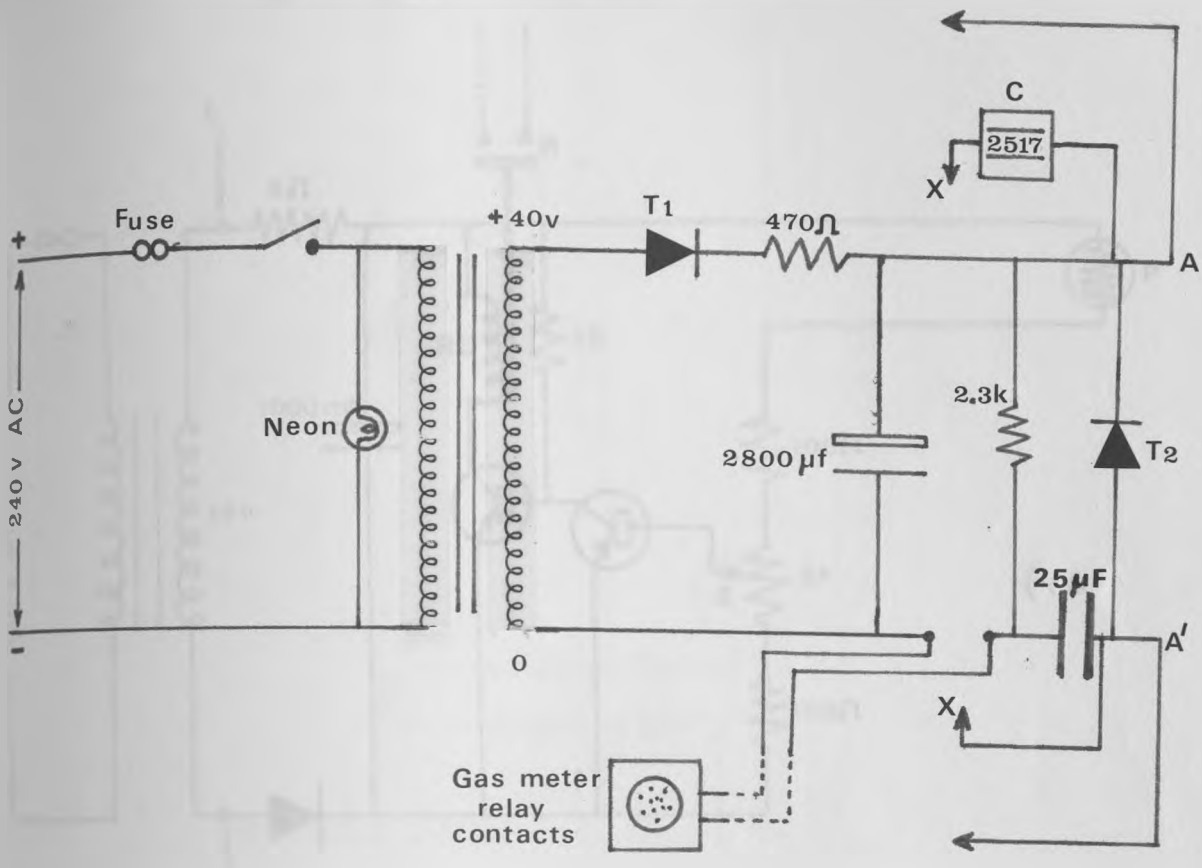
Peristaltic pump

SV Solenoid valve

FIG. (13)

FLOW SHEET — Liquid System

FIG.(14) CIRCUIT DIAGRAM OF THE PULSE COUNTER AND INTEGRATOR FOR THE ETHANE PURGE LINE



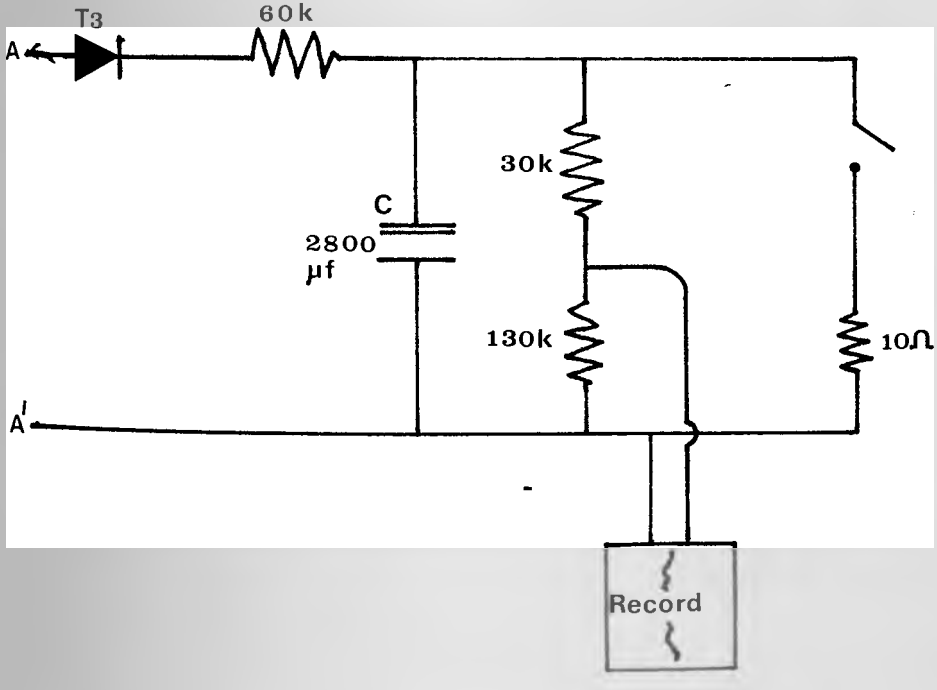
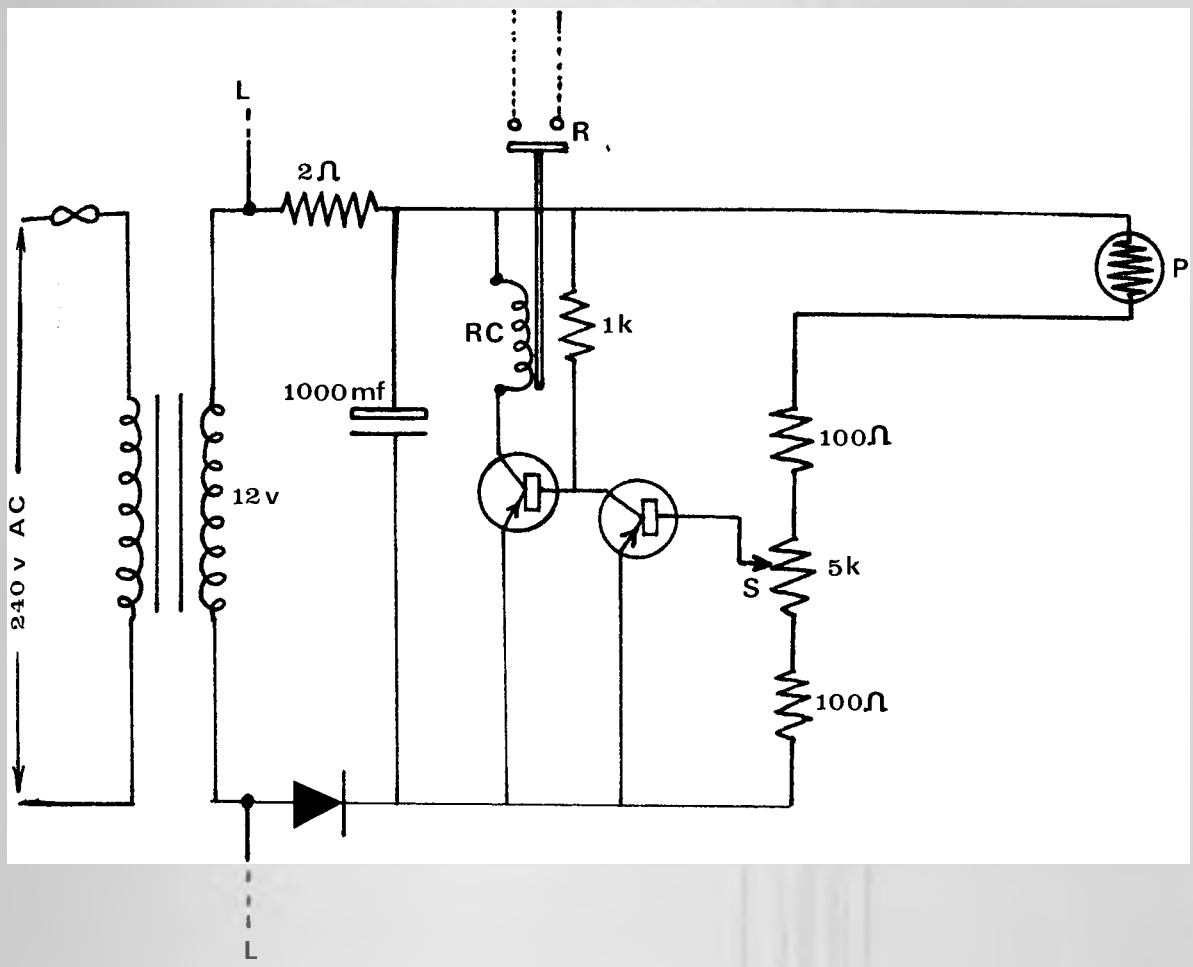


FIG.(15)

GAS METER PHOTOCELL RELAY



KEY

L	12 v supply to light source
RC	Relay coil
R	Relay contacts constituting pulse source for pulse counter/integrator
S	Sensitivity control
P	Photosensitive resistor

FIG.(16) CONSTRUCTIONAL DETAILS OF pH ELECTRODE

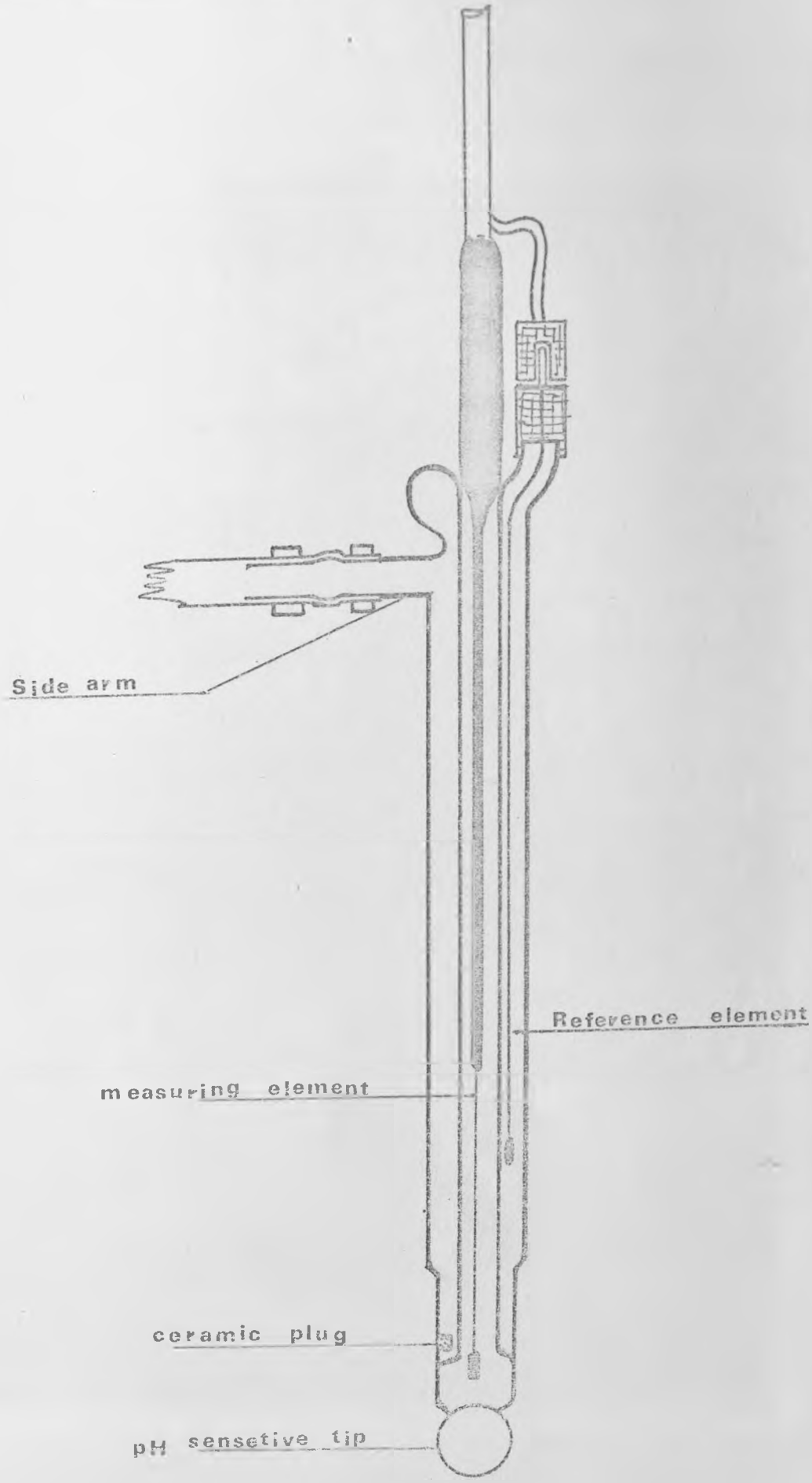
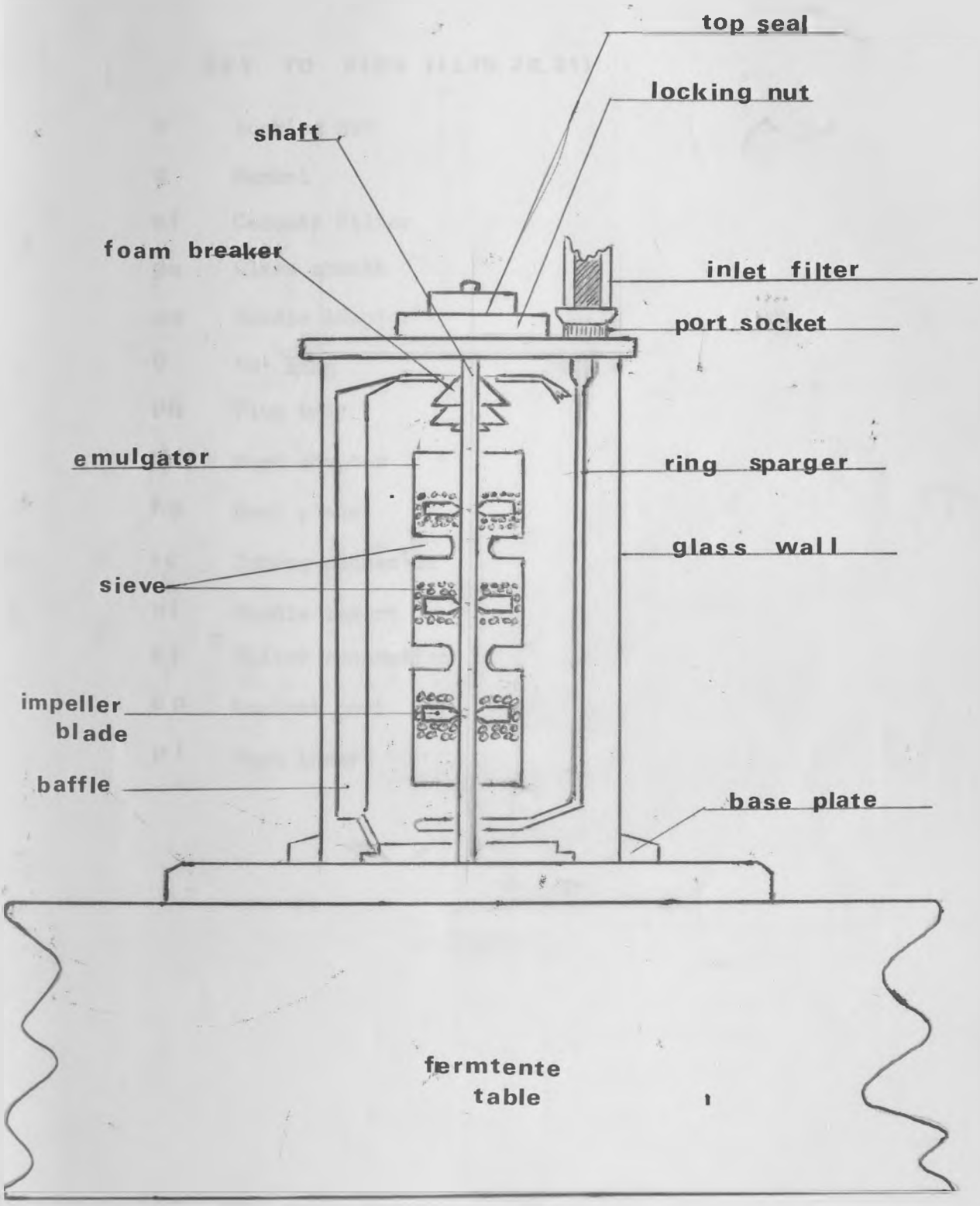


FIG.(I7): LAYOUT OF THE F.Z.7 CONTROL PANELS


AMPERES
SENSITIVITY
HIGH
LEVEL CONTROL FERM. 1
F1 'IN' LINE
F1 pH Pump "OFF"
Auto
Manual
pH TIMED DOSING
OFF TIME
ON TIME
INT
EXT
LEVEL CONTROL FERM. 2

Fig. (18) SCHEMATIC DIAGRAM OF THE FERMENTER DESIGN (NOT TO SCAIE)


top seal
locking nut
shaft
foam breaker
inlet filter
port socket
emulgator
ring sparger
glass wall
sieve
impeller blade
baffle
base plate
fermtente table

KEY TO FIGS (11,19,20,21)

n	Locking nut
g	Gasket
cf	Ceramic Filter
gs	Glass sheath
na	Needle Adaptor
O	'O' Ring
pb	Plug body
ps	Port Adaptor
hp	Head plate
tc	Tubing connector
ni	Needle insert
fi	Filter connection
cp	Coolant port
pi	Port insert

FIG.(11)

SCHEMATIC DIAGRAM OF CHEMAP CERAMIC FILTER

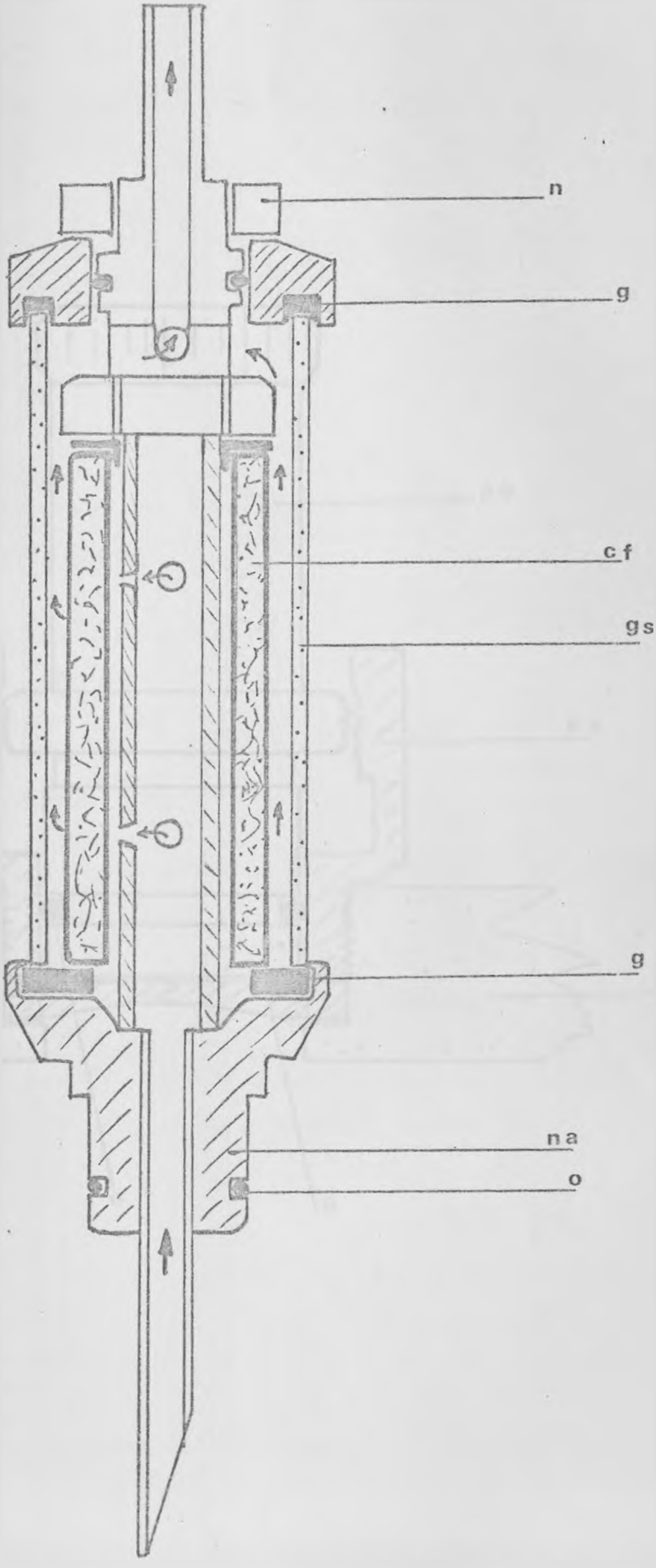


FIG.(19)

SCHEMATIC DIAGRAM OF CHEMAP BLIND PLUG AND SOCKET

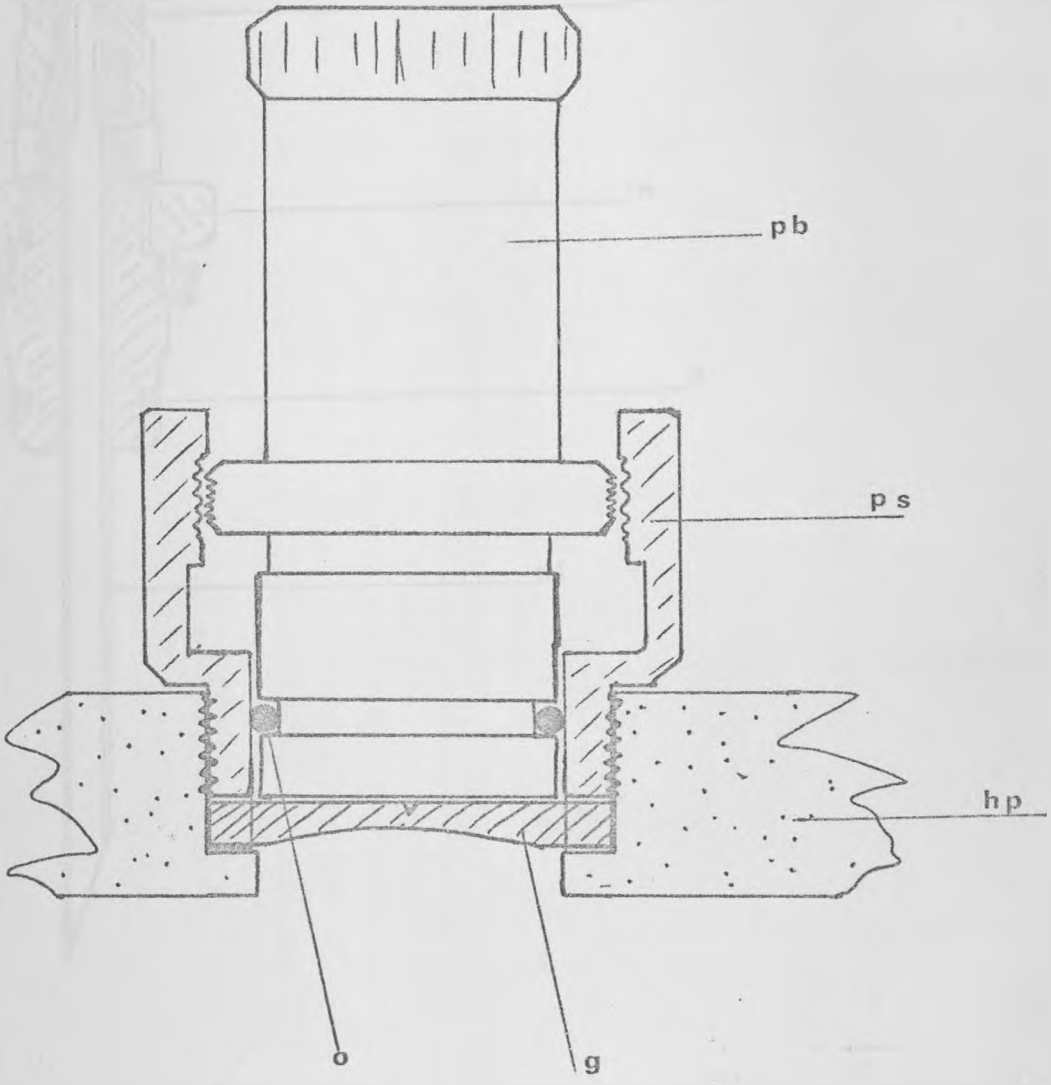


FIG. (20)

SCHEMATIC DIAGRAM OF CHEMAP NEEDLE CONNECTOR

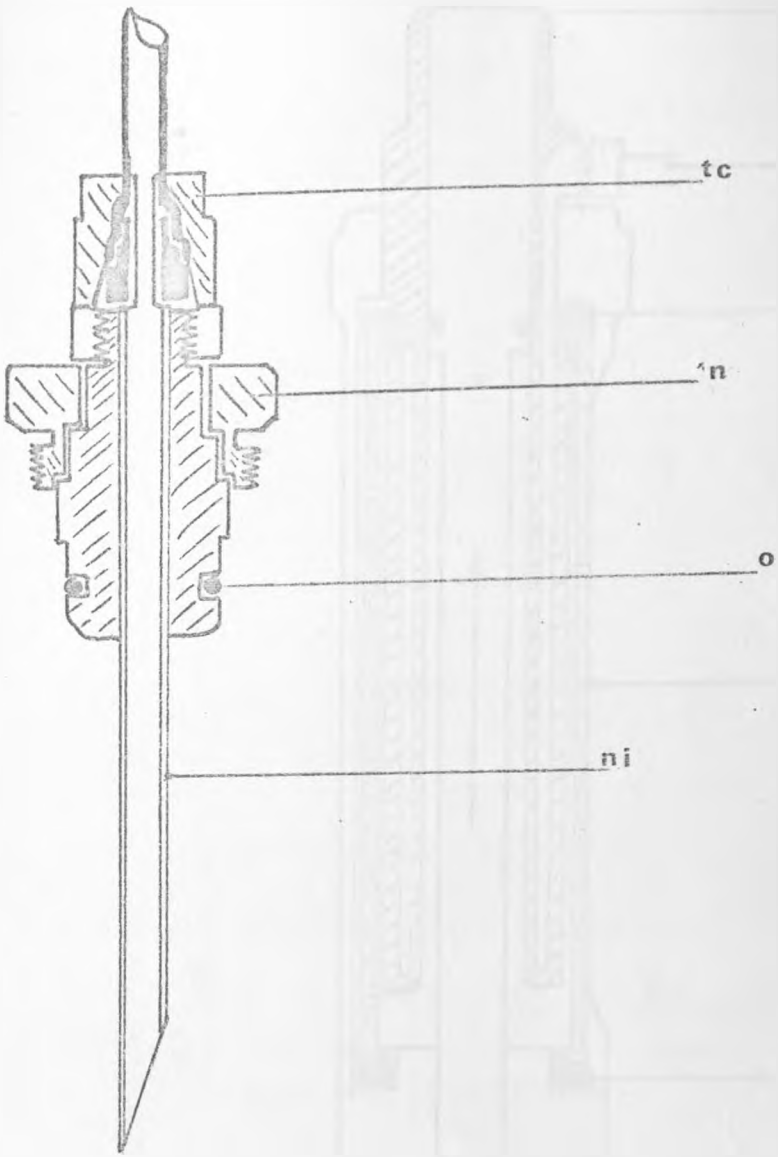


FIG. (21)

SCHEMATIC DIAGRAM OF CHEMAP REFLUX CONDENSOR

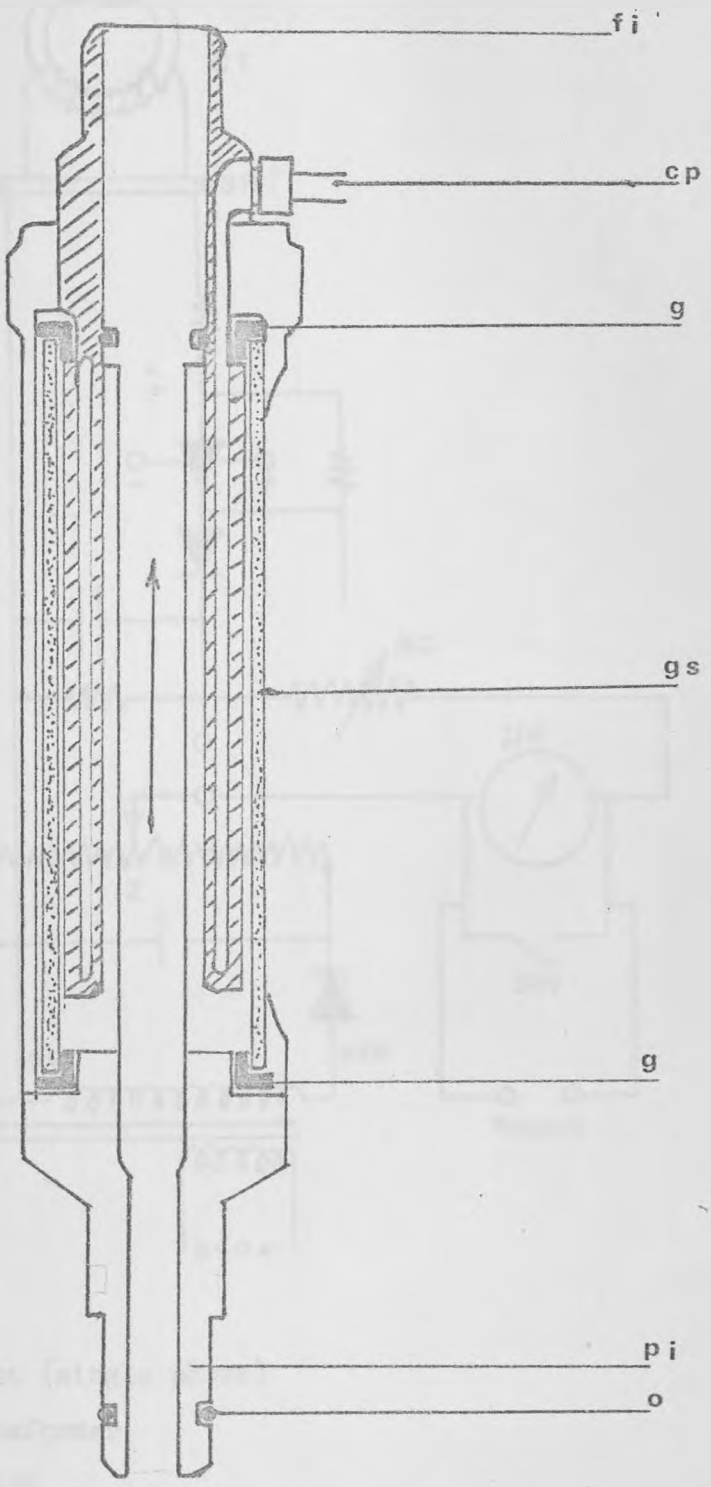
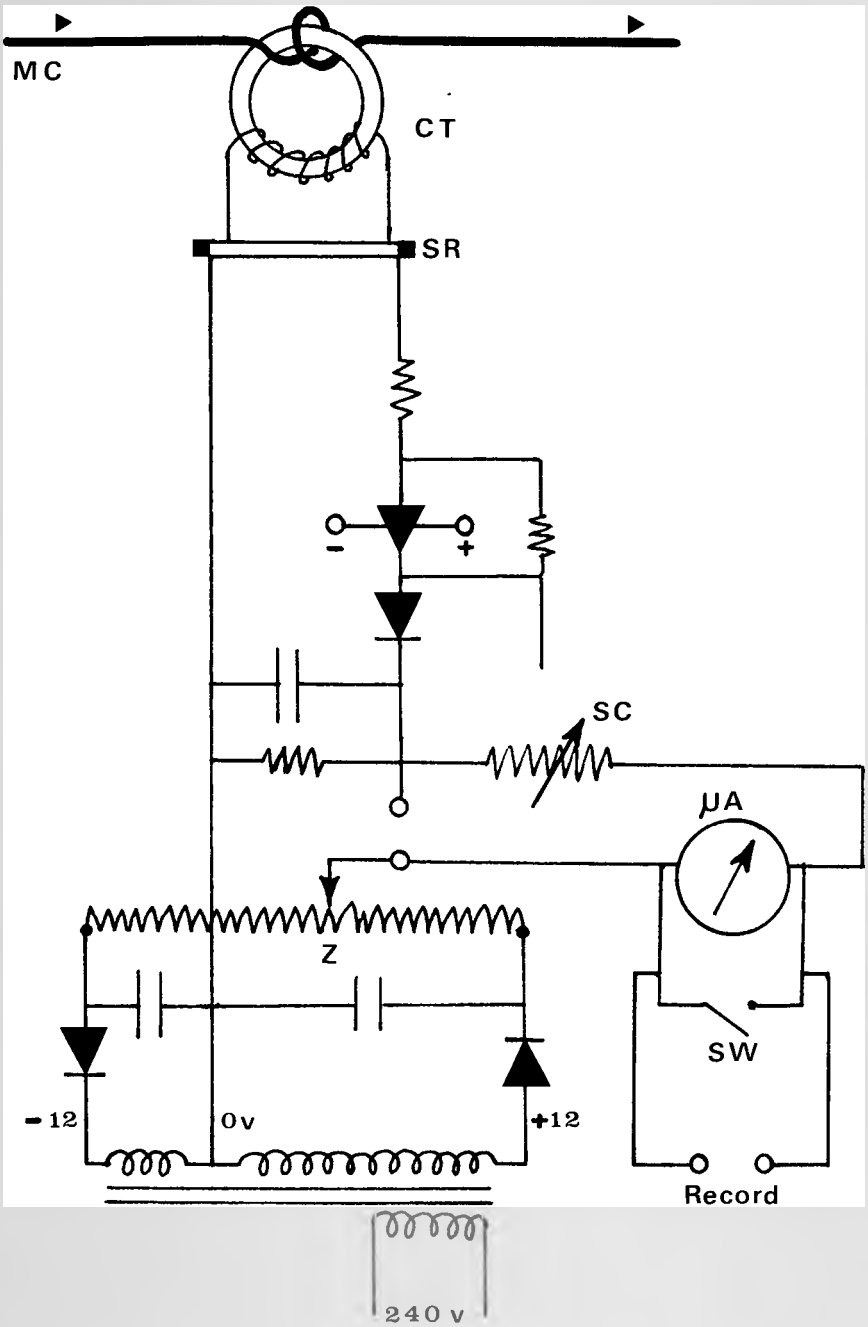


FIG. 22

CIRCUIT DIAGRAM OF CURRENT CONSUMPTION METER



MC	Motor current (single phase)
CT	Current Transformer
SR	Shunt Resistor
SC	Sensitivity Change
SW	Shorting switch for start-up
Z	Zero adjustment for meter

FIG. (23)

CONSTRUCTIONAL DETAILS OF A DISSOLVED OXYGEN ELECTRODE

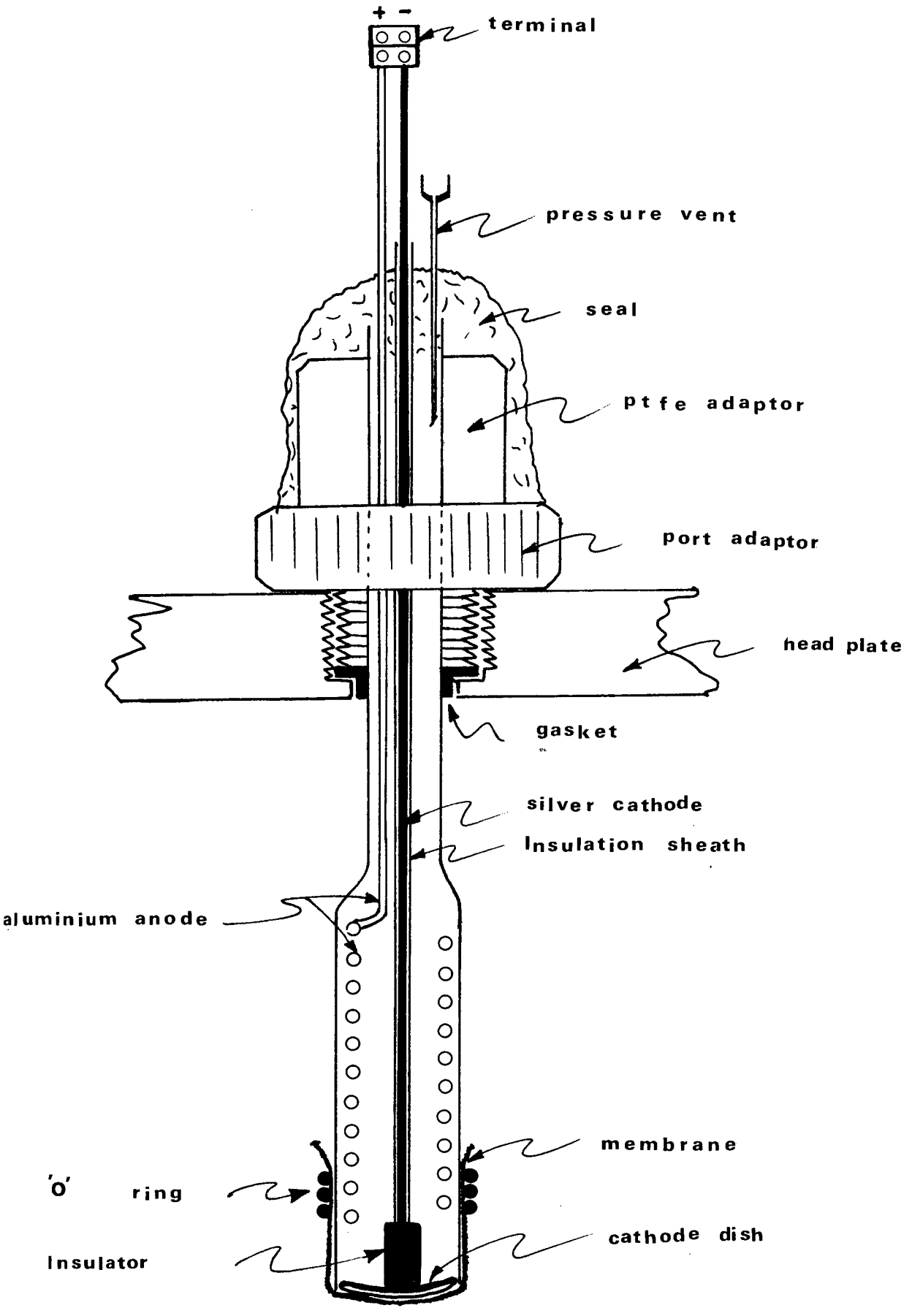
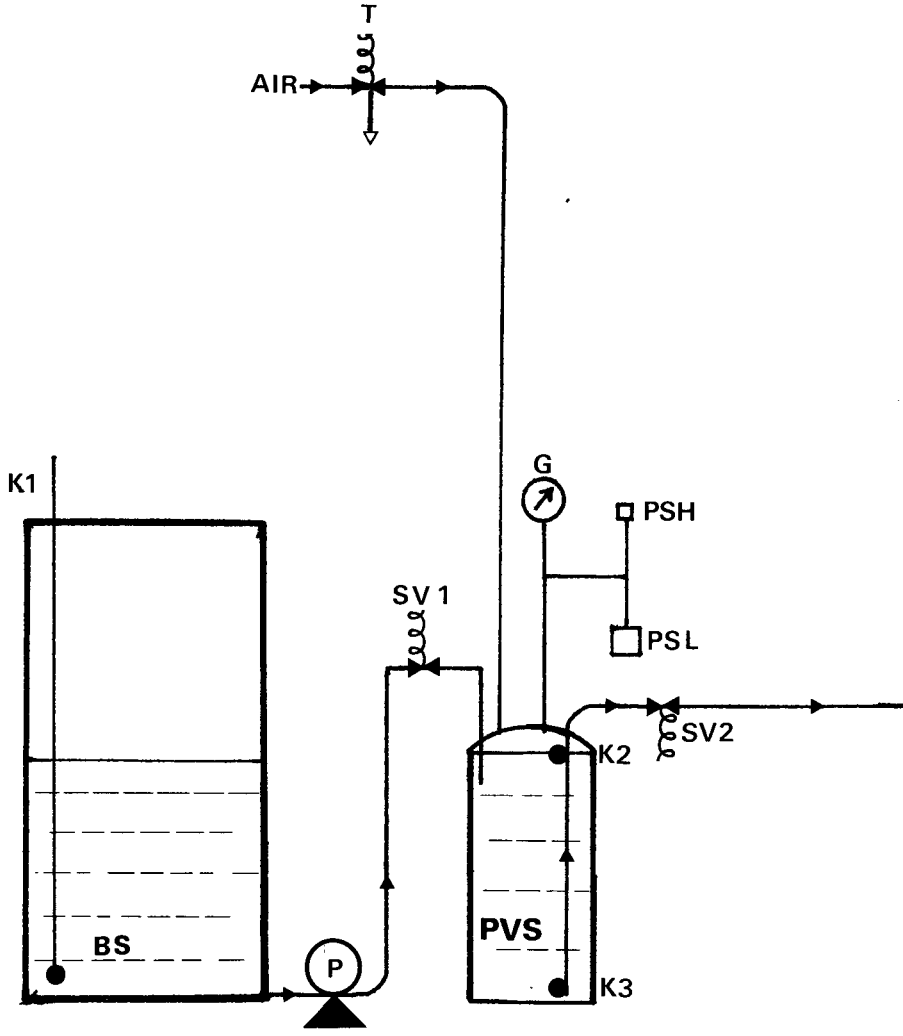

+ −
terminal
pressure vent
seal
ptfe adaptor
port adaptor
head plate
gasket
silver cathode
Insulation sheath
aluminium anode
membrane
'O' ring
Insulator
cathode dish

FIG.(24)

FLOW SHEET OF THE MANUAL AND AUTOMATIC FILTRATION SYSTEM

KEY TO FIG (24)

A, B	Pressure release vents
E, F	Filter Holders
K	Thermistor probe
P	Pump
T	Three way solenoid valve
SV	Solenoid valve
PSH	High Pressure switch
PSL	Low pressure switch
G	Pressure gauge
Q	Quick release coupling
R	Medium Resevoir
1 - 5	Hand valves
PVS	Stainless steel on Pressure Vessel
BS	Bulk storage vessel


T
AIR
K1
G
PSH
PSL
SV 1
SV2
K2
BS
P
PVS
K3

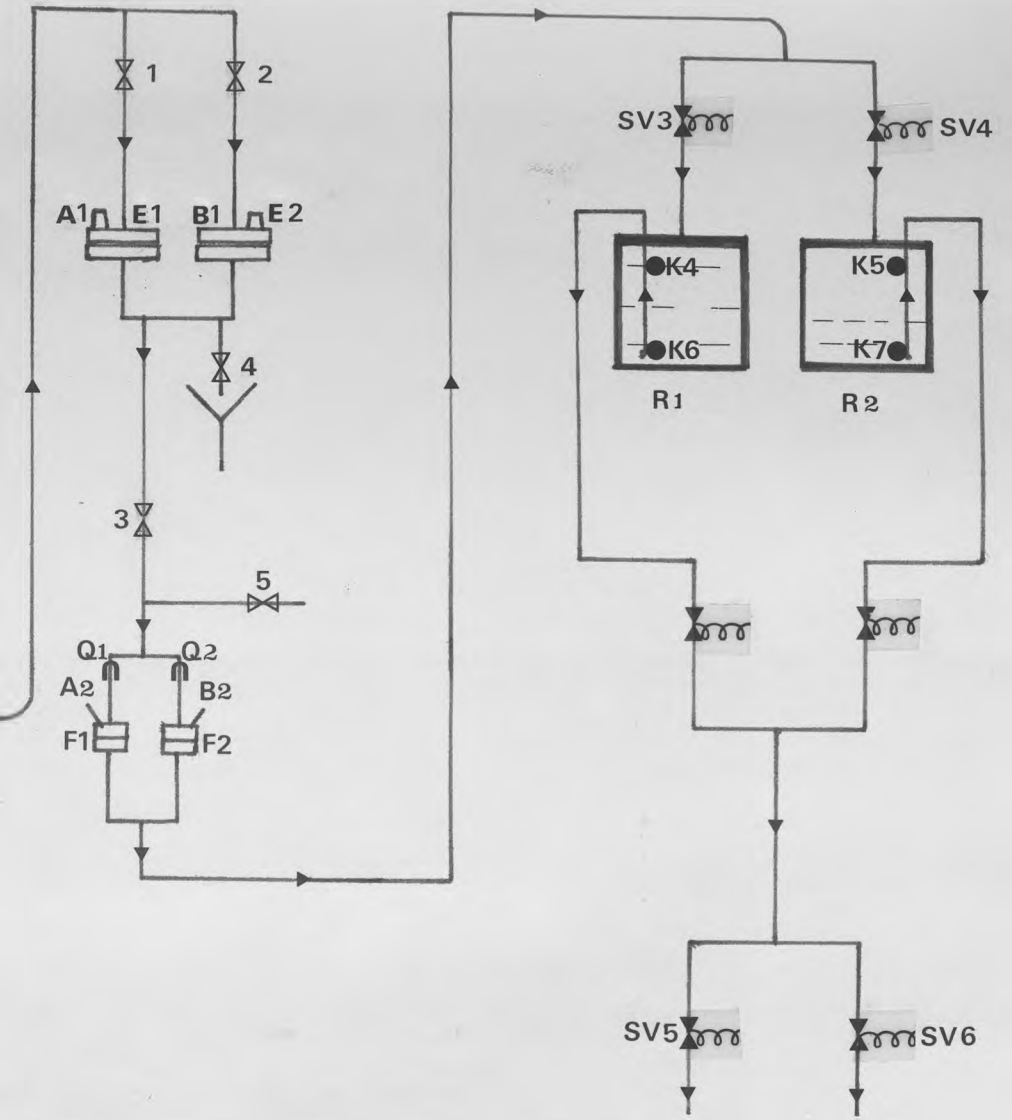

1
2
A1
E1
B1
E2
4
3
5
Q1
Q2
A2
B2
F1
F2
SV3
SV4
K4
K6
K5
K7
R1
R2
SV5
SV6

FIG.(25) LAYOUT OF THE AUTOMATIC/MANUAL MEDIUM FILTRATION RIG.

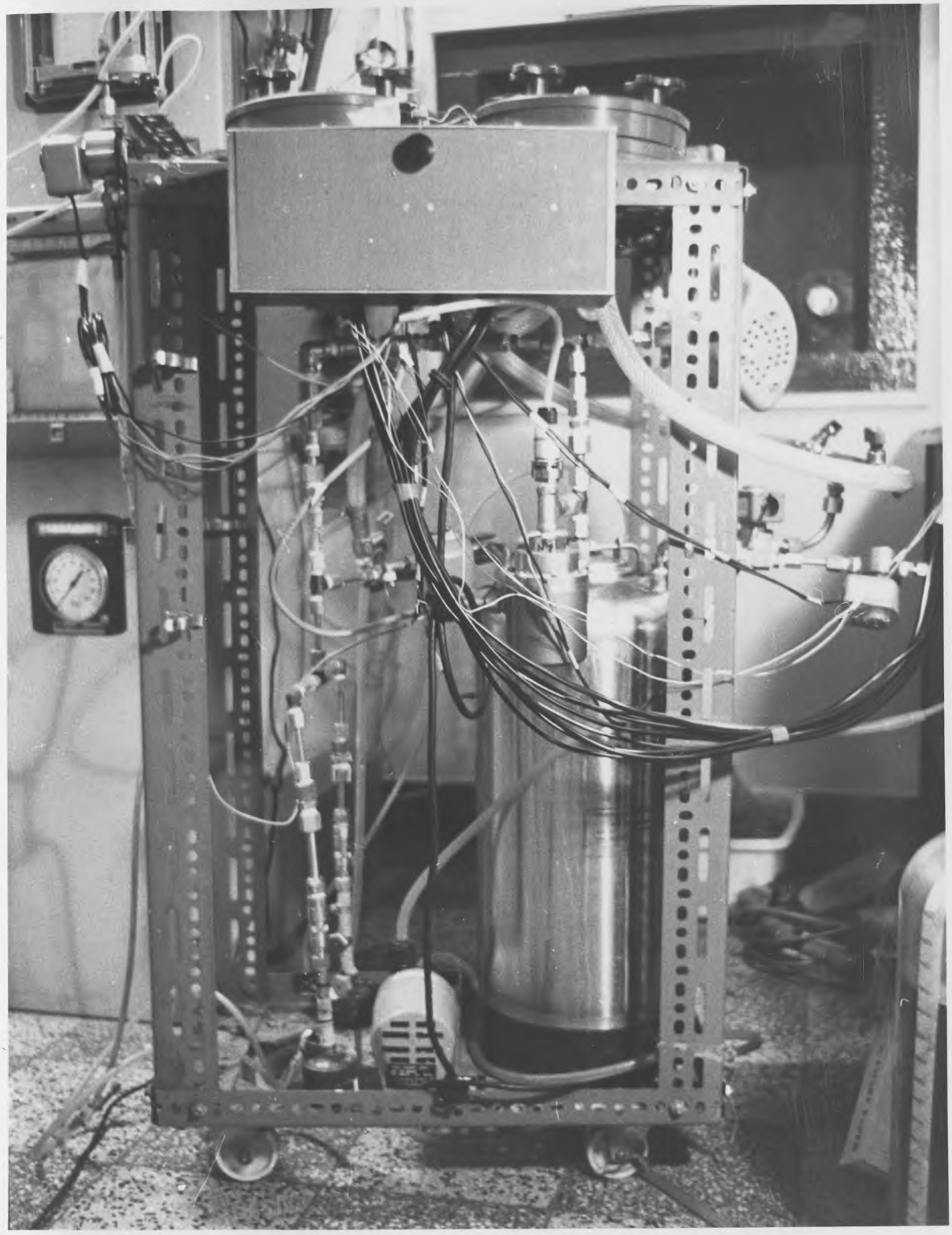


FIG.(27) ROTARY GAS METER ON THE LUTE ESCAPE ROUTE

FIG.(28).ROTARY GAS METER ON THE ETHANE PURGE ROUTE

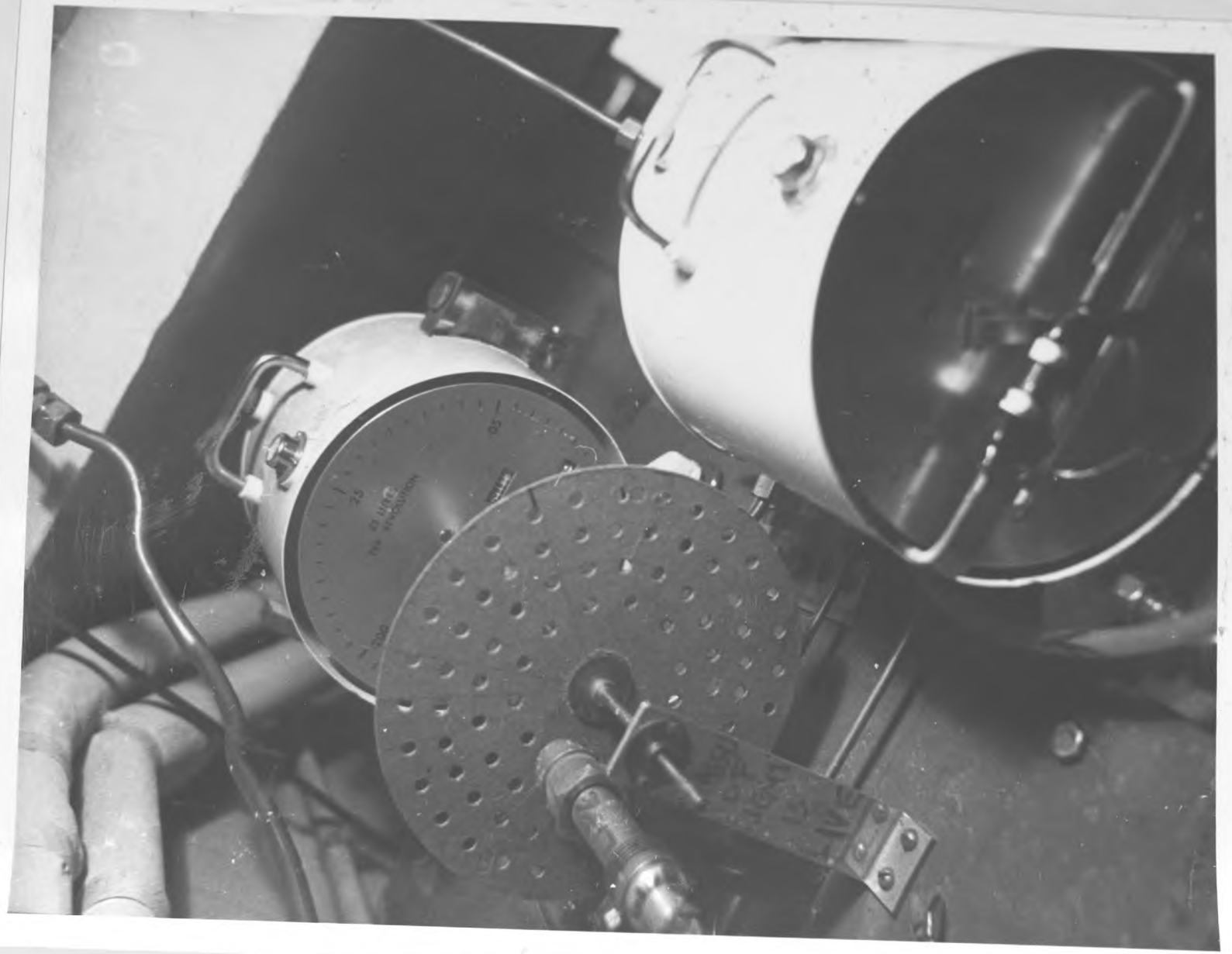
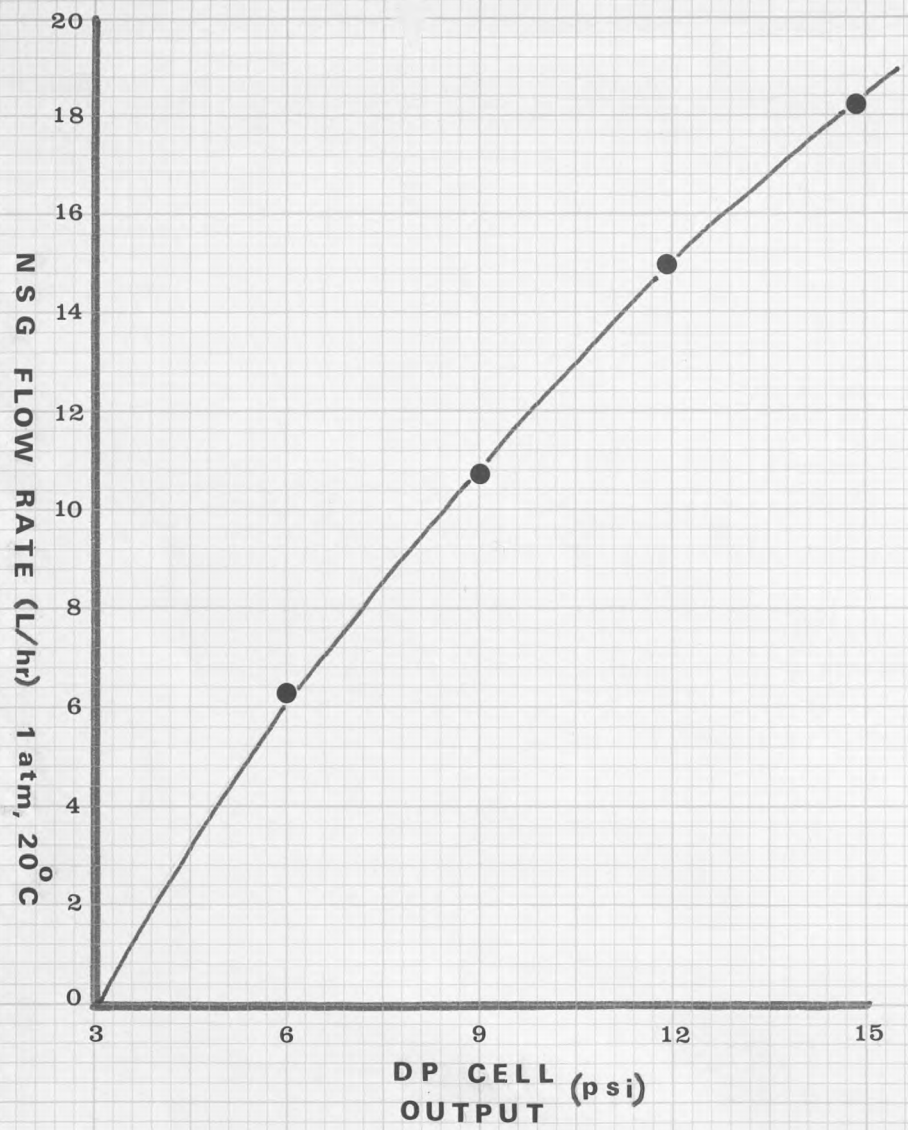
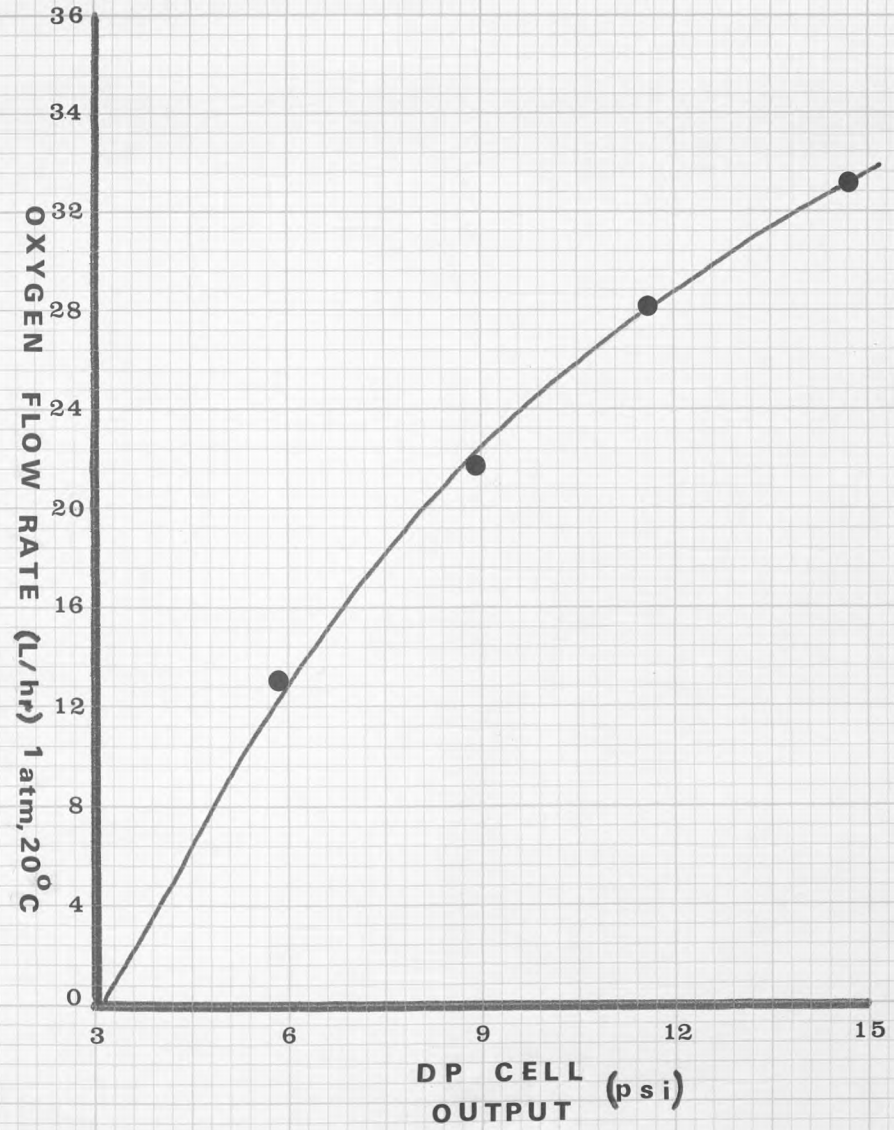


FIG. (29) Fig.(30)

GALIBRATION CURVES OF FISCHER-PORTER D.P. CELL FLOW TRANSDUCERS.

(N.S.G.) (OXYGEN)


NSG FLOW RATE (L/hr) 1 atm, 20°C
0
2
4
6
8
10
12
14
16
18
20
3
6
9
12
15
DP CELL OUTPUT (psi)


OXYGEN FLOW RATE (L/hr) 1 atm, 20°C
0
4
8
12
16
20
24
28
32
34
36
3
6
9
12
15
DP CELL OUTPUT (psi)


UMINPUMP FI
PH CONTROL FI

FIG. (31) PURGED PUMP HOUSING CONTAINING THE PERISTALTIC PUMPS OF THE LIQUID LINE SYSTEM

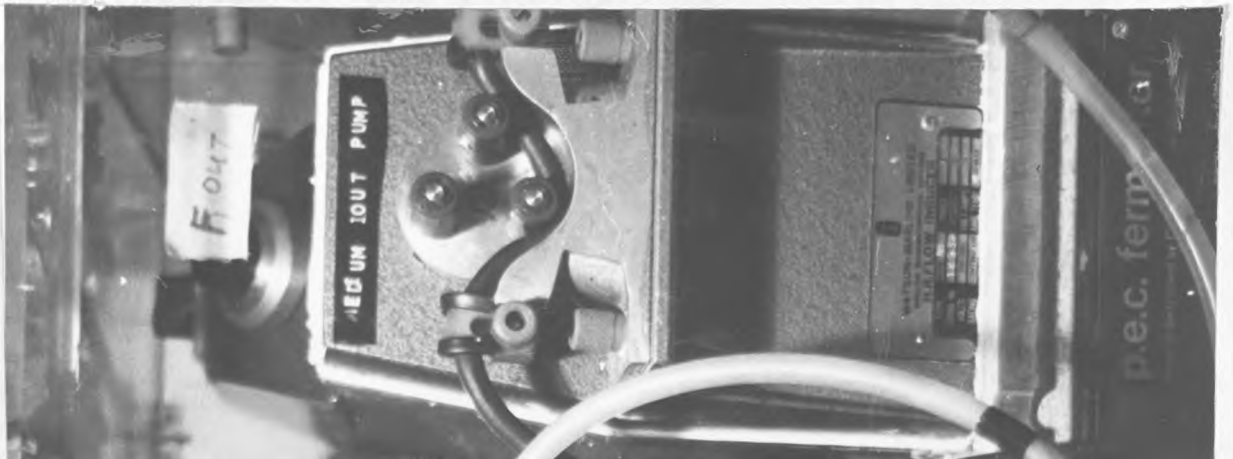
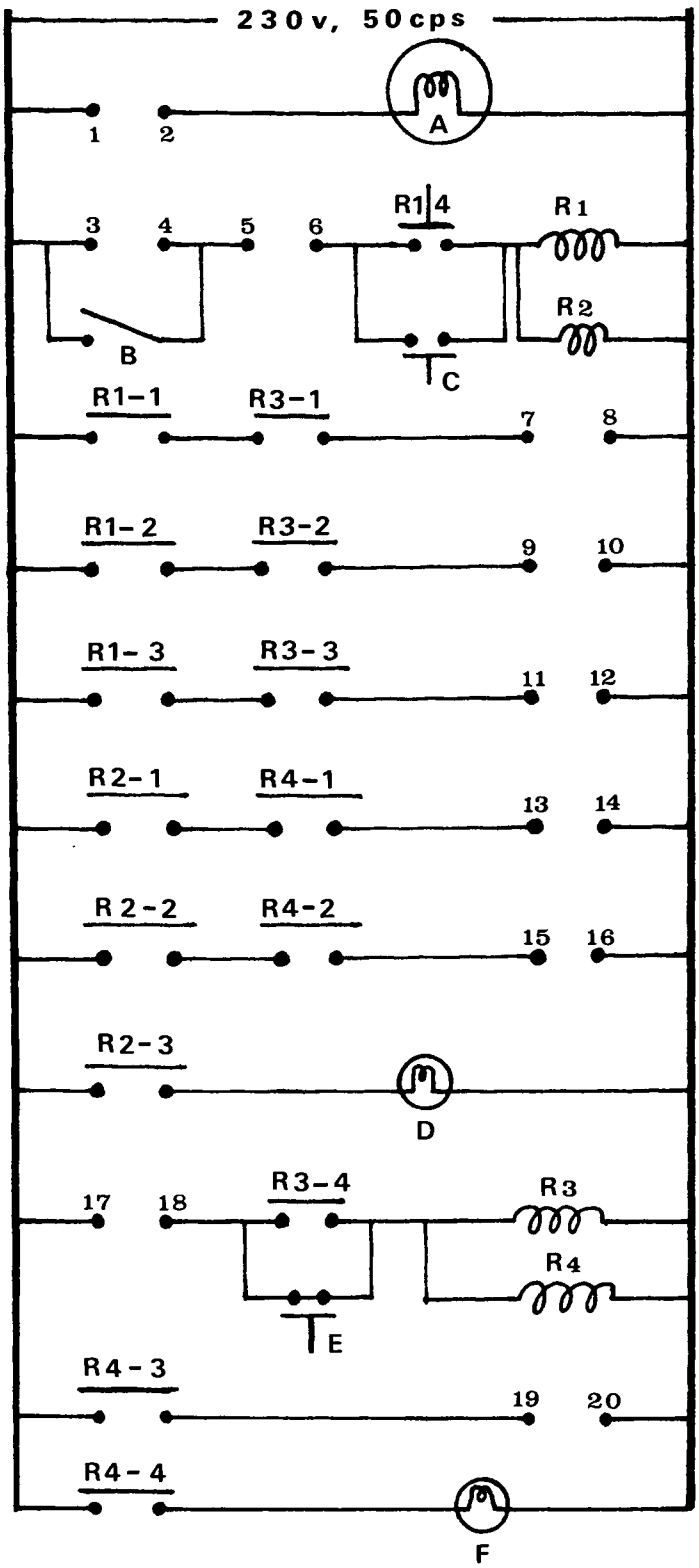


FIG.(32)

RELAY BOX WIRING DIAGRAM



Circ.Gas
HP Alarm Press Switch
Contacts normally open.

Circ.Gas. LP and Ex HP Press Switches. Trips, Contacts Normally closed.

Circ.Gas
Vent Solenoid Valve

OxygenValve
Shut Off Solenoid

NSG Valve
Shut off Solenoid

Circ. Gas Pump

Four Watson and Marlowe Media Pumps

Circ. Gas L.P. and Ex HP Trip light.

Analysers and Pumps Purge Press

Failure Trip

Analysers and PH Pumps
Electrical supply

Purge Failure trip light.

FIG.33 RESPONSE TIMES OF OXYGEN ELECTRODES IN FERMENTERS CONTAINING 5 l. OF WATER AT 40°C; 3000 RPM

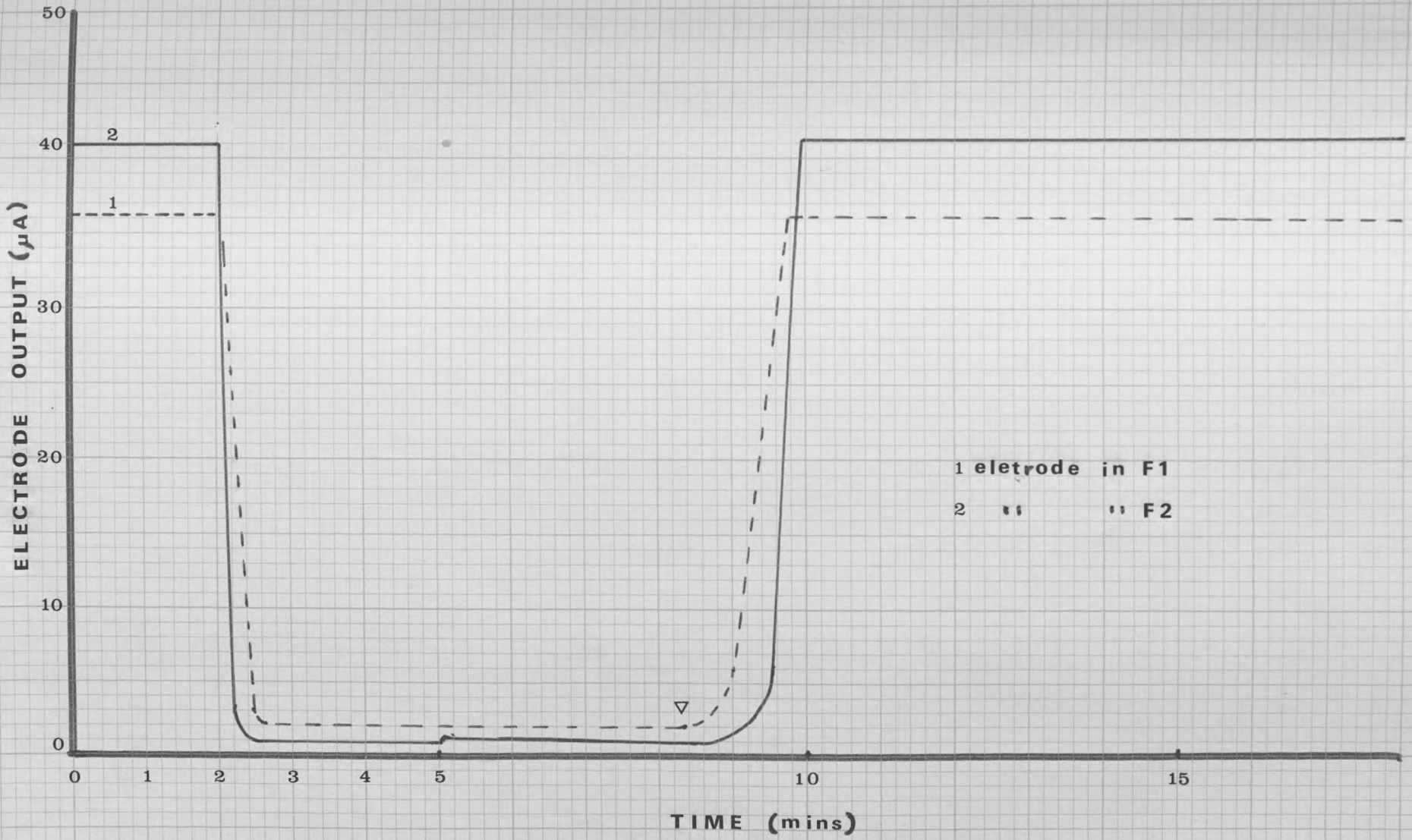

50
40
30
20
10
0
2
1
ELECTRODE OUTPUT (μA)
0
1
2
3
4
5
10
15
TIME (mins)
1 eletrode in F1
2 " " F2

FIG. (34)

THE EFFECT OF TEMPERATURE ON THE OUTPUT OF DISSOLVED OXYGEN ELECTRODES

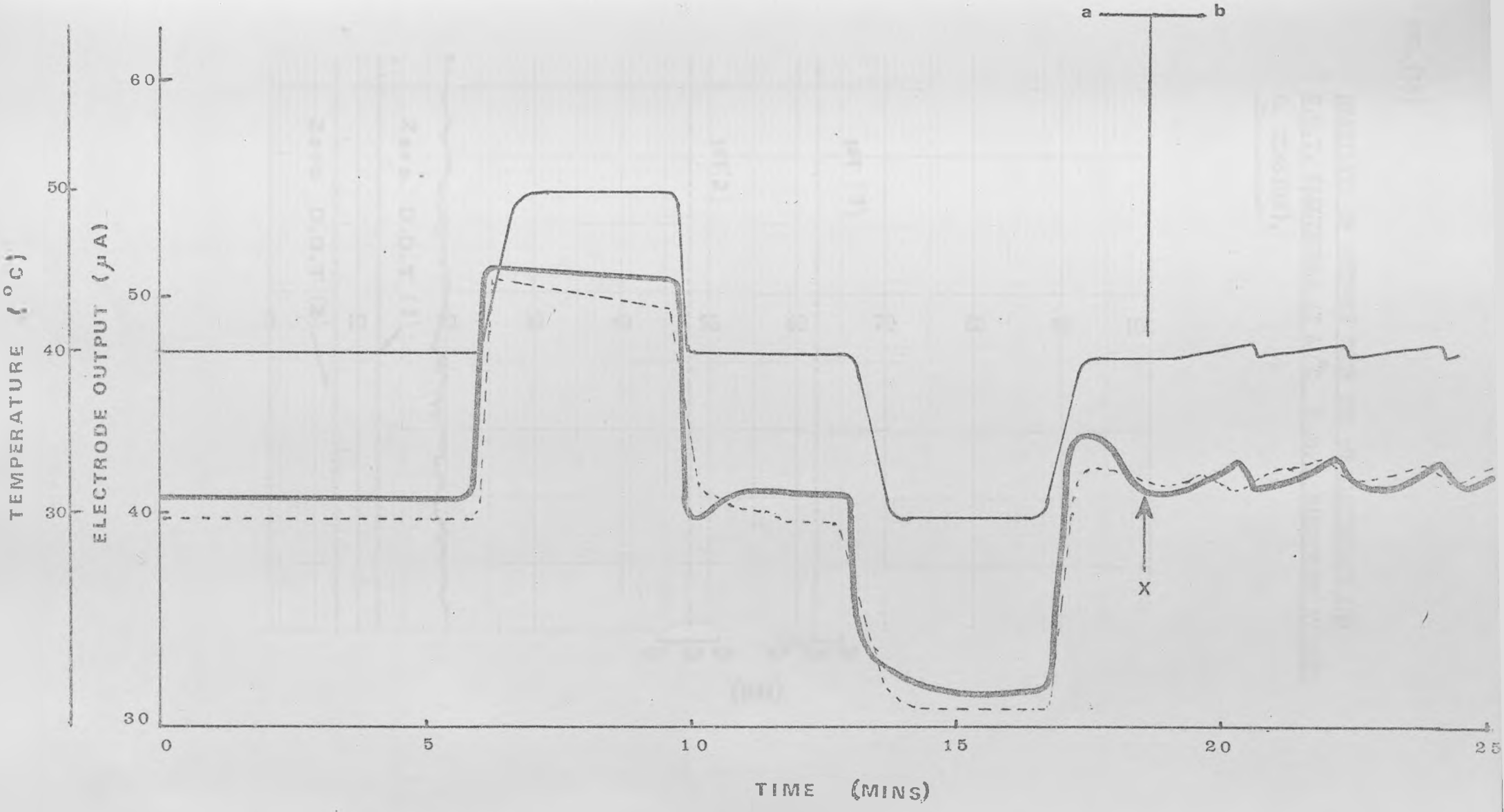

a
b
60
50
40
30
50
40
30
TEMPERATURE (°C)
ELECTRODE OUTPUT (μA)
X
0
5
10
15
20
25
TIME (MINS)

FIG.(35)

STABILITY OF OUTPUTS FROM THE pH ELECTRODES AND D.O.T. ELECTRODES AT 40°C (D.O.T. ELECTRODE AT ZERO O_2 TENSION).

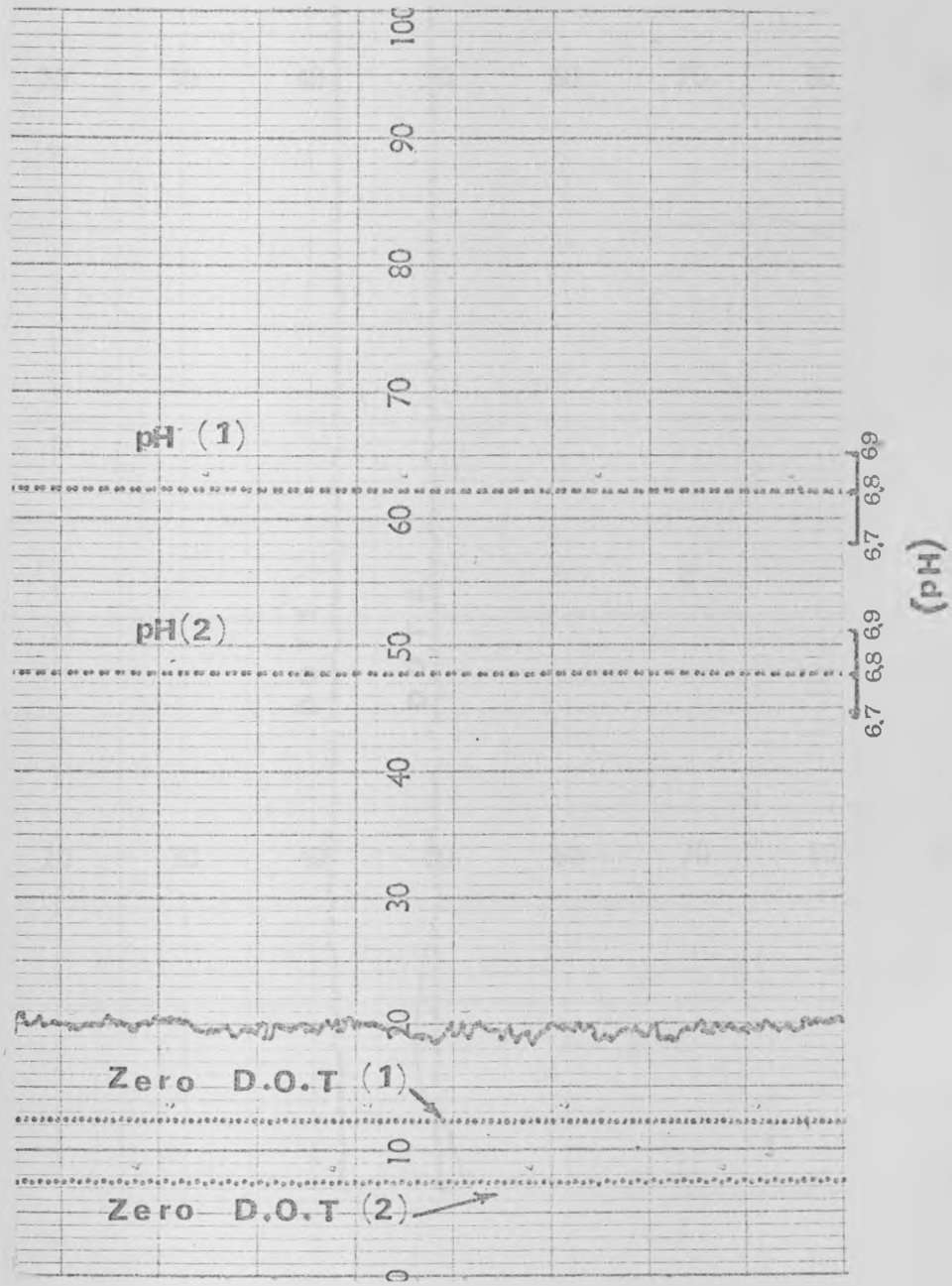
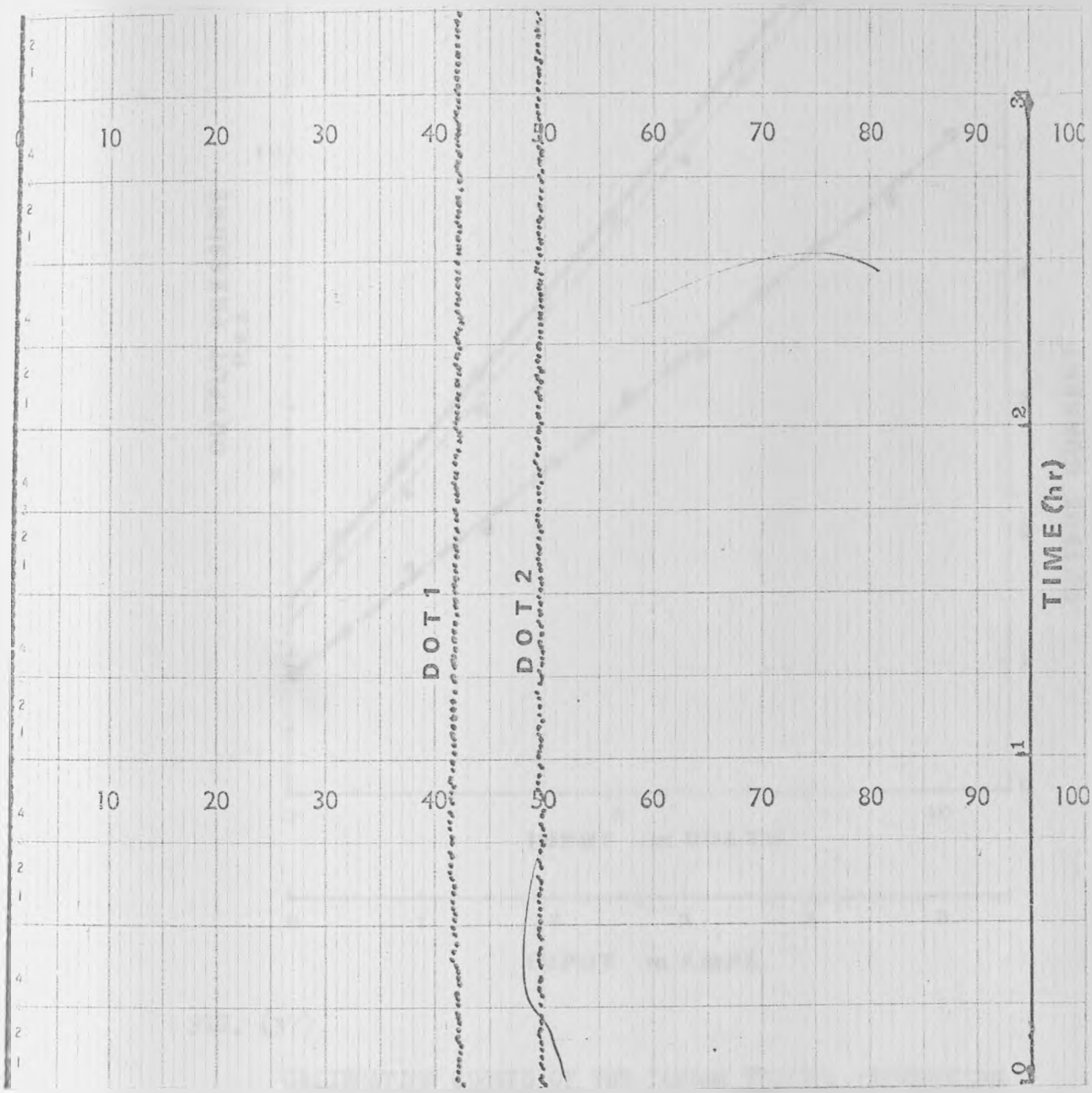


FIG. (36)

STABILITY OF DISSOLVED OXYGEN ELECTRODES AT 40°C AND OXYGEN PARTIAL PRESSURE OF 14.5% (V/V) IN THE GAS PHASE



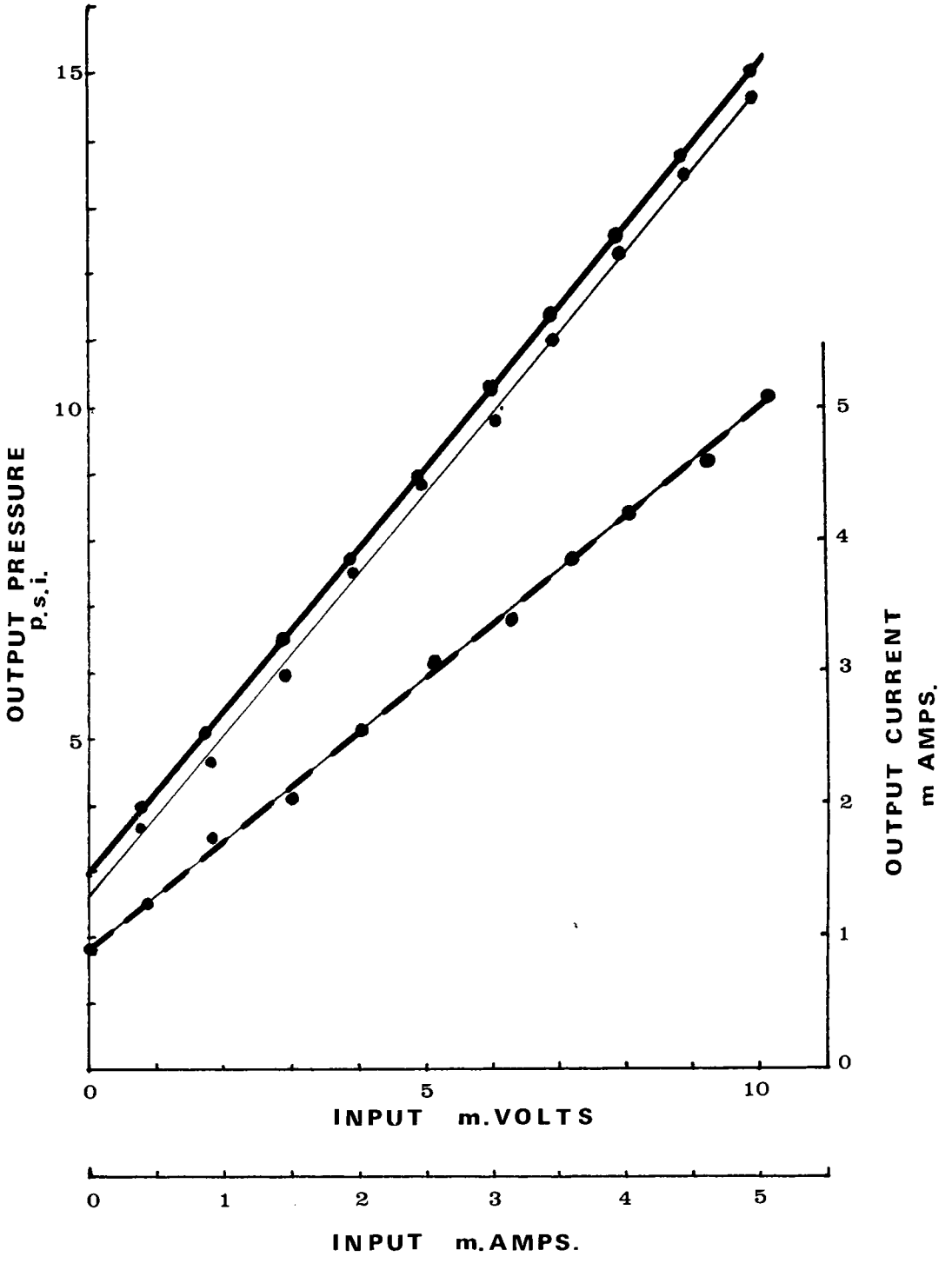


FIG. (37)

CALIBRATION CURVES OF THE TAYLOR 701/721 TRANSDUCERS

FIG. (38)

THE RELATIONSHIP BETWEEN VOLUME AND CURRENT CONSUMPTION BY THE FERMENTERS AT 1500 AND 2000 RPM (F2 ONLY)

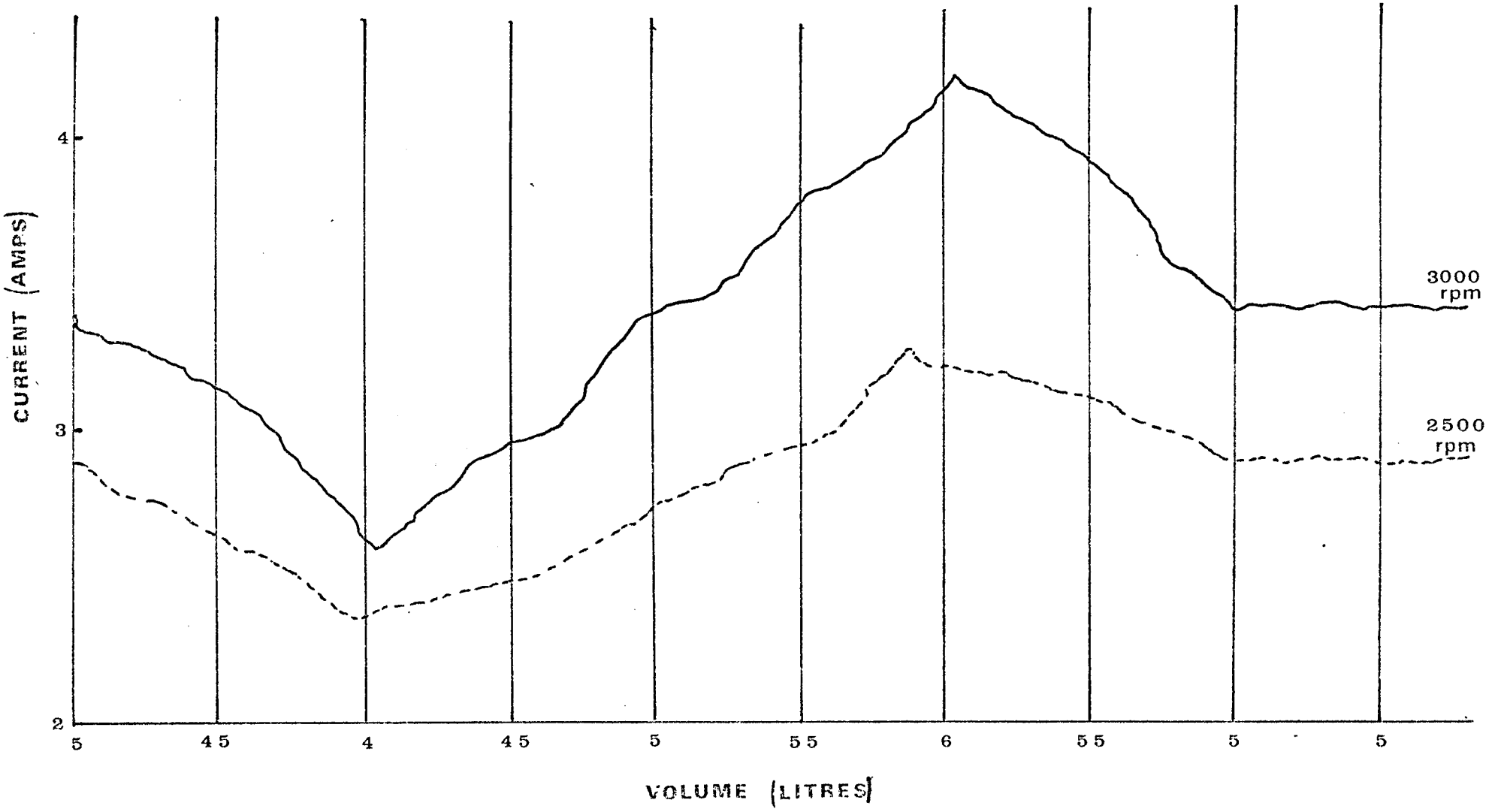
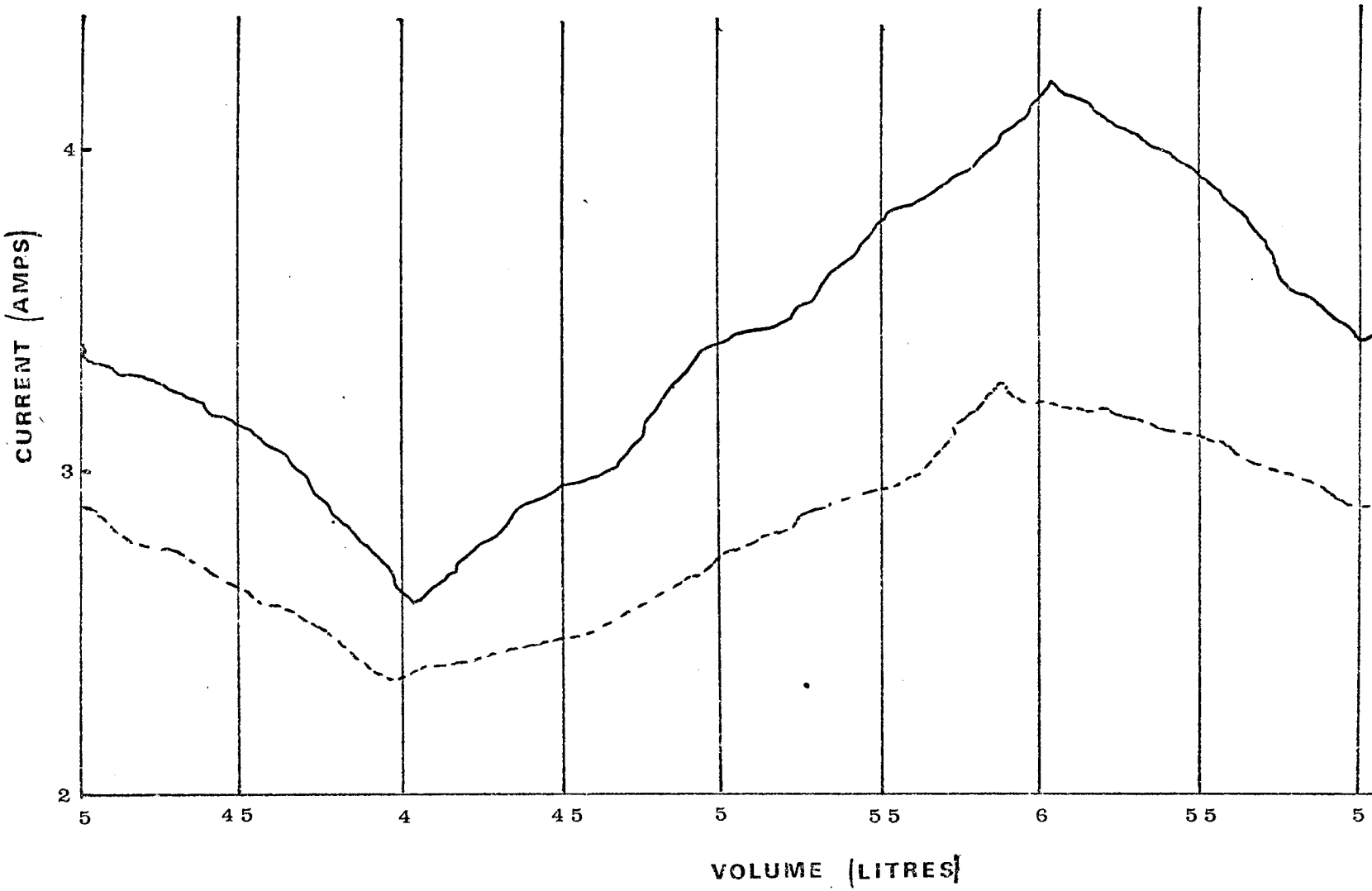

CURRENT (AMPS)
4
3
2
3000 rpm
2500 rpm
5
45
4
45
5
55
6
55
5
5
VOLUME (LITRES)

FIG. (39)

THE RELATIONSHIP BETWEEN VOLUME AND CURRENT CONSUMPTION BY THE FERMENTERS AT 2500 AND 3000 RPM (F2 ONLY)


CURRENT (AMPS)
4
3
2
5
45
4
45
5
55
6
55
5
VOLUME (LITRES)

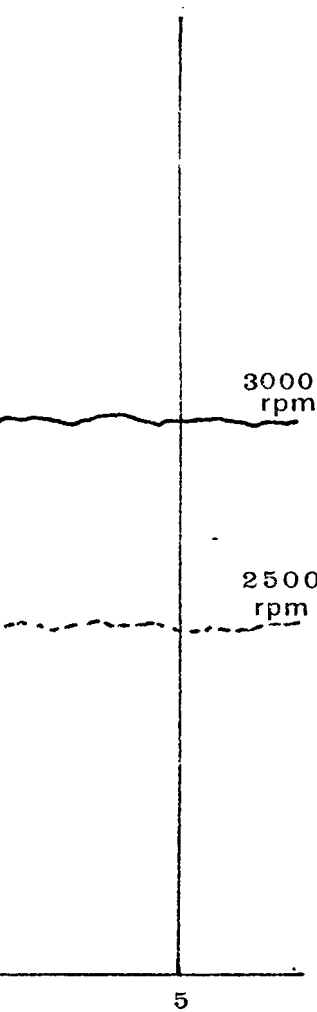

3000
rpm
2500
rpm

FIG. (40)

STABILITY OF CURRENT CONSUMPTION BY THE FERMENTERS AT 3000 RPM AND A CONSTANT VOLUME OF 5 LITRES

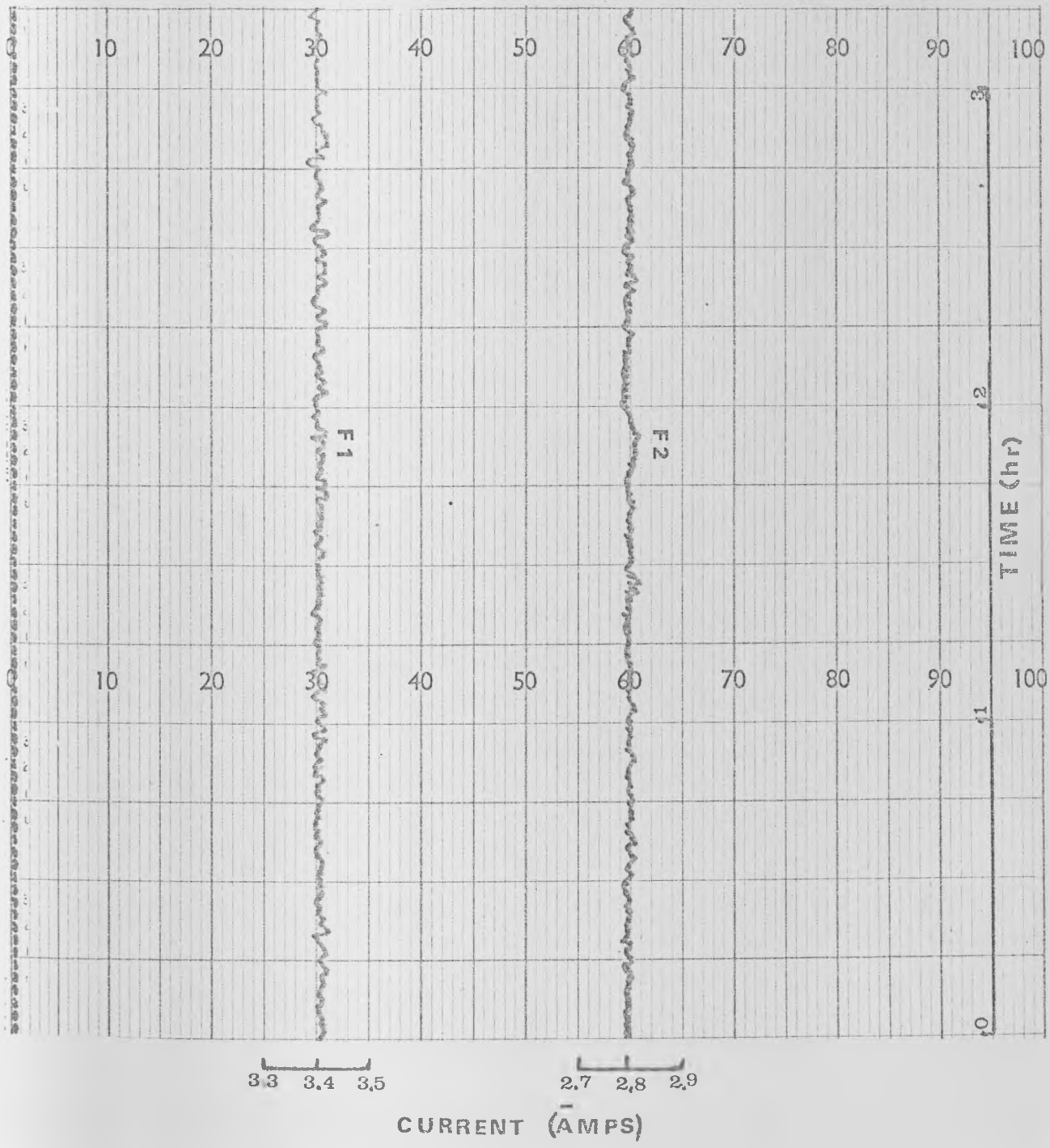


FIG. (41)

CONTROL OF FEMENTER VOLUME OVER A 120 hr.PERIOD

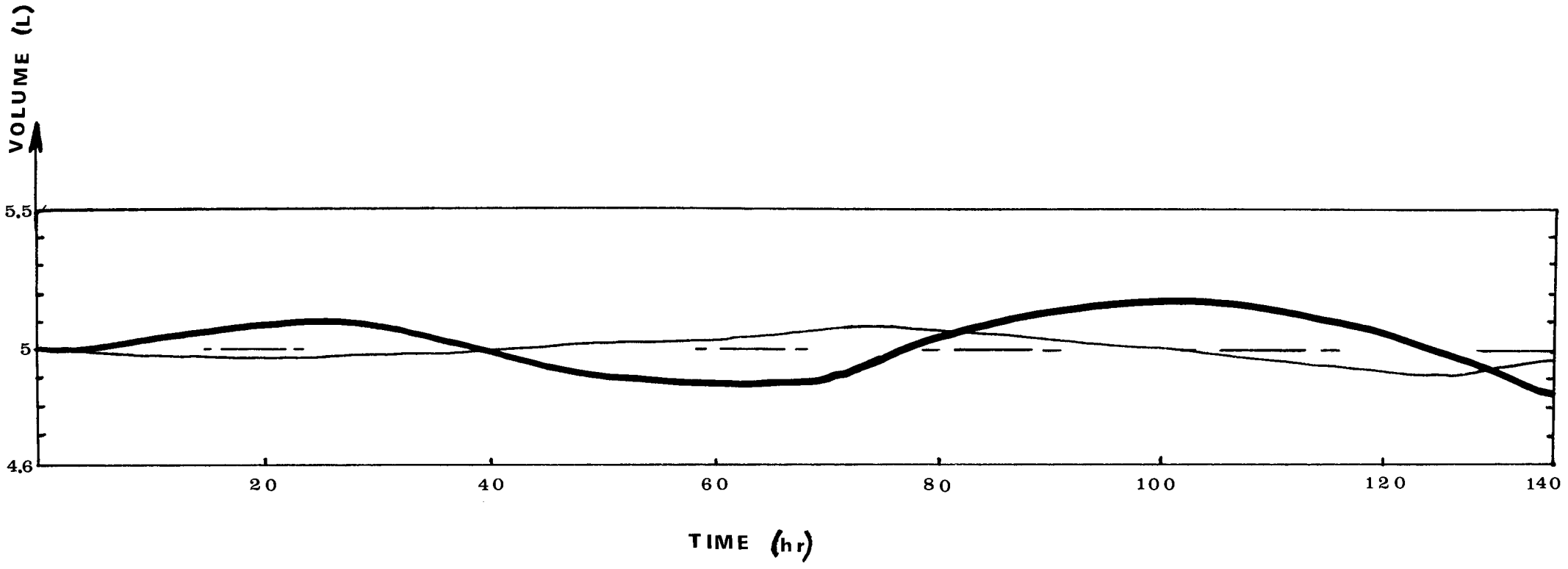

VOLUME (L)
5.5
5
4.6
20
40
60
80
100
120
140
TIME (hr)
F 1
F 2

FIG. (43)

CONTROL OF O_2 CONCENTRATION IN THE CIRCULATING GAS MIXTURE

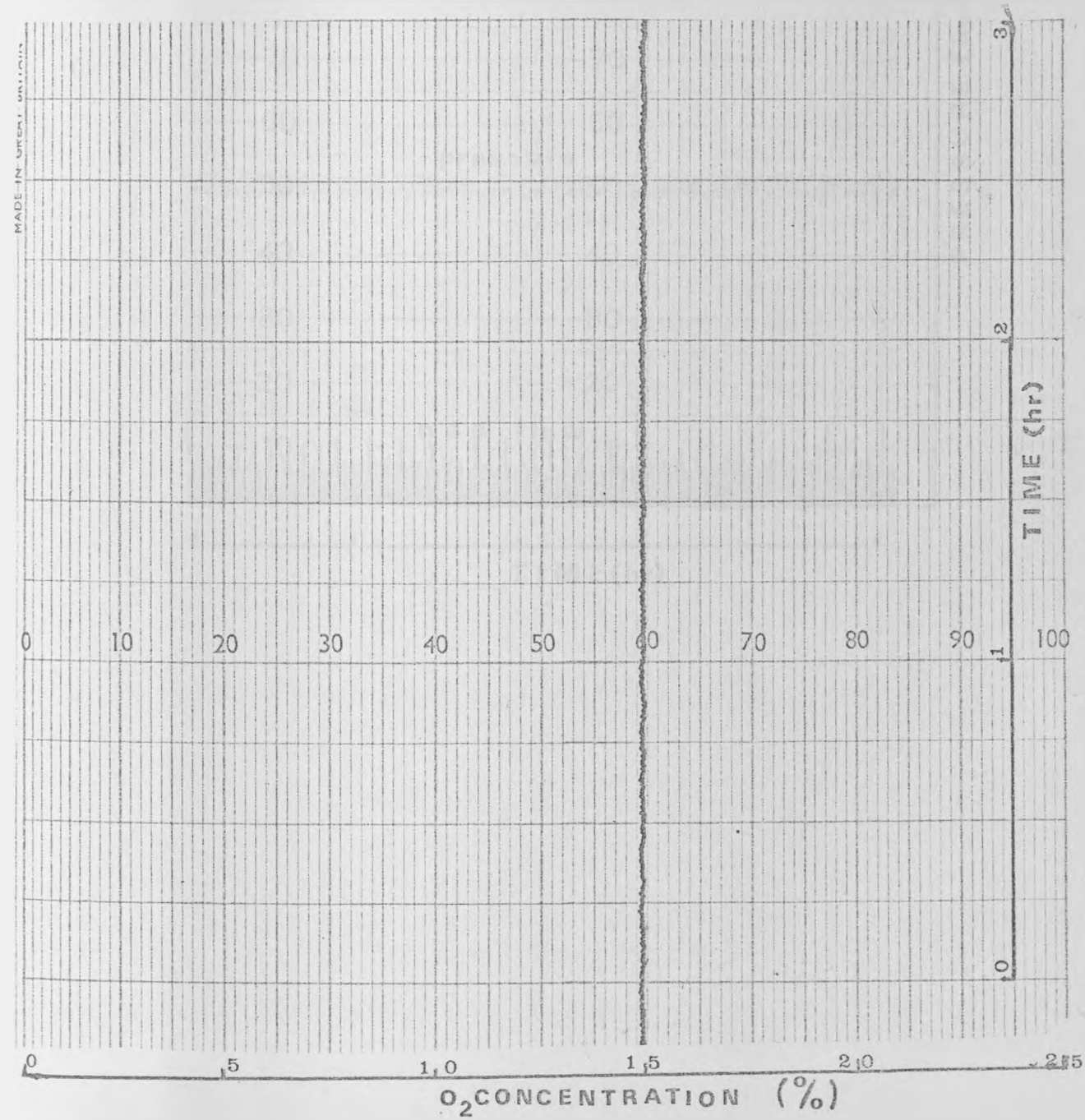


Fig. (44)

CONTROL OF PRESSURE AFTER DECONTAMINATION OF CONTROLLER (6)

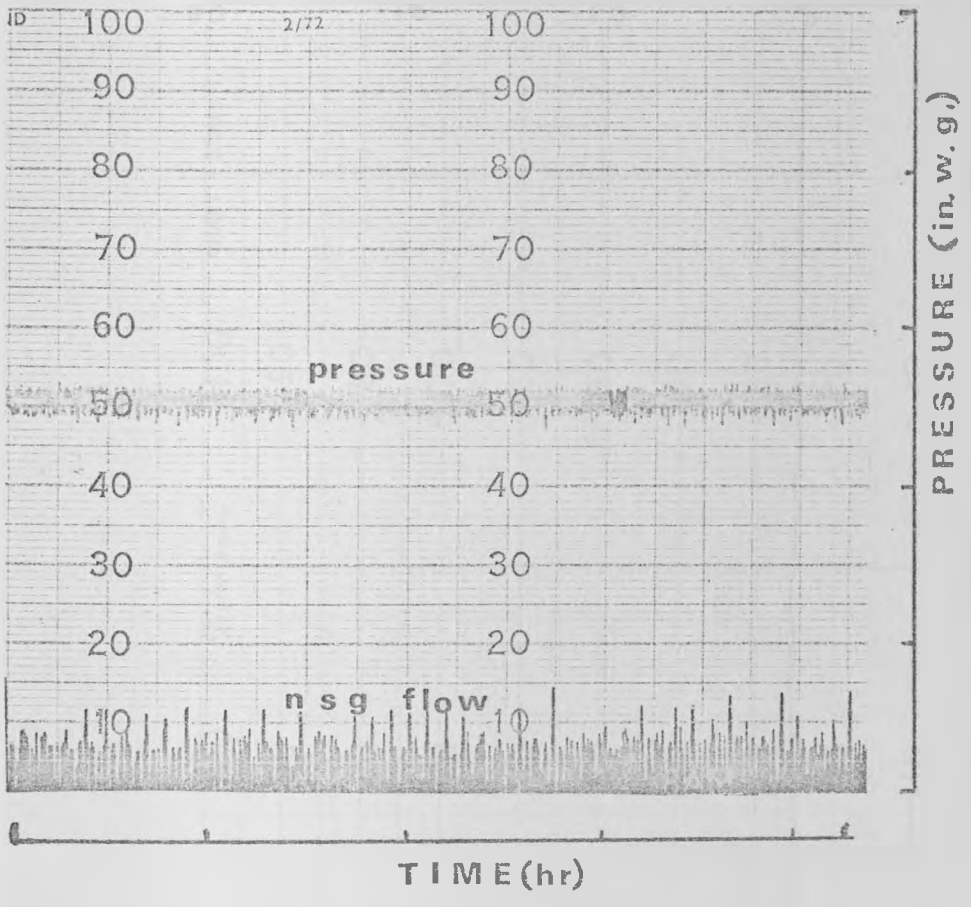


FIG.(45)

CONTROL OF ETHANE CONCENTRATION IN THE CIRCULATORY GAS MIXTURE

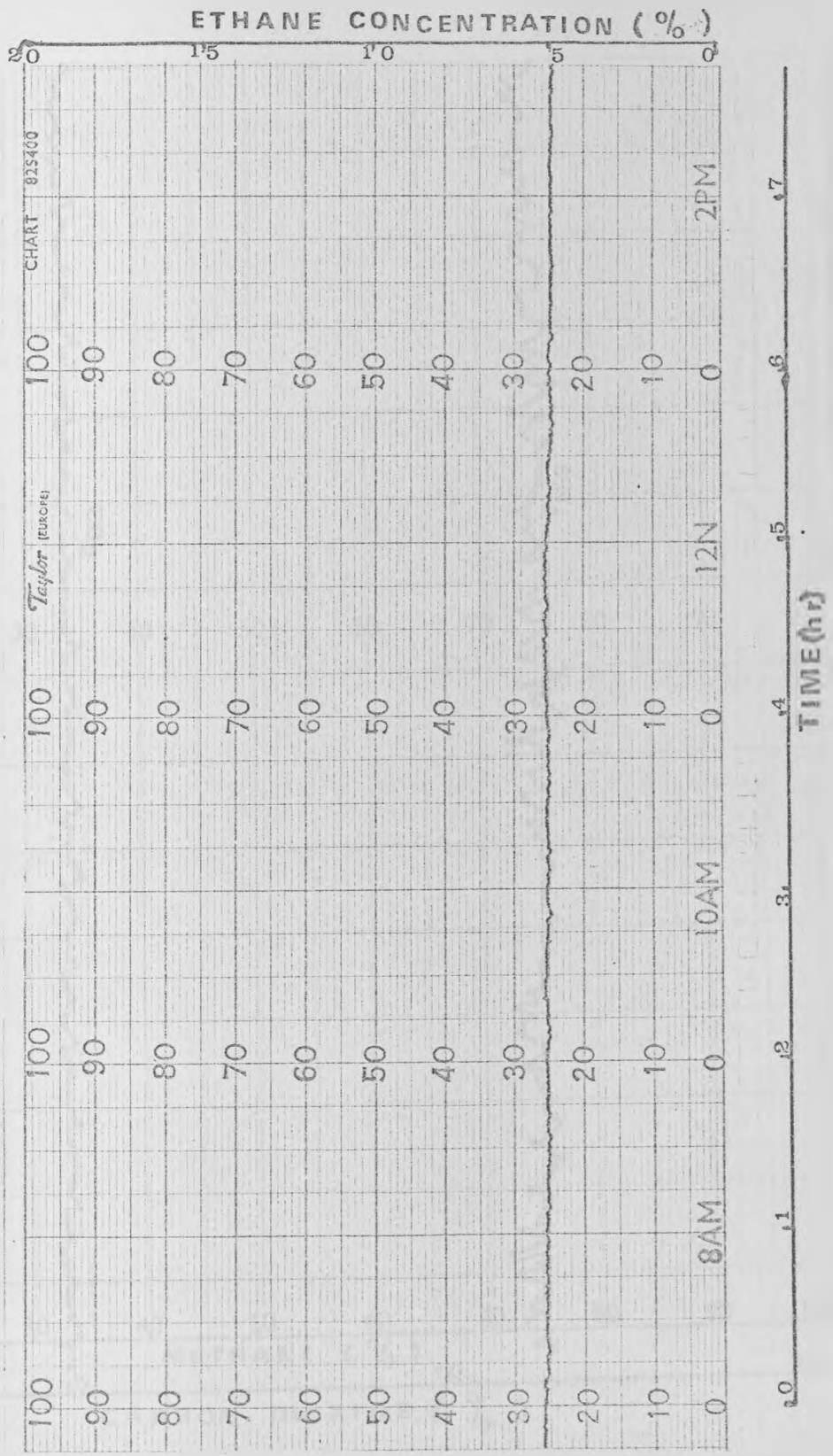


FIG.(46)

CONTROL OF CO_2 AND CH_4 CONCENTRATION IN THE CIRCULATING GAS MIXTURE

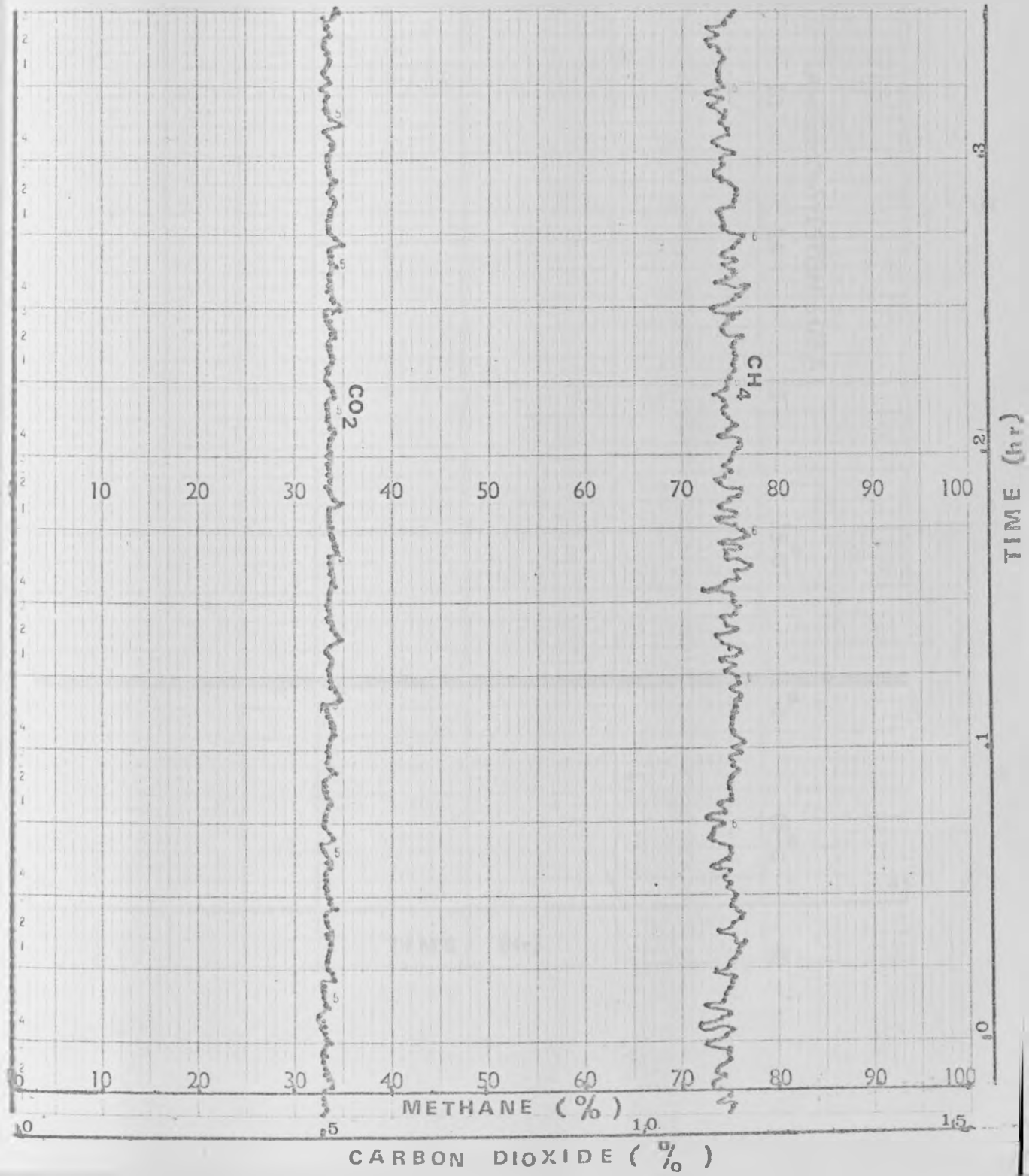
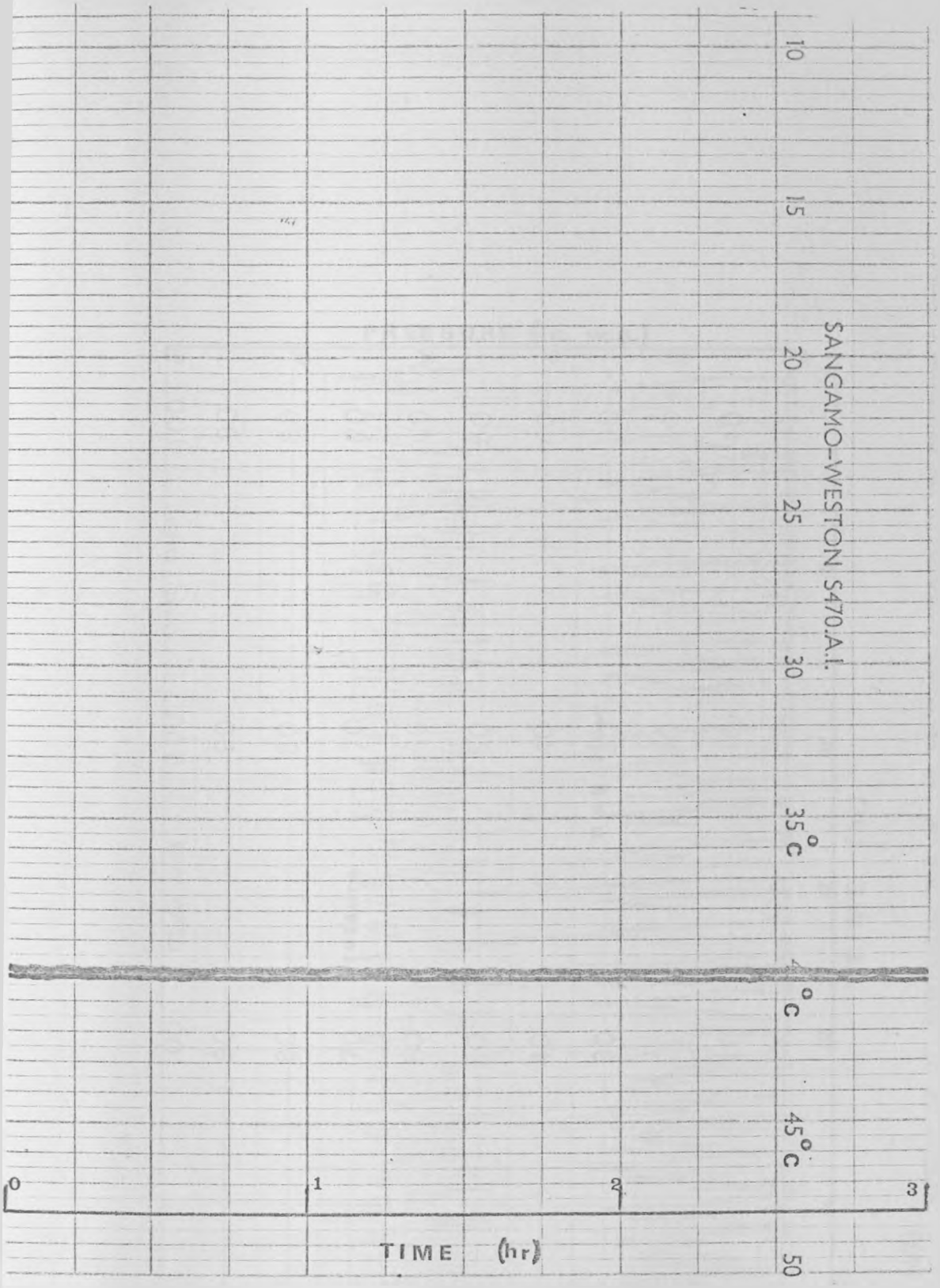


FIG. (48)

FERMENTER TEMPERATURE CONTROL AT 3000 RPM



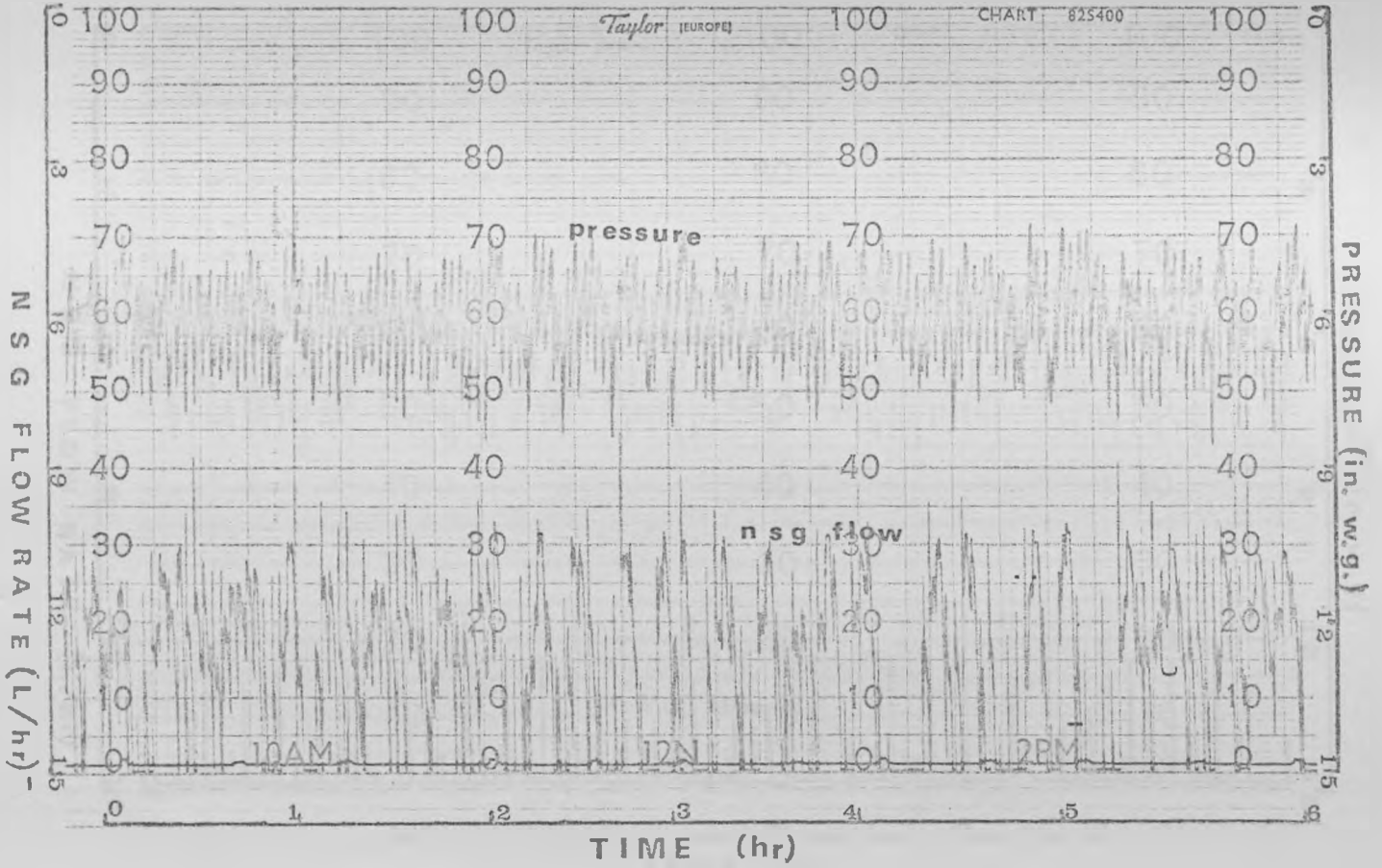
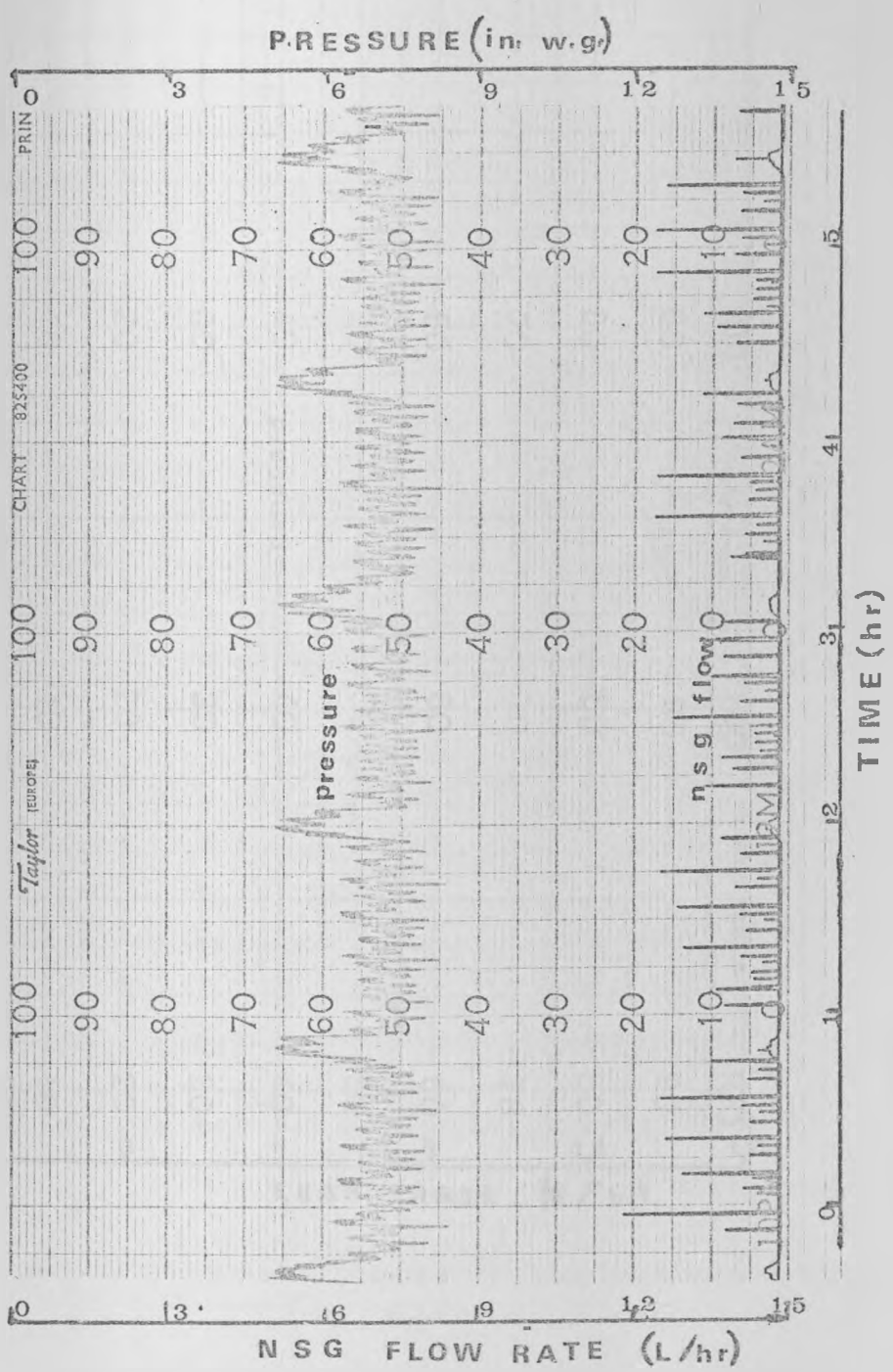


Fig. (49) EFFECT OF OIL CONTAMINATION OF THE PRESSURE CONTROLLER ON PRESSURE CONTROL

FIG.(50)

THE EFFECT OF OIL CONTAMINATION OF THE PNEUMATIC CONTROL VALVE ON PRESSURE CONTROL



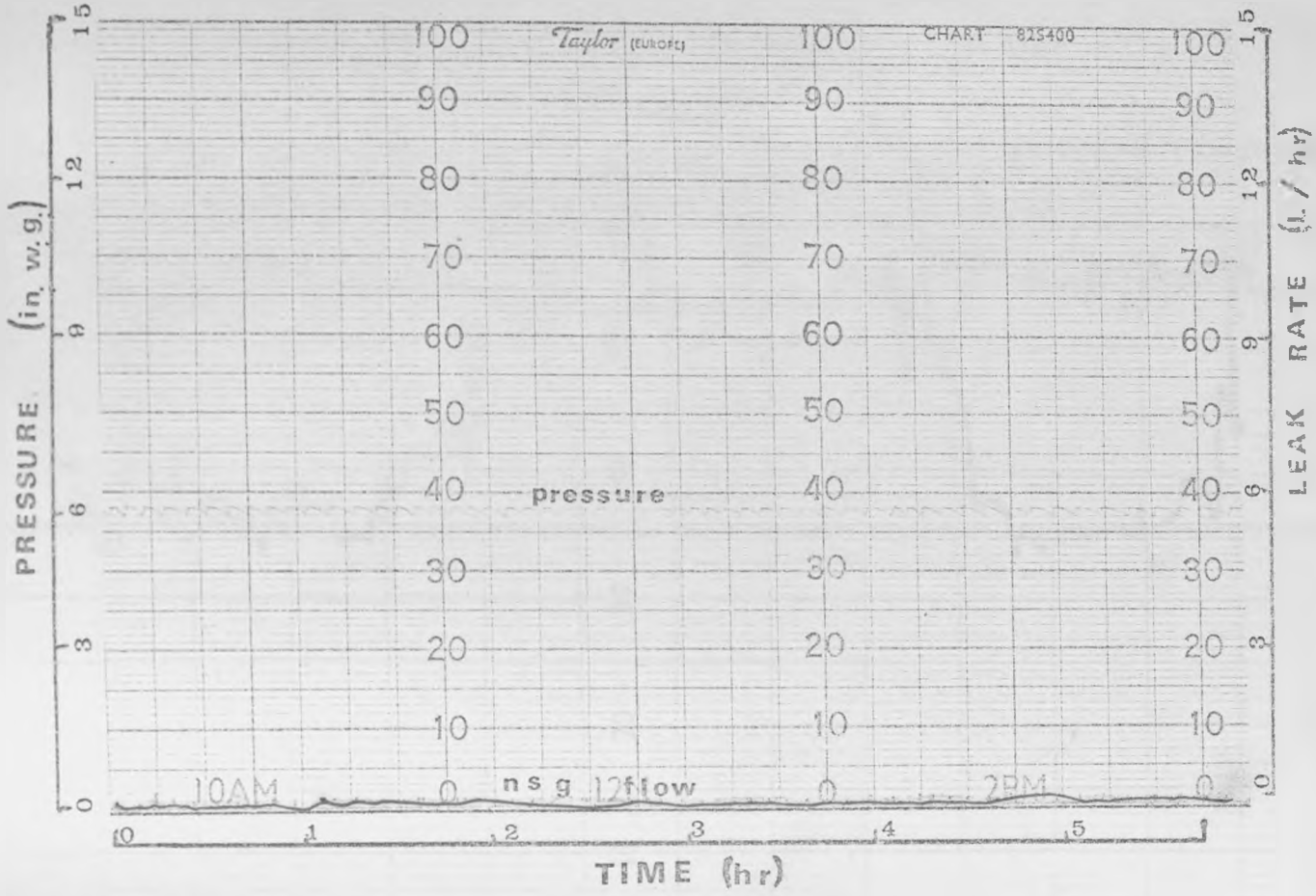


FIG. (51)

THE RATE OF GAS LEAK FROM THE SYSTEM AT THE START OF THE COMMISSIONING RUN

FIG.(52)

FERMENTER VOLUME CONTROL AT 3000 RPM BEFORE REPLACEMENT OF THE HYDRAULIC VARIATORS

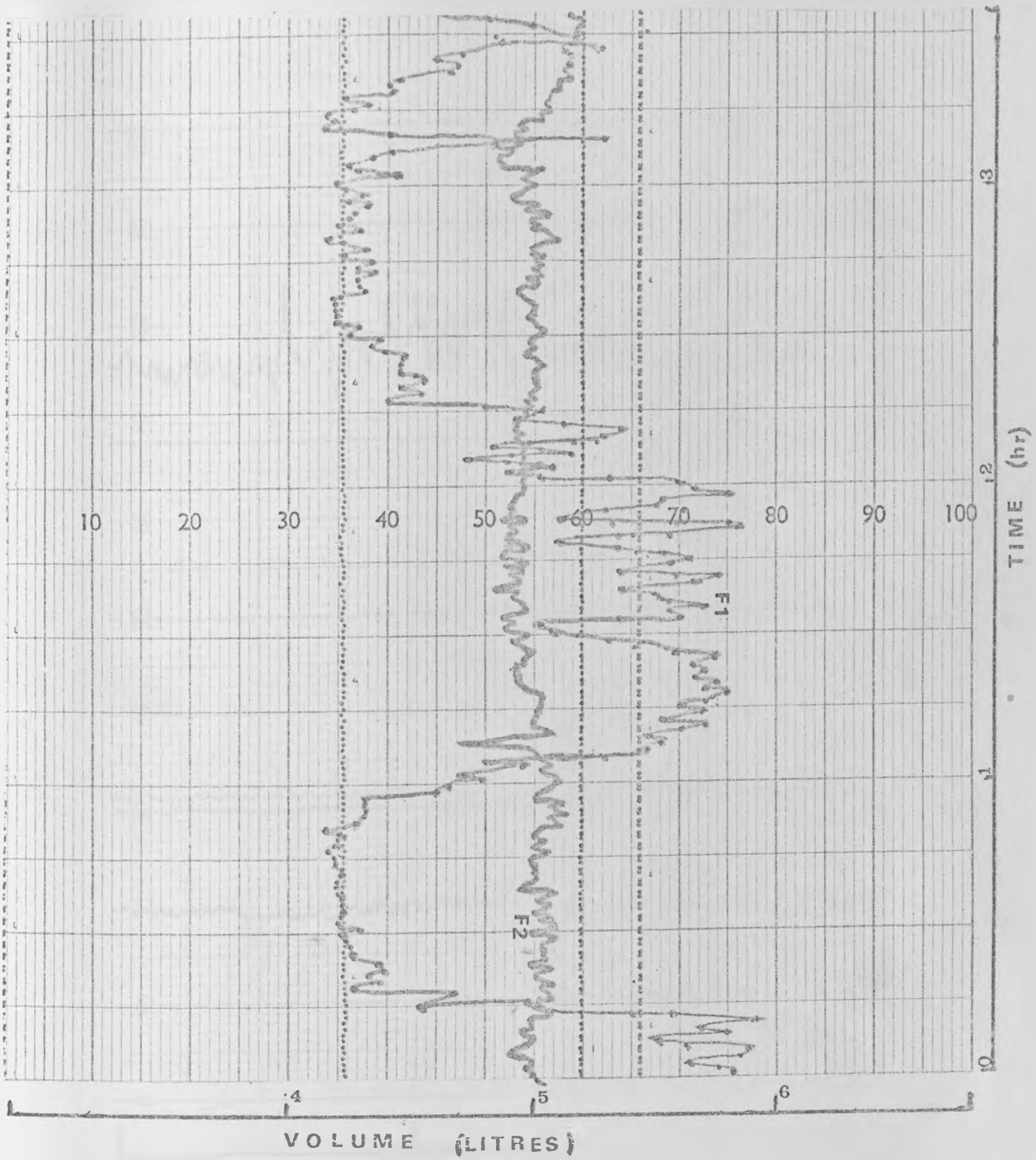


FIG.(53)

FERMENTER VOLUME CONTROL AT 3000 RPM AFTER REPLACEMENT OF THE HYDRAULIC VARIATORS

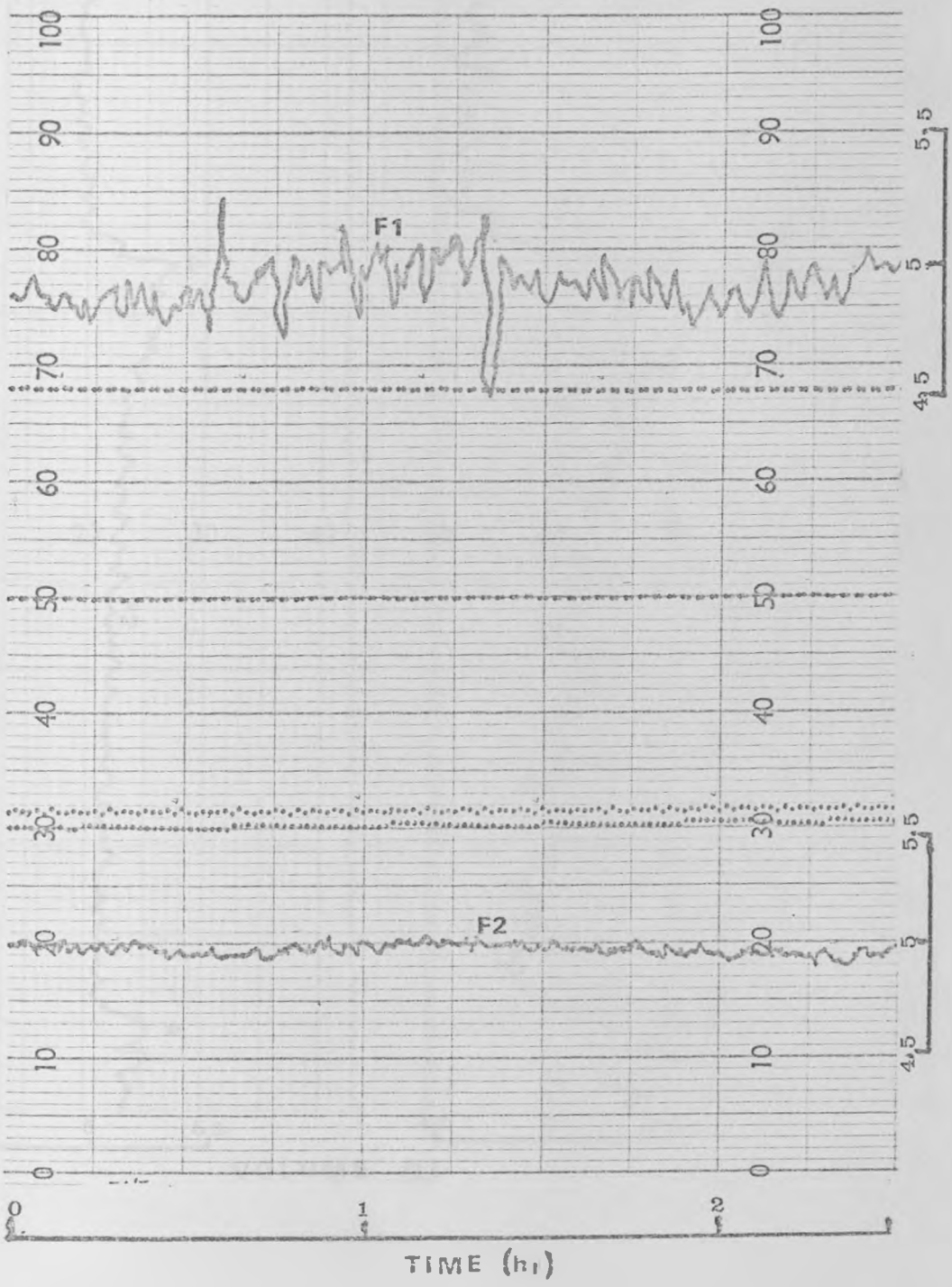


FIG.(54)

THE EFFECT OF VOLTAGE SURGES ON FERMENTER VOLUME CONTROL AT 3000 R.P.M.

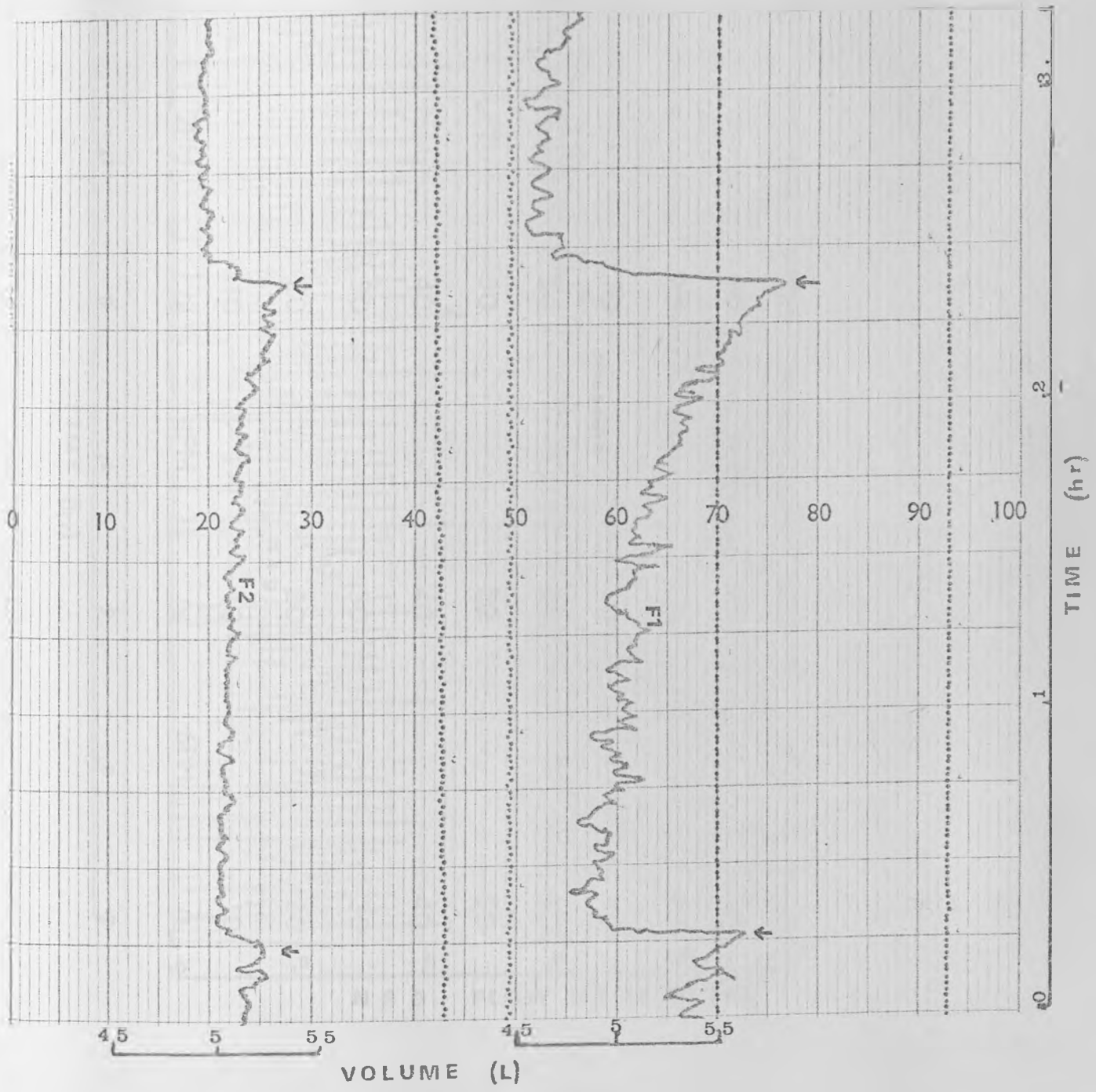
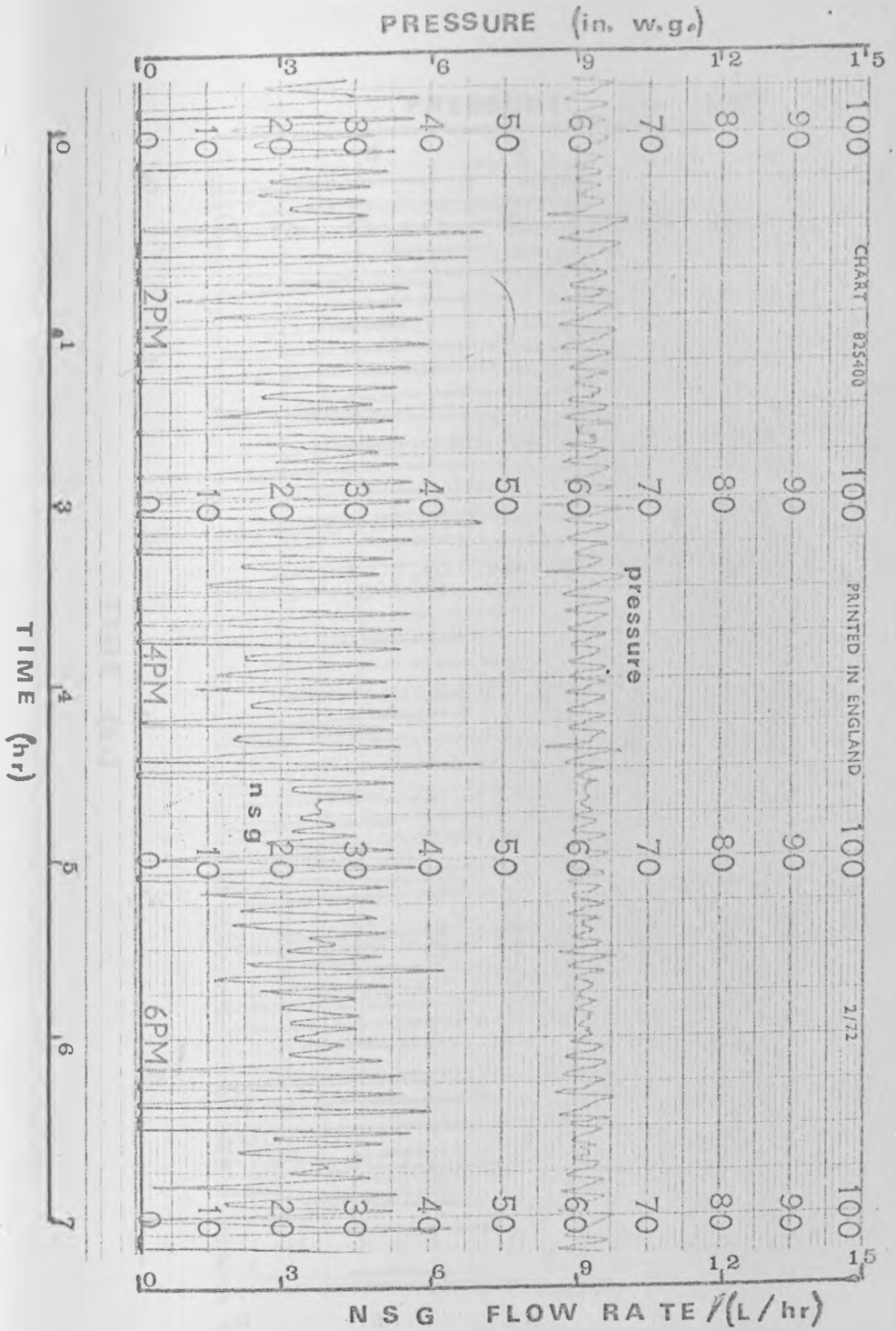


Fig. (55)

THE EFFECT OF HIGH RESET RATE ON THE CONTROL OF SYSTEM PRESSURE



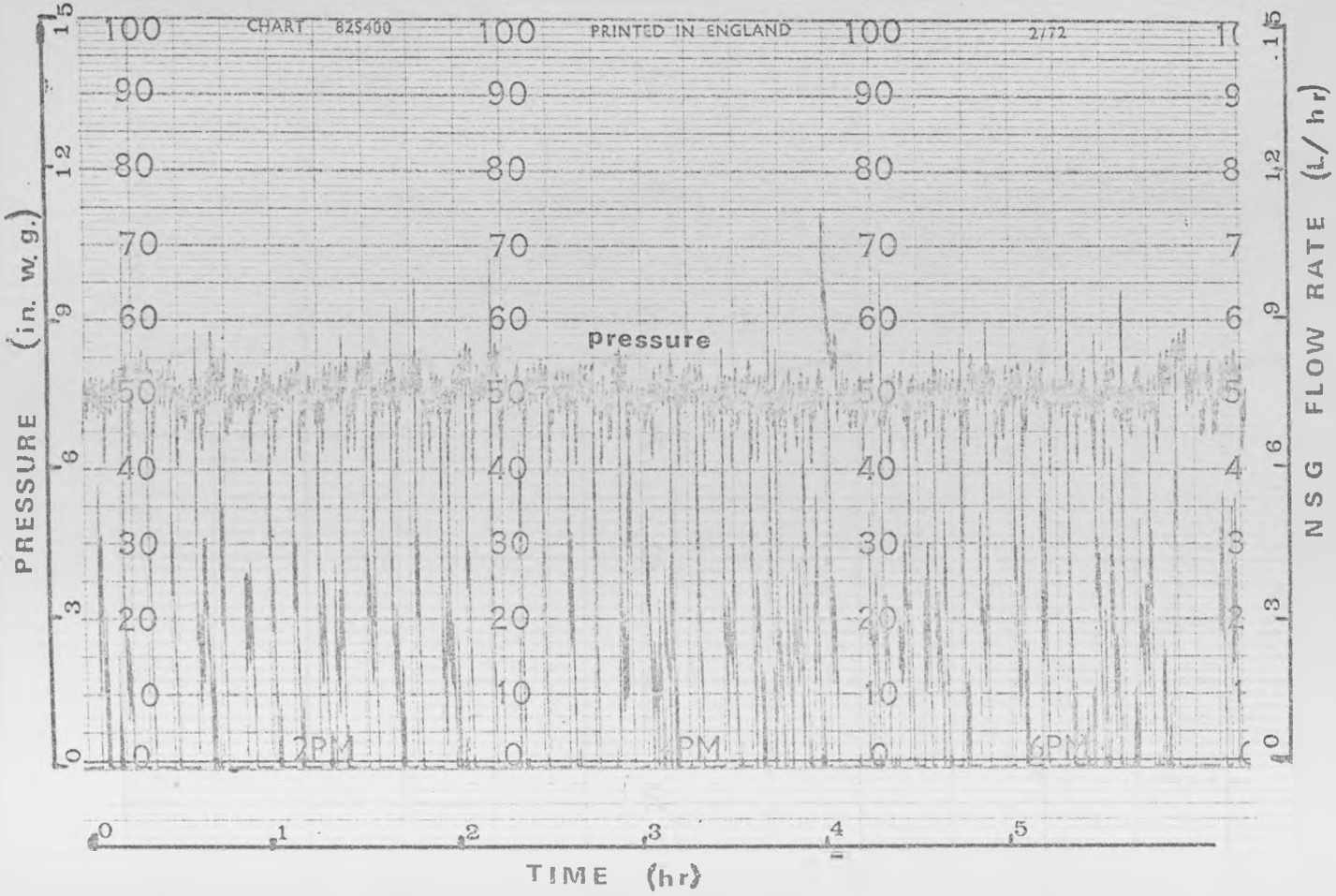
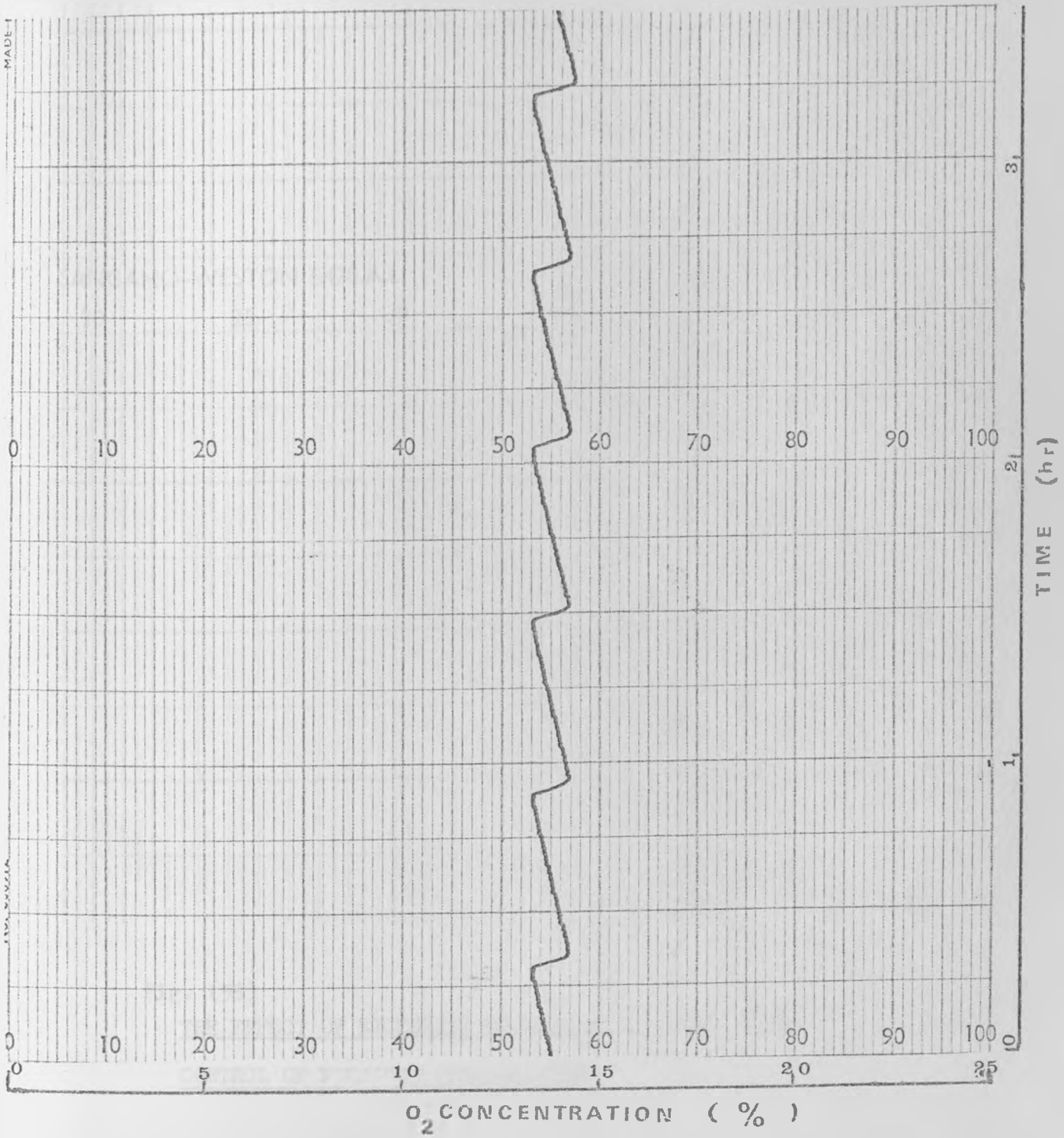


Fig. (56) THE EFFECT OF LOW PROPORTIONAL BAND WIDTH ON THE CONTROL OF SYSTEM PRESSURE

FIG. (57)

THE EFFECT OF LOW PROPORTIONAL BAND SETTING ON THE CONTROL OF OXYGEN CONCENTRATION IN THE CIRCULATING GAS MIXTURE



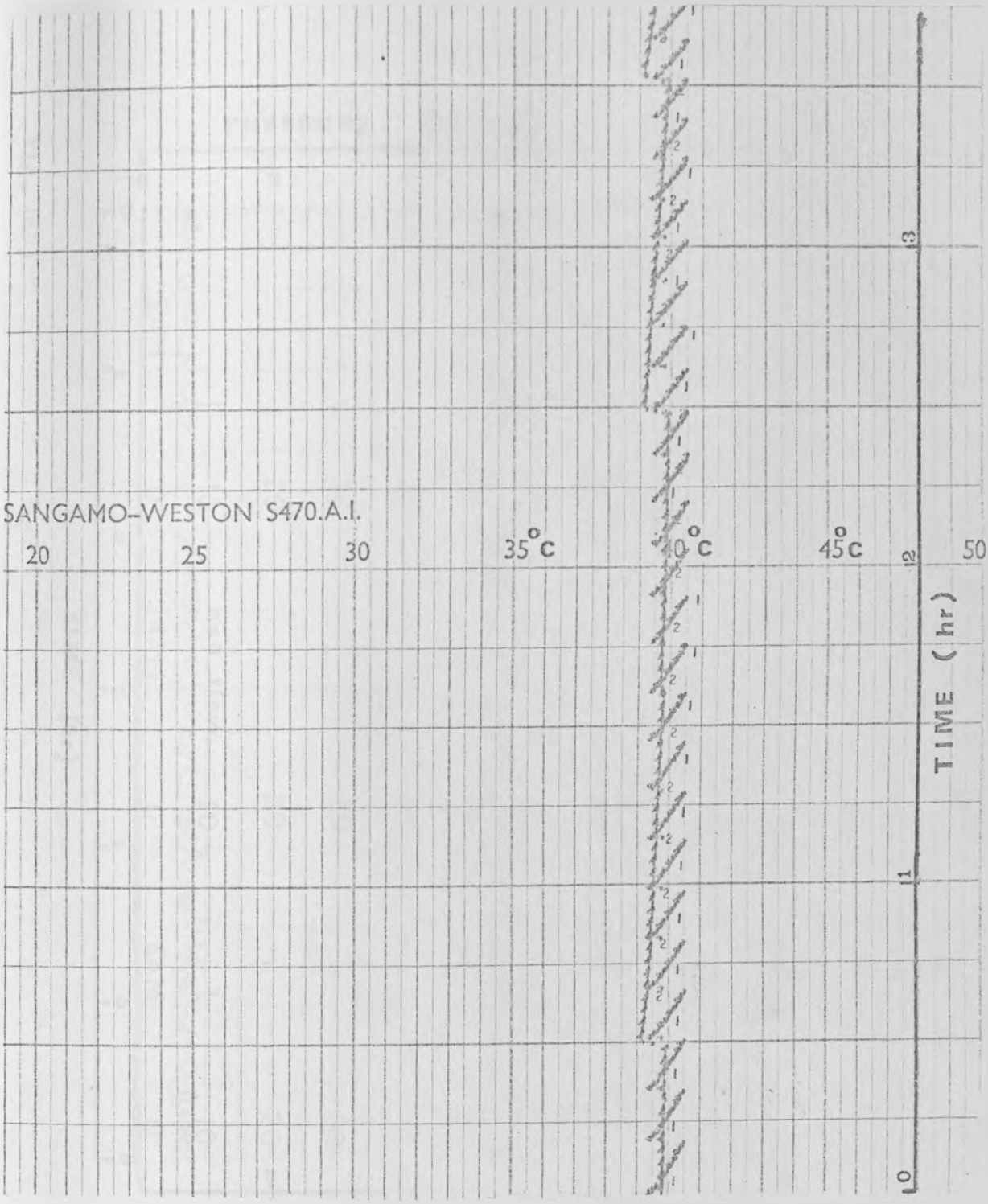


Fig. (58)

THE EFFECT OF EXCESSIVE COOLING WATER FLOW ON THE CONTROL OF [illegible] TEMPERATURE

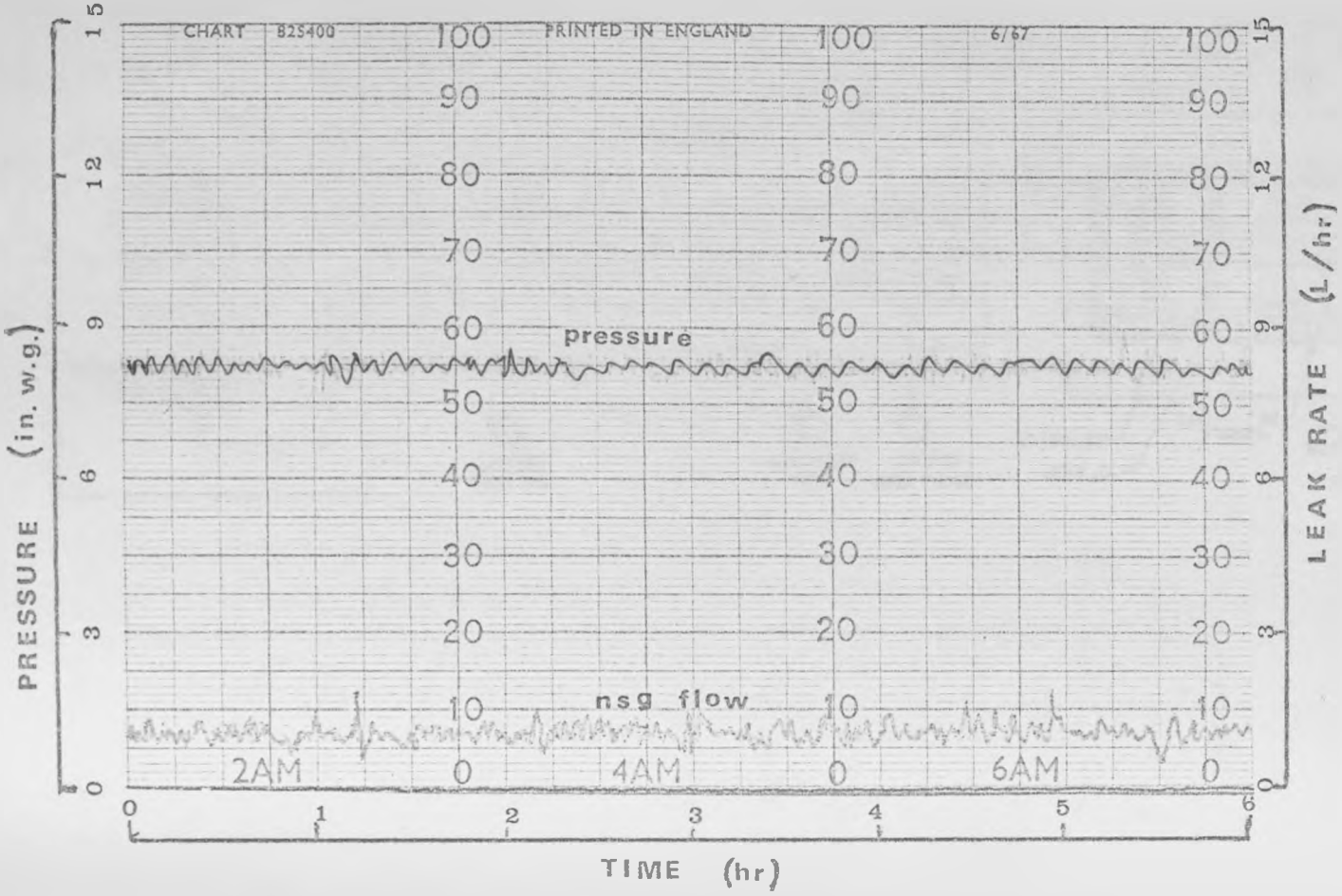


Fig. (60)

RATE OF GAS LEAK FROM THE SYSTEM AFTER 6 MONTHS OPERATION

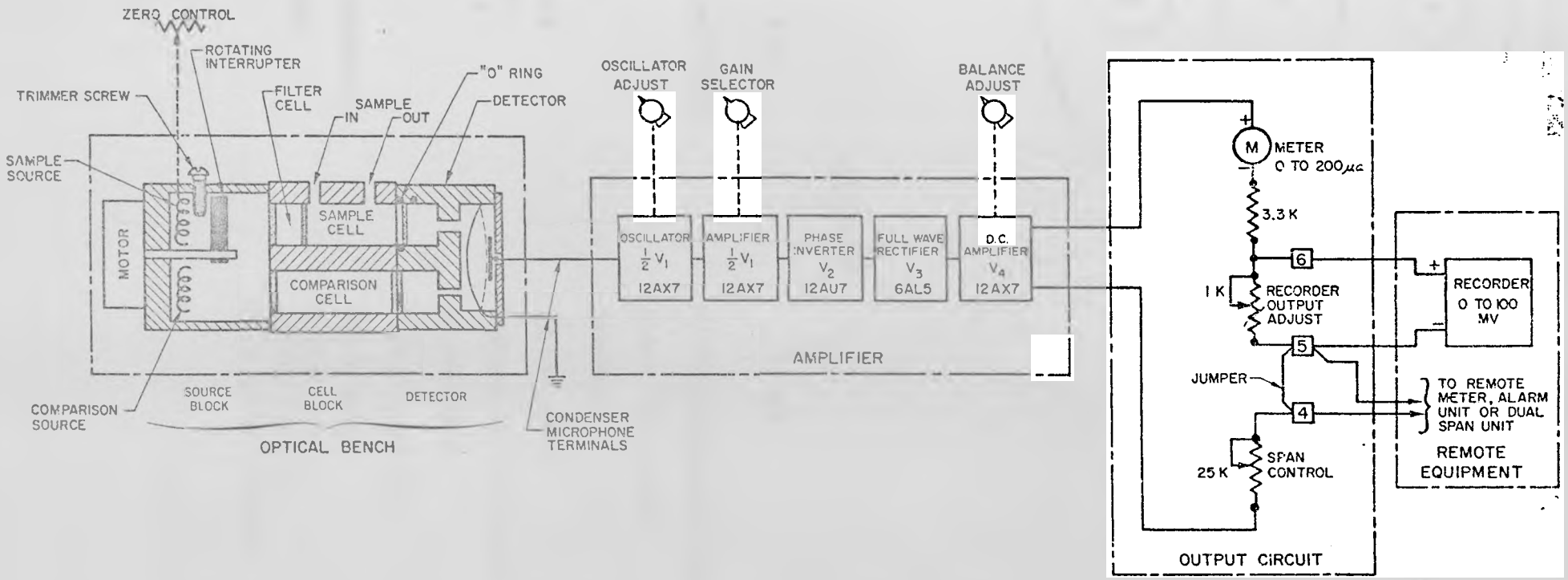


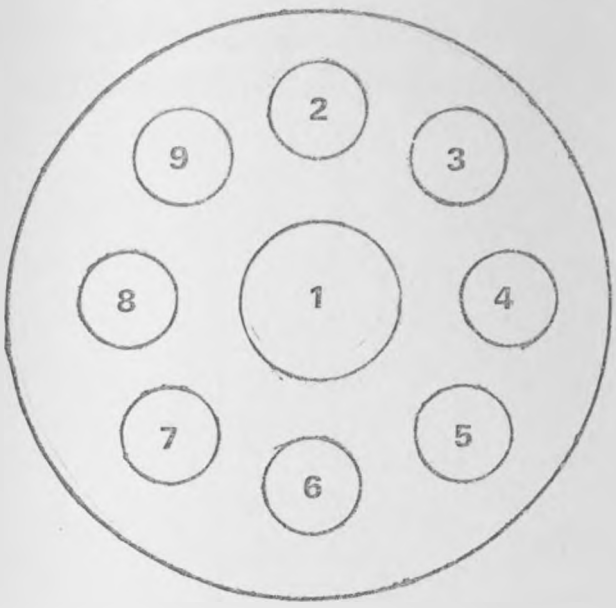
FIG 61 SCHEMATIC DIAGRAM

M-S-A® LIRA® INFRARED ANALYZER

FIG 61

Fig. (63).

PLAN OF THE FERMENTER HEADPLATES



PORT NO.	FUNCTION	
KEY	F1	F2
1	Condenser/Outlet Filter	Condenser/Outlet Filter
2	Sampler	Glass Thermometer
3	O_2 Electrode	Sampler
4	Culture Effluent	pH electrode
5	Alkali Delivery Medium Delivery	Resistance Thermometer
6	Inlet Filter	O_2 electrode
7	pH electrode	Culture effluent
8	Resistance Thermometer	Inlet Filter
9	Glass Thermometer	Alkali Delivery Medium Delivery Multistage function

Fig. 64.

THE CHANGE IN OPTICAL DENSITY AND DRY WEIGHT
OF THE CULTURE OVER 36 HOURS IN BATCH CONDITIONS

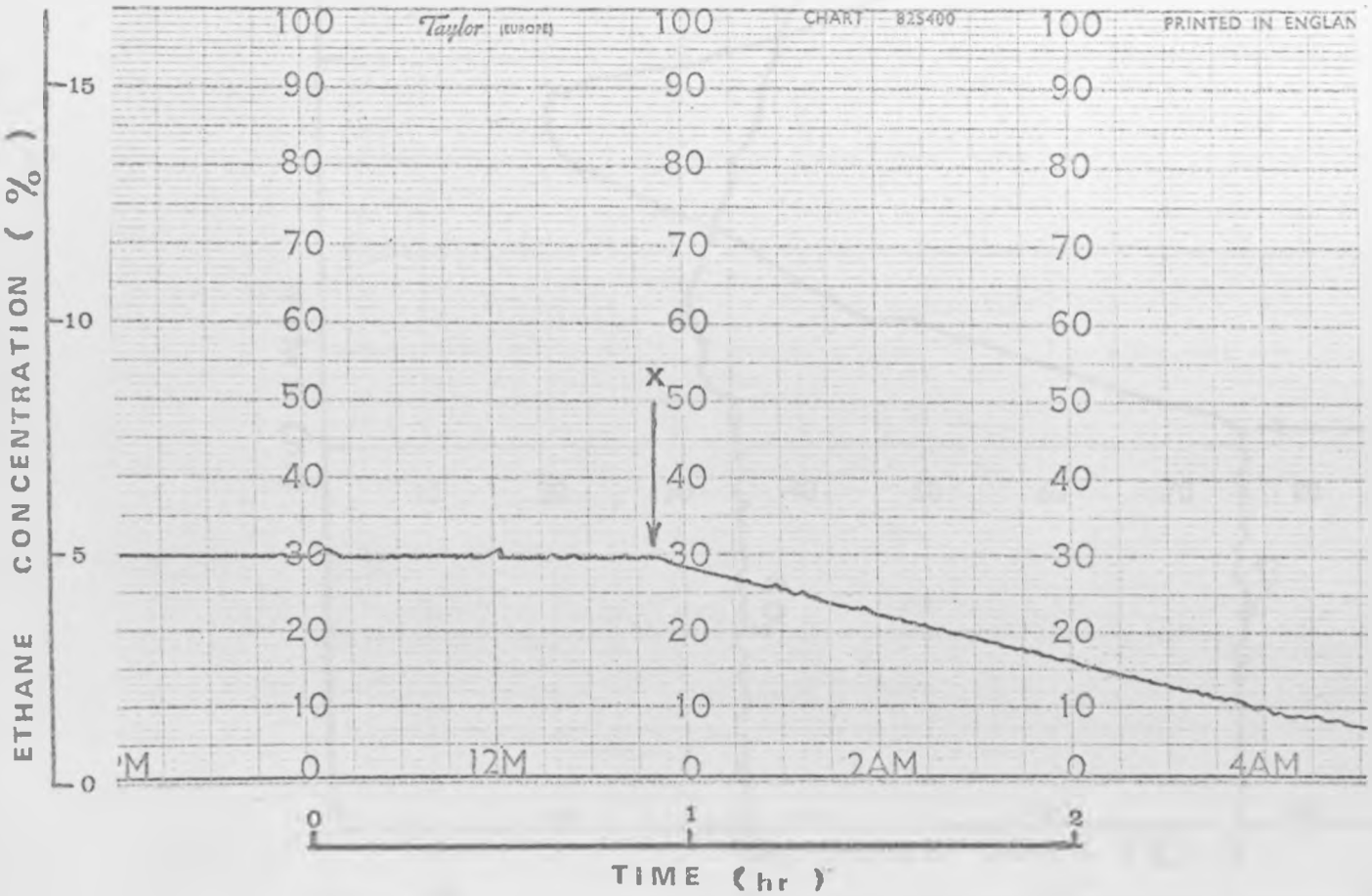


Fig. (65) CHANGE IN ETHANE CONCENTRATION DUE TO GROWTH OF THE CULTURE

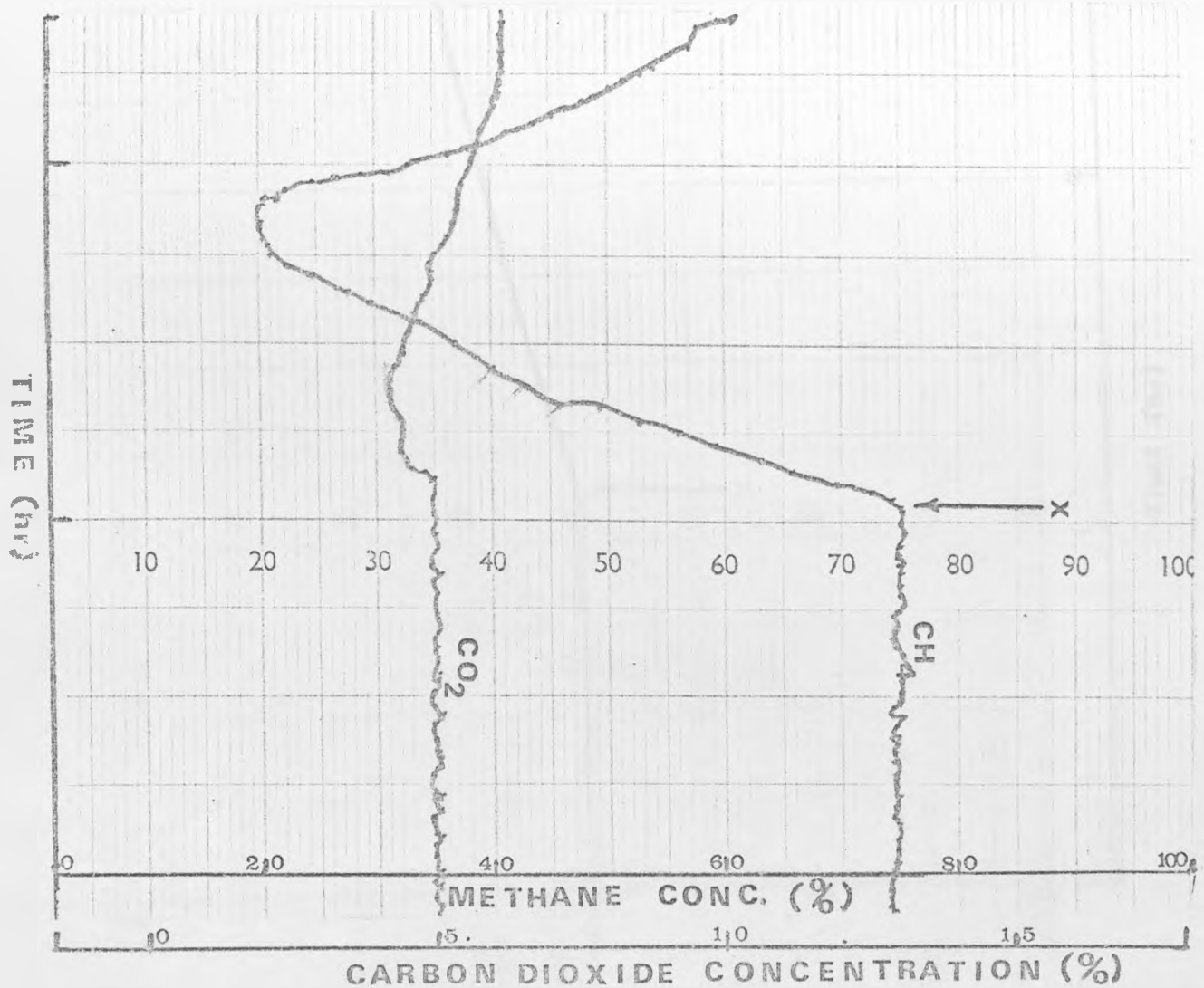


FIG.(66) CHANGE IN CH_4 AND CO_2 CONCENTRATION DUE TO GROWTH OF NSG OXIDISER

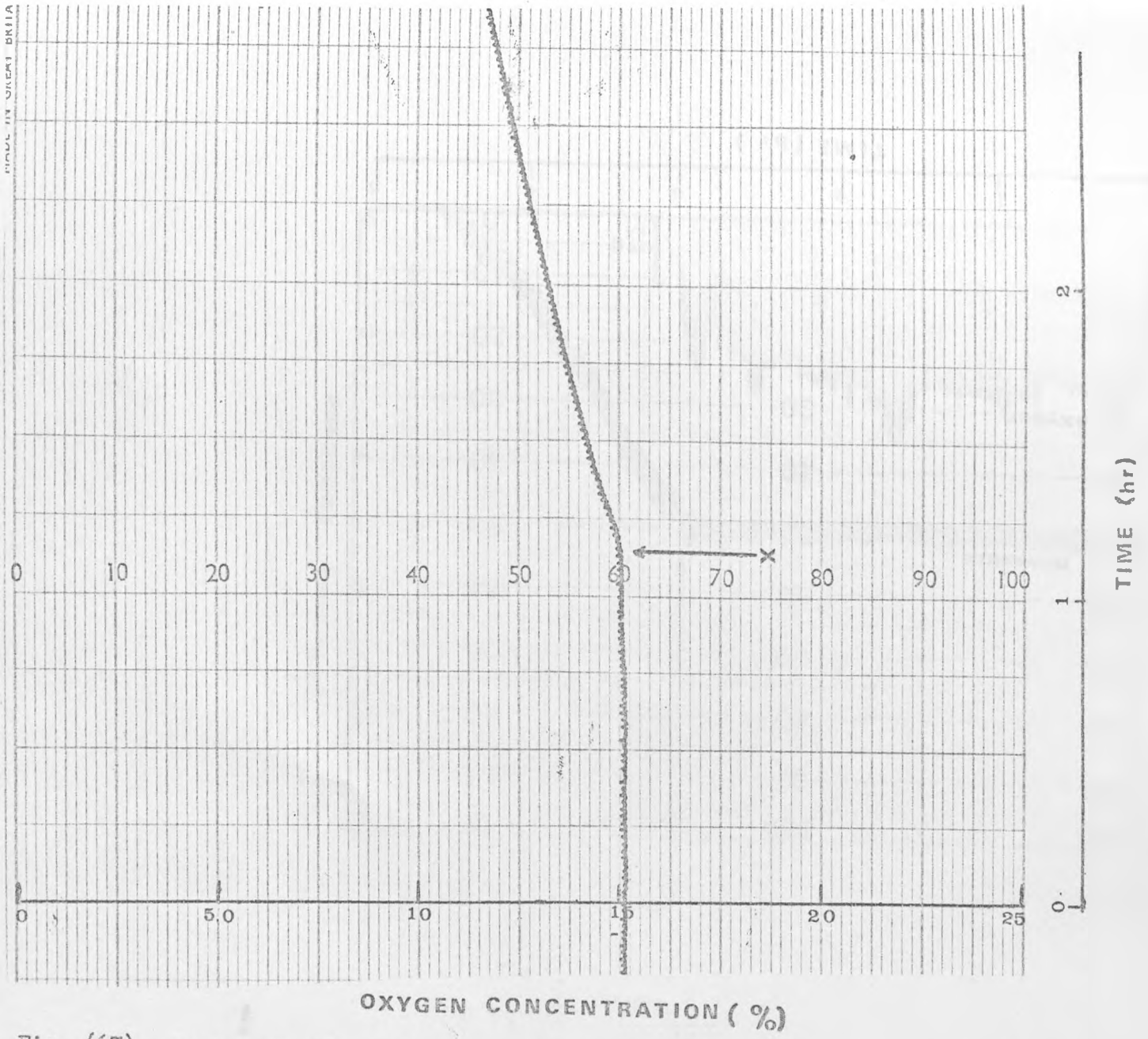


Fig. (67) CHANGE IN OXYGEN CONCENTRATION DUE TO CONSUMPTION BY THE CULTURE

Fig. (68)

CHANGE IN SYSTEM PRESSURE DUE TO GROWTH OF THE CULTURE ON NSG AND OXYGEN

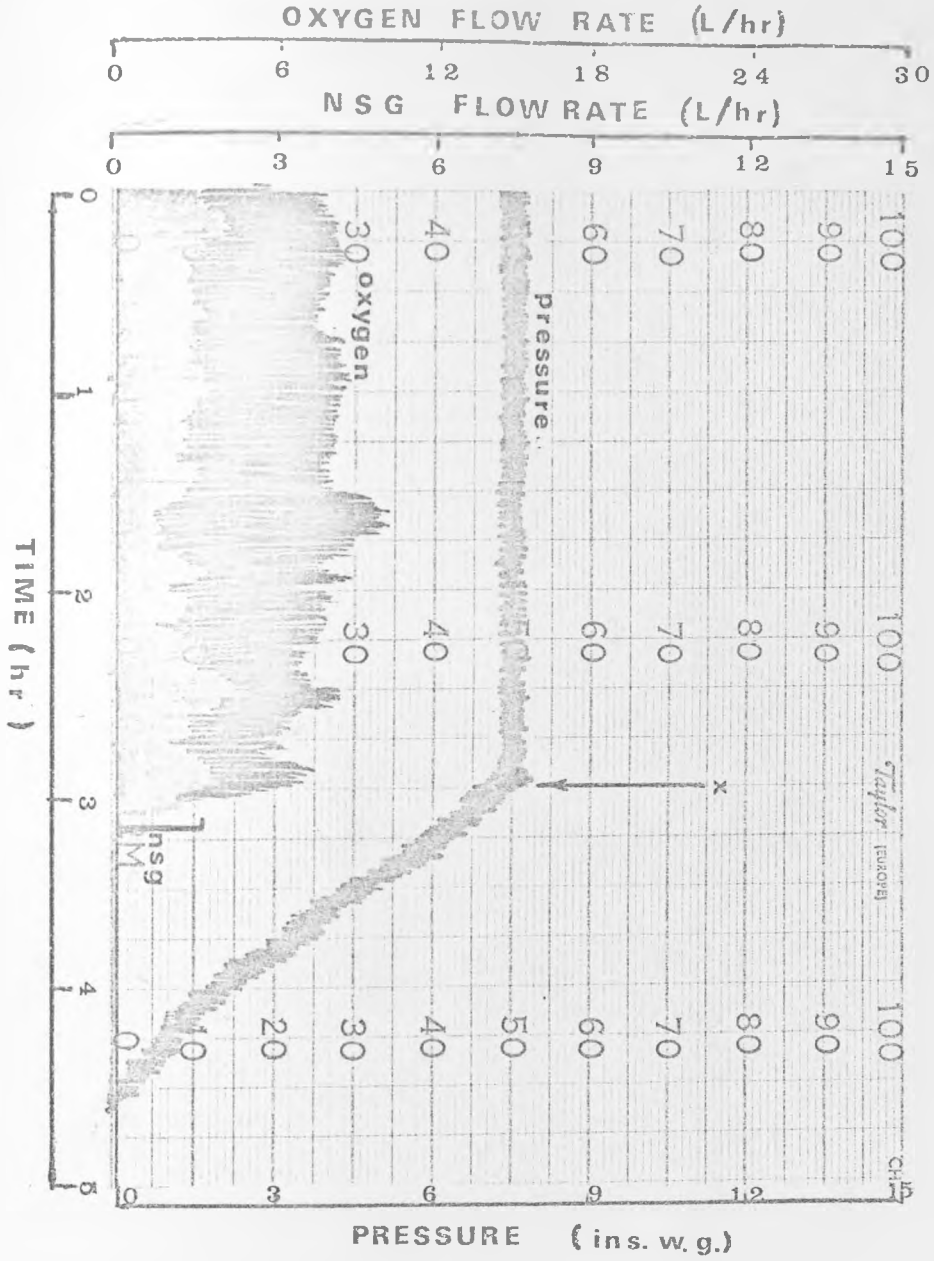
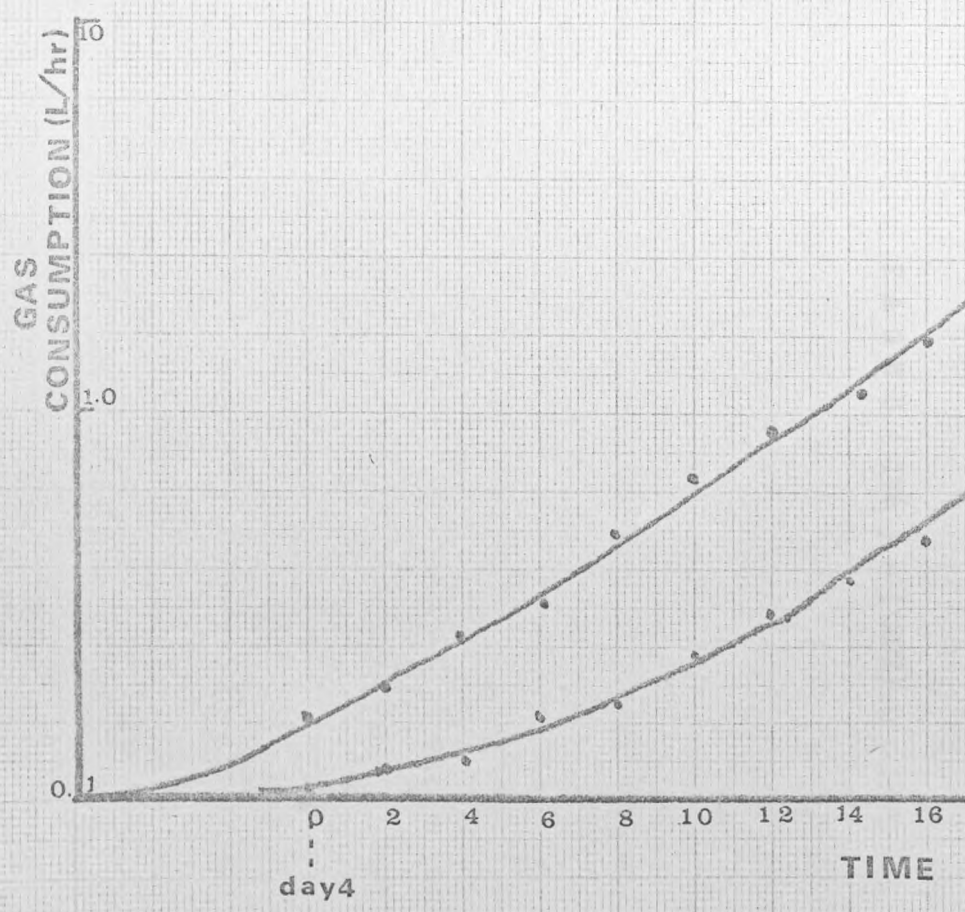
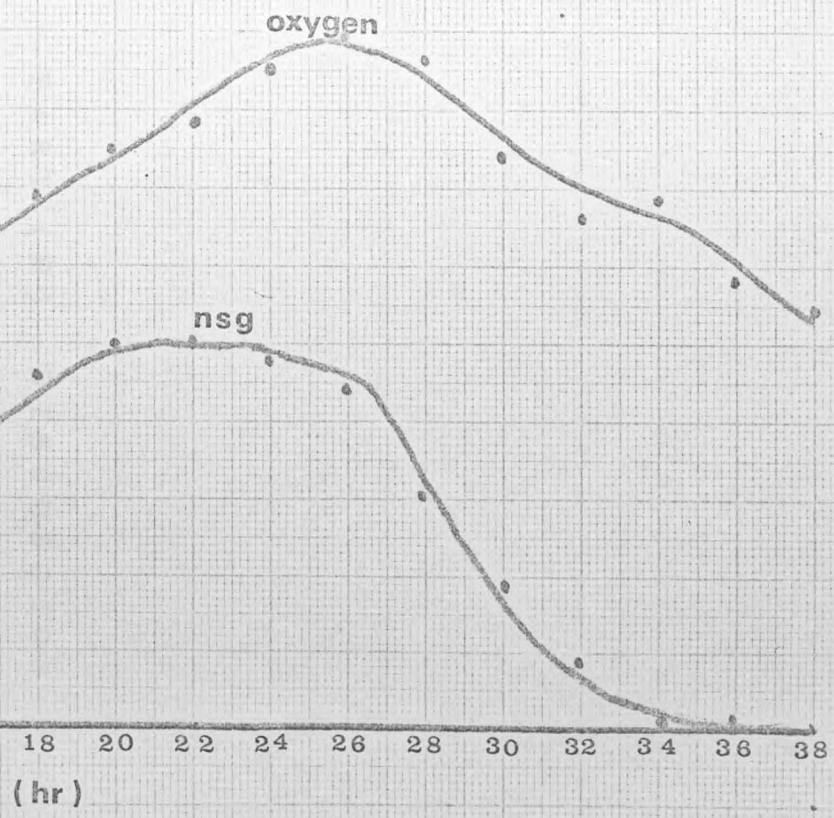


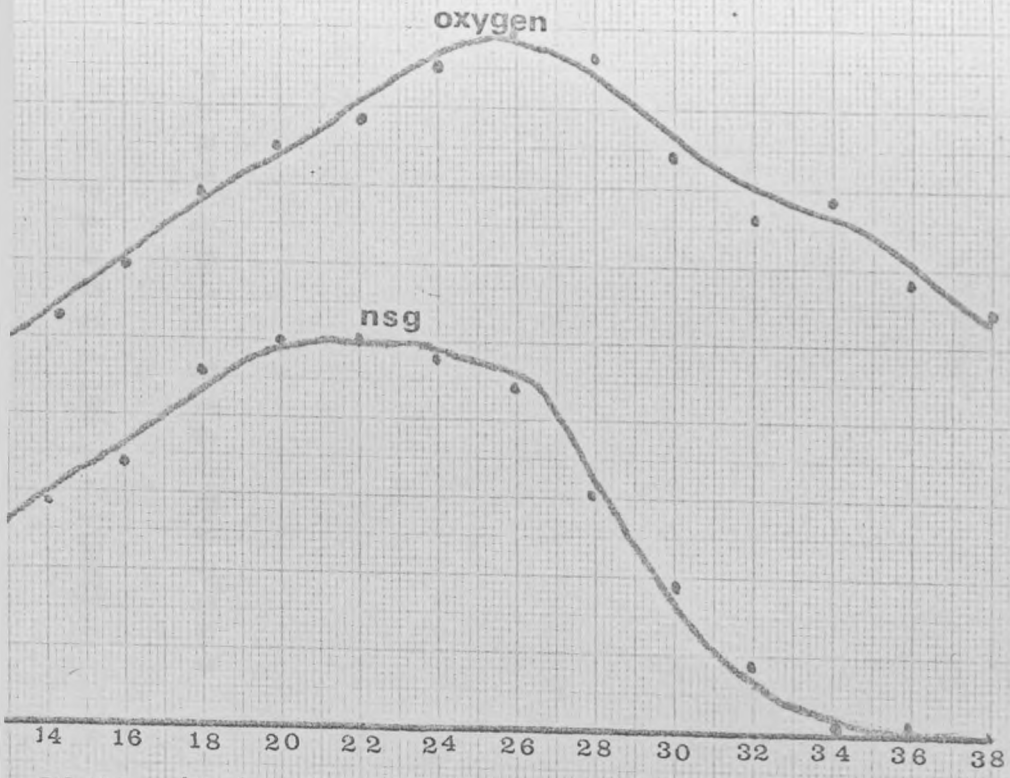
Fig. 69:

CONSUMPTION OF NORTH SEA GAS AND OXYGEN
BY THE CULTURE DURING BATCH GROWTH


GAS
CONSUMPTION (L/hr)
10
1.0
0.1
0
2
4
6
8
10
12
14
16
TIME
day4


oxygen
nsg
18
20
22
24
26
28
30
32
34
36
38
(hr)

GAS
CONSUMPTION (L/hr)
10
1.0
0.1
0
2
4
6
8
10
12
day4


oxygen
nsg
14
16
18
20
22
24
26
28
30
32
34
36
38
TIME (hr)

P A R T (F) D I S C U S S I O N

P A G E S (176) - (202)

(1) THE STATUS OF SINGLE CELL PROTEIN (BIOMASS): A REASSESSMENT:

During the last three years a series of political and economic upheavals have shifted the world situation in terms of the distribution of resources, and for this reason a reassessment of the status of biomass as a potential source of food or feed is called for.

The need for an additional or supplementary source of protein remains as crucial as ever. There has been no new advance in food production on the scale and intensity of the 'Green Revolution'. It remains true that world population figures are still increasing and therefore the per capita production of food has gone down rather than up. (See Table (1) F.A.O; 1967).

What has changed is that the distribution of need has enlarged to encompass the industrialised societies in addition to the developing areas. This is because the dependency of the Western world on the natural resources of the Third world has been recognised in a general sense. Thus it is well known that Britain and most of Europe imports approximately half of its food and, most importantly, the materials to produce the remaining half. As developing countries become more industrialised their need for the same raw materials goes up. The most important resource is, of course, oil, and the current political situation in the Middle East has highlighted the extreme reliance which countries such as Holland place on oil from developing areas. It is a moot point as to which areas of the world are the richest. The West has the technology and expertise but little fuel to sustain it whilst the third world possesses the majority of those resources essential to industrial life.

It has been made clear that not only is the protein gap a problem

of/

of the developing world but also a feature in the continued existence of industrialised society. Agriculture in the West is energy consuming to an extreme degree. It has been argued by Goldsmith (1973) that America cannot afford to continue its agricultural policy much longer. When the cost in terms of resources, energy and environmental damage becomes widely recognised a more sane approach to food production may be adopted. Perelman (1972) estimated that American farm machinery consumes about 8 million gallons of fuel per year; this is equivalent to the energy value of the total amount of food crops consumed per year. Perelman argued further that farmers in American consume 2.5% of all electricity in The United States and that five times as much energy is used by farmers than is consumed as equivalent energy in food. This does not count the energy used to manufacture farming equipment nor that consumed in transport and storage of food. Interestingly, Perelman pointed out that Chinese wet rice agriculture produces about 53.5 BTU of energy per BTU of human energy expended so that, in effect, Chinese agriculture is 250 times more efficient than that of the United States.

It is doubtful if the world can sustain American agricultural practices for much longer but, as Goldsmith remarked, the products of American agriculture - wheat, grain and vegetable oils - are at present essential to the poorly nourished areas of the world. It is a paradox with uncomfortable implications.

It is generally known that as per capita incomes increase then so does grain consumption. With the increasing industrialisation of the developing world, a gradual increase in per capita income can be expected so that the overall world demand for grain will increase. Competition in the free market for available grain by these ever richer countries (rich/

(rich in terms of resources as well as capital) will increase the burden on industrialised countries - especially Europe. As a result of these economic and political factors it seems likely that the demand for unconventional sources of protein by the industrialised sectors of the world will be as great as, or greater than the demand from already under-nourished areas. One of the major disadvantages of many biomass production processes is that they tend to be energy consumptive. Reference to Appendix (IV) indicates that the plant described in this thesis had a high energy requirement, and it may be that in the long term such energy 'wasting' processes will have no part to play in the provision of protein for food or feed. In the short term, say 50 years, providing that a sensible energy policy with world wide acceptance is developed it is likely that technology dependent food production methods which directly convert energy into protein, i.e. biomass techniques, will be most needed in the already industrialised countries and not the less technologically advanced areas. This is a reversal of the role usually envisaged for biomass and single cell protein. It is probable that those areas of the world which have a high degree of technical sophistication with a small percentage of the population on the land will find it easier to maintain per capita protein and calorie intake through biomass than by conventional agricultural practices, considering the present population trends.

In certain respects the adoption of biomass by the more affluent countries is essential to world wide acceptance. If biomass can be given a prestige value initially then its general acceptance will be made easier. In connection with prestige value, De Maeyer (1968) suggested that high prices should be charged for biomass derived foods. If this became the case then, in part, the high cost of the energy input in terms of capital would be offset as well as providing a psychological 'bonus'.

Whatever/

Whatever the political or economic condition of the world it is true that persuading people to eat biomass rather than their usual fare presents immense problems to food technologists. Preparation of the final product into a marketable form requires the techniques of spinning, drying, flavouring, colouring, texturising and advertising. Psychologists have a role to play in the development of biomass. McKensie (1969) has stated that, in general, people's eating habits are not rationally determined. Galliver (1969) pointed out that certain existing biomass products had suffered in recent years from irrational attitudes to foods. Silverston (1972) and Harper (1961) have discussed the psychological aspects of food acceptance at length.

The project described in this thesis has not developed the ancilliary techniques associated with biomass production. These include harvesting and collection of cells, and the treatment of these to derive an edible product. It was not thought that any useful contribution could be made to these areas of study since the principles of harvesting and treatment have largely been worked out. Papers by Wang (1969); Carepa et al (1972); Labuza et al (1970); have dealt extensively with these topics.

(2) PRELIMINARY TROUBLE-SHOOTING PROBLEMS:

When the project was initiated it was not realised how complex the plant would be nor how difficult it would be to translate the theoretical design into a working unit. Some of the problems encountered in this phase of the work have been discussed in relation to the need for an adequate technical backup to a microbiological laboratory engaged in complex fermentation work.

(1) The Fermenters; The most serious limitation to the progress of the work was a result of problems directly and indirectly associated with/

with the fermenters supplied by Chemap of Zurich.

(i) Initially the operating instructions sent were 4 months overdue and were in German. A further 3 months wait was necessary before the English versions arrived. The fermenters themselves were late in arriving.

(ii) Wiring diagrams were similarly overdue and when obtained did not relate to the actual fermenters ordered. Fault finding and rectification was extremely difficult.

(iii) Contrary to claims made by Chemap none of the equipment had been tested thoroughly before despatch. Had it been tested then few of the described problems would have arisen.

(iv) The equipment supplied was not adequately rated for British 240/250 voltage supply. It was found that over-voltage caused damage to the F.Z.7 units, for example, resistor pre-sets and relay coils burned out. A transformer should have been incorporated into the fermentation system before despatch. The lack of a wiring diagram did not facilitate self-help.

(v) One of the GF007 fermenters delivered was in the following condition;

(a) The maximum stirrer speed obtainable was 1000 r.p.m. with a great deal of vibration at this speed.

(b) A worn top bearing had to be replaced.

(c) A faulty steam trap prevented steam sterilisation. The trap was also incorrectly sited.

(d) Much time was wasted before the needle valve in the cooling water channel to the fermenter was found to be blocked.

(2) The F.Z.7 Detecta control units. Again delivery was 9 months late and the units were not in working order. It was necessary for a technician/

technician to be sent from Switzerland to service these instruments which were found to be incorrectly wired; the diagram in the manual did not relate to the correct wiring. The over voltage effect caused the resistors supplying the panel neons to burn out on both F.Z.7 units.

(3) Fermenter Volume Control. Initially the control of fermenter volume was designed so that changes in volume would cause a change in the torque generated by the hydraulic variators. A torque gauge registered the change and a photocell arrangement on the F.Z.7 allowed liquid volume control via peristaltic pumps (See section 2 Part C). At no stage was control by this method possible. The torque gauges supplied were designed to measure torques equivalent to about 12 British Horse power or approximately 9K watts. The range of torque generated by the agitation system was a maximum of 1.5 K watss and thus these gauges were useless for the purpose of volume control, proving also that the rig had never been tested under working conditions before despatch.

Problems with both hydraulic variators, were never fully identified and ensured that the control of volume could not be achieved using changes in torque. Both variators were eventually replaced after months of negotiation, but it was not possible to develop the control of volume to a sufficient degree of sensitivity. For this reason a method based on the consumption of motor current was designed in these laboratores and the hydraulic system of control was discarded entirely. Unfortunately the mechanical deficiencies in the hydraulic variators were not resolved entirely. Fig.(52) shows the effect of the variator malfunction on volume control based on current consumption. It became necessary to repair the new variators once more. Malfunction was cured in the case of fermenter (2). Only fermenter (1) gave a persistently erratic current consumption/

consumption with constant volume and could not be run at agitation speeds in excess of 1500 r.p.m. In these conditions control of liquid volume was achieved by a conventional weir-overflow.

It is felt that the problems affecting the hydraulic variators resided in the oil seal. A persistent leak of oil has been observed in both fermenters although in fermenter (1) it was more severe. When oil was replaced to the recommended level, the current drawn at constant volume has a smoother trace at 2500 and 3000 r.p.m. In the case of fermenter (1) oil spurted out of the overflow connection for several minutes and the current consumption became increasingly erratic. Fermenter (2) leaked oil at a much slower rate and the effect on current consumption was not noticeable for several months. Strathclyde experts (Hydraulics Dept., Mechanical Engineering) were convinced that these variators were not designed for vertical operation, as in our units. Denials of this point came from both Chemap and the Italian manufacturers of the Variator. The Italian company flew an engineer to Strathclyde at our insistence, but neither this engineer nor the service department of their U.K. agents could suggest a solution to the problem. The Italians in any case felt the responsibility lay with Chemap as the original suppliers of their equipment. The conflict between experts remains unresolved but has been the major cause of project delays. This problem must be resolved before long term fermentation runs can be contemplated. A discussion of the results of volume control in the commissioning runs is given later.

Trouble-shooting of the type described above is an inevitable part of the design and commission of any complex plant, but it soon became evident that successful completion of the project depended in no small measure on the/

the technical expertise of the mechanical, chemical and electrical engineering departments of this University. It is doubtful if the plant could have been completed at all without this help and the lesson to be learnt is that such work should not be contemplated without first ensuring that such assistance is available.

(4) RELIABILITY OF THE LIRA GAS ANALYSER:

It has already been mentioned (Section 2 Part C) that some of the components of the infra-red analysers deteriorated and had to be replaced. Some loss of performance can be expected with any instrument with prolonged use. The choice of LIRA over IRGA analysers was made on the basis of cost. It is interesting that later models of the LIRA do not have valve amplifiers and that the capacitors are of a different design. The unreliable chopper arm motors seem to be unchanged. Choice of instrumentation on the basis of cost should be the last criterion to be applied and not the first as in this case.

(5) THE NEED FOR AN ADEQUATE SERVICES BACKUP:

Compressed air in a clean dry oil free state was essential to the continued operation of the fermentation rig. Similarly mains water and electricity supplies were absolute requirements.

It has been mentioned (Section part D) that contamination of the pneumatic instruments occurred. This was traced finally to a workman in the maintenance department of the University inadvertently loading the main compresser with oil in such a way that the distribution pipes were severely contaminated. Before the rig was installed a guarantee of oil and water free compressed air was obtained from the maintenance department and this was accepted with disastrous results. Once again the dependence of/

of the project on outside technical assistance was highlighted. All of the pneumatic instrumentation was expertly overhauled but the work was set back by about 9 months. The pipework had to be degreased and cleaned, as had filters, flame traps, valves, flowmeters and the air supply lines; followed by 6 weeks of leak-testing.

To prevent such an occurrance jeapordising the work again, two small compressors were installed with the appropriate driers and filters in line. A filter was installed in the compressed air line feeding the instruments and analysers. In hindsight, such preventative measures should have been taken at the outset.

(6) THE PROBLEM OF LEAKAGE OF GAS FROM THE SYSTEM:

A certain degree of gas leakage was inevitable from a system as complex as that described and it was found to be impossible to eliminate totally all sources of gas loss. Fig. (51) shows the leak rate before oil contamination and dismantling of the system. It was noted that each time a coupling was made or broken it became progressively more difficult to arrange a gas tight seal. In fact at one stage it was felt that the leak rate was so high as to mask any gas consumption by the culture.

Perhaps the chief disadvantage of a gas recycle incorporating automatic control of the gas concentrations is that to be fully effective, leaks must be reduced to the minimum. It is doubtful if a leak rate of greater than 1% of the consumption rate could be tolerated. The exact threshold rate would depend on the consumption rate due to microbial growth. It has been noted that the elimination of leaks was impossible and should these leaks enlarge it may become necessary to replace many of the couplings, or even discard gas recycle entirely. Lack of experience in the matter of pipebending and handling compression couplings may have contributed in some measure to the persistent leakage.

(7) /

(7) AN ASSESSMENT OF THE OVERALL DESIGN OF THE FERMENTATION PLANT:

At the end of three years during which the fermentation plant described was constructed and commissioned, the approach adopted has not been duplicated elsewhere and therefore a comparative assessment has not been possible.

(A). The fermenters; In spite of the deficiencies described in section (2) of the discussion, it must be added that the fermenter vessels were of a very high standard of design and manufacture. This statement applied also to the associated filter units and attachments. The condensers supplied by Chemap were an essential feature of the fermenters and whilst they removed water vapour effectively from the exit gases at first, they seemed extremely susceptible to blockage. On several occasions the plant was shut down to clear blocked condensers. Accumulated debris from coolant water caused most of the restriction and as this built up it was finally impossible to circulate coolant at the required flow (about 1 litre/hour). Even after thorough cleaning in various solvents only partial clearance could be attained. Eventually a more powerful circulation pump was resorted to, involving more expense and labour. The basic fault lay in poor design of the condensers; significantly, Chemap no longer produce this model of condenser.

The fermenters, whilst of a high standard of finish, were extremely inconvenient to dismantle and reassemble. (See appendix III); Careful attention had to be paid to this operation since the carbon seals of an incorrectly assembled fermenter were in danger of irrepairable damage. This happened on one occasion and the fermenters were deposited with a carbon dust from the worn bearings. This was dangerous in oxygen service and also made optical density readings difficult to interpretate.

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The blind plug and socket arrangement together with the needle inserts used on the liquid line system were excellent. Aseptic connections were simple to perform and with practice it was possible to make these connections whilst the system was pressurised with only an insignificant loss of system pressure. The simplicity and speed of this arrangement was instrumental in preventing shut down on several occasions.

(B) Multipoint recorders; Extensive use was made of multi point recorders in the design. The multipoint recorder is obviously a means of recording information which otherwise would require a separate recorder for each section of information. Economy is therefore obtained but those recorders used in this project were of the sequential print out type and therefore a delay of about 30 seconds occurred between two successive printouts for the same recording.

Often a multipoint recorder would not be used as a part of a control station because of the delay, but the most useful feature of multipoint traces is that related or inter-dependent parameters can be recorded on the same chart. For example in the work described, pressure and the flow of north sea gas required to maintain the pressure at the set point were recorded on the same chart. Similarly the outputs from both pH electrodes and both dissolved oxygen electrodes were displayed on the same chart. Interpretation of data is facilitated by this arrangement. At the same time the presence of several traces on one chart lead to confusion particularly if the traces are grouped closely together.

(C) Flow meters and rotameters; Except for the Fischer-Porter differential pressure flow meters, all of the flow meters were calibrated with a compromise gas mixture of 50% ethane/50% oxygen at 40°C. Errors were introduced when gas compositions and temperatures other than these/

these were used but absolute flow measurements were needed from these instruments and comparative errors were acceptable.

The rotameters used to measure flow of gas to the gas analysers were of a particularly poor design. The float was exceedingly small and tended to stick in the rotating coil at the top of the calibrated tube. Although the analysers were relatively insensitive to flow variations, it was essential to control the flow of gas to the analysers to a value close to that at which they were calibrated. This was especially true of those analysers (oxygen, ethane and carbon dioxide) which were components of control loops.

(D) Safety aspects of the plant; To some extent the complexity of the fermentation plant was due to the many safety features which had been incorporated. It may appear that an undue emphasis was placed on the safety aspects of North Sea gas fermentation. It is true that there are no degrees of safety. Either the plant is safe or it is not and, once embarked on committment to safety, must be total. Although gas concentrations used were in the non-explosive range (see Section part C) the complex nature of the control loops allowed the possibility of an explosive gas mixture circulating in the system due to control malfunction. Any source of ignition present would be liable to cause an explosion at any time and therefore the attention paid to safety was justified. In the plant described, only the gas composition after make-up could be guaranteed outside explosive limits - elsewhere it was highly likely that 'pockets' existed which were within the explosive range. One extreme condition would have been 100% oxygen and an additional safety feature to prevent such a situation from arising is a microswitch on the oxygen recorder to cut the O_2 supply above a certain limit, e.g. 14.5% oxygen. This was not included in the plant but on reflection would have been a useful addition.

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In any process using explosive gas mixtures there is no excuse for ignoring simple safety precautions. The most important of these is that as many sources of ignition should be removed or negated as is possible. Extensive flame proofing is difficult to arrange, but models of most instruments can be obtained for use in hazardous areas. Often intrinsically safe instruments can be obtained off the shelf or can be modified to a safe condition, e.g. by air purge lines.

One of the major safety features used was that relating to high and low pressures in the system. In the worst case, two cylinders of gas deliver a total of 45 L/hr into the system and the lute escape route is sufficient to cope with such flows. Similarly a loss of pressure due to system leaks can only continue until the gas cylinders are empty. How dangerous these leaks are depends on the environment into which the gases leak. In the present system the danger from outside ignition sources was not high; the fermenter motors were brushless and the pump housing cabinet was air purged. Only the gas circulating pump was not intrinsically safe. When large volumes of gas are used or when on line oxygen and north sea gas are available then fail safe devices become essential. In this event the experience gained with such instruments in small scale plant will be useful when applied to industrial scale operations.

The fermenters were situated in a safety cubicle with explosion windows and a heavy door whilst the analysers O_2, CO_2 and N_2 gas cylinders were in an identical adjoining cubicle. Ideally these cubicles should have been about twice the size. It was difficult to work in the cramped conditions and the consequent battering dealt out to the pipework may have been responsible for the persistent leaks noted earlier.

(E) /

(E) Pipework and liquid lines; Inspection of fig.(4) shows the complexity of the pipework. The plant itself was crammed into two small cubicles which made leak testing difficult. Even tracing particular pipes from source to end required a great deal of mental concentration and perhaps colour coded pipes should have been used.

The extensive liquid lines consisting of pH delivery and resevoir refill lines, medium delivery lines and effluent take off, and the delivery lines for the filtered medium caused confusion at crucial times. At the same time autoclaving of these lines was a major operation. Subsequent to autoclaving these lines had to be unravelled and laid out with no constrictions prior to insertion in the fermenters. Other than employing permanent in line delivery tubes with sterilisation facilities it is difficult to see how this could have been avoided.

The majority of the gas pipework was in stainless steel. The alternative would have been plastic or copper tubing. It was felt that plastic tubing would not have conformed to the industrial standards of operation and design which were imposed from the start. Copper could have been used but there is the possibility of oxidation products contaminating the gas lines, filters and analysers. Copper ions are toxic to micro-organisms and in the event of condenser failure on the exit gas lines, contamination of the fermenter contents was a distinct possibility. For these reasons stainless steel was used in preference to other materials.

Stainless steel piping is expensive and in hindsight much less stainless steel pipework could have been used. The most important areas where stainless steel was needed were the line from the oxygen cylinder to the main recycle line and the sections of tubing connecting the two fermenters. In all other sections copper tubing could have been used with a consequent reduction in capital outlay.

Swagelok quick/

Swagelok quick release connections were installed on both the gas inlet and exit filters with the corresponding female coupling on the recycle pipework. Without these couplings ordinary compression couplings would have been necessary with the result that each time the fermenter filters were removed or the fermenters dismantled each coupling must be broken and remade, possibly several times. It has been noted that such treatment of the couplings may have been responsible for the persistent system leak. Although the quick release couplings were expensive (See appendix IV) it was felt that the convenience they afforded justified the investment.

It has been mentioned that the outer diameter of the pipework was $^{3}/_{8}$" in the gas recycle lines and $^{1}/_{4}$" elsewhere, (See section 2, part C). This allowed a working volume which was almost at the limit for control purposes and although at the same time the volume of potentially explosive gas mixture was kept to the minimum in this way, it is probably true to say that control of the methane concentration by changes in pressure would have been less susceptible to system leaks had the volume been larger. Pipes of $^{3}/_{4}$" (O.D.) and $^{1}/_{2}$" (O.D.) would have been preferable.

(8) AN ASSESSMENT OF THE PERFORMANCE OF THE FERMENTATION PLANT IN THE COMMISSION RUN:

(1) THE FERMENTERS:

(i) General; Both fermenters were able to run at up to 3000 r.p.m. Fermenter (1) was observed to generate carbon dust at this speed but this was not due to incorrect assembly. Eventually the fault was traced to the supply of a slightly warped stirrer shaft and it is possible that the erratic current consumption observed in this fermenter is attributable to this cause. A bent shaft was only one fault accounting for the/

the symptom since a similar erratic current consumption was recorded for fermenter (2) in which the shaft was not bent; the fault was rectified by replacement of the hydraulic variator in this instance.

(ii) Gas leaks from the fermenter; At no stage was gas leakage from the fermenters observed providing that they had been assembled correctly and that the needle inserts and connections had been fitted properly. The high standard of manufacture of the blind plugs and fittings supplied by Chemap was responsible for the ease with which gas tightness in the fermenters was attained. When dealing with potentially explosive agents it was comforting to have confidence in the equipment used. For this reason the decision to buy top quality fermenter vessels was justified.

(iii) Foam control; A major patented feature of the Chemap fermenters was the standard mechanical defoamer. The performance of this part of the fermenters was excellent, allowing foam control at maximum gas flow rates and agitation speeds. Thus in another aspect the choice of Chemap fermenters was vindicated. The foam breakers appeared to have an important role in level control. This is discussed in sub-section (v) below.

(iv) Chemap filters; The filters were tested for percentage retentivity and dirt handling capacity, (See table 22,23) since no figures were supplied with these units. The tests performed were designed only to give some indication of the capability of these filters under working conditions of gas velocity and particle (organism) concentration and although no long term tests were conducted it was felt that the filters were adequate for the requirements of the plant.

In terms of dirt holding capacity of a filter it was seen that the condenser/

condensor was essential for prolonged gas sterilisation (Fig.(23). Blockage with deposited organisms completely prevented gas flow within hours when the condensers were not functioning. It is difficult to assess the Chemap filters in that most depth filters of this dimension could be expected to exhibit a greater dirt handling ability and it was concluded that the mechanism of entrapment of particles in these filters was not according to the principles worked out by Humphrey and Gaden (1955). It is suggested that the mechanism of entrapment of the ceramic filters depended on factors intermediate between those operating for membrane filters and those for conventional depth filters. The high degree of sterility observed and the poor dirt handling capacity indicates that they functioned more in the manner of a membrane filter than a depth filter.

(v) Volume control (level); A prerequisite of continuous culture is a constant fermenter volume, and many of the arrangements devised to ensure this are often unable to control volume to better than 20% (Solomons, 1969). The system based on current drawn by the fermenter motors related to the volume of the fermenter contents, and in essence the success of this method was limited by the sensitivity of the units which measured changes in current drawn and by the threshold current below which volume changes in the fermenter had no significant effect.

It was noticed that the foam breaker cones contributed greatly to the total current drawn. The total current drawn was influenced by the density of the emulsion striking the cones which in turn was dependent on the volume of liquid in the fermenters. At higher agitation rates the force of the emulsion striking the cones was greater and at the same time was affected by changes in the density of the emulsion to a greater extent.

It was decided that fermenter (2) should be run at not less than

2500 r.p.m. both in batch and continuous culture. Fermenter (1) was run at 1500 r.p.m. and served as a means of isolating cultures under conditions of reduced gas transfer. It was not possible to run fermenter (1) at less than 1500 r.p.m. because of a heating element failure. Control of temperature during the commissioning run with fermenter (1) depended on heat of agitation and heat evolved during growth. Below 1500 r.p.m. the heat generated was not sufficient to maintain the temperature at 40°C.

The mal-function in the hydraulic variators noted in section (3) was instrumental in almost preventing any form of level control based on current consumption. In retrospect the design of the drive for the fermenters seems clumsy and unsophisticated. At the time of construction of the plant, this seemed to be the only way of obtaining a variable speed drive for use in hazardous conditions. It is unlikely that this system will be employed again in these laboratories.

The control of volume achieved in the early stages of the commissioning run, fig. (41), using both fermenters although not as accurate as hoped, was good enough to maintain the dilution rate within acceptable limits for continuous culture. About ±10% were felt to be the acceptable limits.

(vi) Gas transfer; The fermenters supplied by Chemap were available with a choice of agitational systems designed for specific applications. It was claimed that the emulgator arrangement (see section part C) was most suitable for hydrocarbon fermentation, (Muller 1972), particularly methane fermentation. The comparative tests performed on the gas transfer ability of the emulgator system with the conventional impeller arrangement seemed to indicate that whilst there was an increased rate of gas transfer (as measured by the sulphite oxidation technique of Cooper/

Cooper Fernstrom and Miller 1944) with the emulgator in place, it was hardly great enough to warrant the excessive claims of Chemap. The influence of the emulgator was more noticeable at higher gas flow rates and at higher rates of agitation. It was thought that the emulgator enabled a greater proportion of gas to be transferred for a given power input. In other words, little gas was lost through entrainment along the agitator shaft when the emulgator was in place.

Entrainment was visibly more apparent at high gas flows when the emulgator was removed. The status of the emulgator has not been established unequivocably so far. It is felt that the efficiency of the conventional impeller disc arrangement is great enough to obviate the use of the emulgator.

Criticism has been levelled at the sulphite oxidation test as a measure of gas transfer capability because in many cases there have been large differences between values obtained with sulphite oxidation tests and those determined in actual fermentation runs. Phillips and Johnson (1961) observed that the presence of micro-organisms raised the mass transfer coefficient above that derived from sulphite oxidation. Although the sulphite method is one of the most common methods of determining the mass transfer coefficient of fermenters it is unlikely that the chemical reactions occurring during sulphite oxidation can bear any resemblance to those taking place in a fermentation process with a living culture.

Sulphite oxidation is sensitive to impurities in the reagents used. For example, the presence of organic traces reduces the reaction rate but heavy metal ions accelerate it. For this reason cobaltous (or cupric) ions are used as a catalyst. Copper ions have been found to reduce the reaction/

reaction rate whilst iron or phenol had no effect. (deWaal and Okeson 1966). Similarly Wesselinghand van 't Hoog (1969) reported that cuprous ions caused a 4% decrease in reaction rate. Deionised water has been observed to give an accelerated rate compared to tap water. (Yagi and Inoue 1962).

pH has been shown to affect the rate of oxygen transfer as measured by sulphite oxidation (Roxburgh 1962). Indeed an almost linear relationship between pH from 7.5 to 9 with increasing transfer rate was noted. Between pH9 and 10 the rate dropped sharply. During sulphite runs the pH drops and for the results to be meaningful some form of pH control must be employed. Often this aspect of sulphite oxidation experiments is ignored.

It has been reported (Linek and Tvrdik, 1971; Y agi and Inoue, 1962; Wesselingh and van 't Hoog 1969) that there is a linear dependence of oxygen absorption rate on the square root of the concentration of the cobalt catalyst in the region of 5×10^{-6} to 2×10^{-3} g mole/litre. For this reason cobalt was preferred as a catalyst to copper.

The convenience of the sulphite oxidation method was that it enabled a rapid comparative assessment of gas transfer rates either between types of agitational system or the same system under different conditions of gas velocity, temperature and partial pressure. Several attempts with the method were necessary before a standardised technique was perfected. Once this had been achieved, the sulphite oxidation method for measurement of oxygen transfer rates could be used confidently in comparative studies.

(2) INSTRUMENTATION AND CONTROL LOOPS:

(i) Analysers; The difficulties encountered with the infra red/

red analysers have already been dealt with in section (4) of this discussion. It was evident that the most stable analyser was the methane analyser. This was attributed to the fact that a new amplifier had been fitted immediately prior to the test. The ethane, carbon dioxide and oxygen analyser did not drift significantly until the 48 hour period and it was decided that all the analysers should be recalibrated every day. The method of calibration was simple and rapid so that this did not cause any inconvenience. (See section 3 of Part D).

The performance of the analysers was considered to be acceptable for the requirements of the fermentation plant. A constant gas environment was a major feature of the plant and apart from the general unreliability of the LIRA components all four analysers functioned in a satisfactory manner.

(ii) pH electrodes; Over three year period only minor operational difficulties were encountered with the pH electrodes supplied by Activion Ltd. (See appendix V). The removable element type of electrode was a considerable advance on the more usual sealed variety of electrode so that recalibration was not necessary after each *in situ* sterilisation. The only way in which these electrodes could be faulted was that they were extremely fragile and for use in an industrial context, a protective support sheath would be advisable.

(iii) Temperature probes; Throughout the entire working life of the plant the temperature probes gave entirely satisfactory service. One fault which was noted was that at extremely high rates of agitation (2500 r.p.m. or above) the screws holding the output leads into the terminal strip tended to work loose because of the vibration unless forcefully screwed down. The connections to the terminal strip were checked regularly to ensure that this did not affect the measurement of temperature.

(iv) Dissolved oxygen electrodes; The stability of response

was/

was excellent. The nylon/silicone rubber membrane was the only major innovation in the electrodes. In terms of design the electrodes were a little clumsy and difficult to handle and the 'O' ring seals did not always produce a complete seal. It was found that silicoset dissolved in cyclohexane and applied liberally to the membrane around the stem usually ensured a complete seal. This brought difficulties in itself because it was then impossible to remove the electrodes without first peeling off the membrane.

The silicoset plug used to seal the electrode body at the top was also inconvenient since dismantling the electrodes ruined the plug and a new layer of silicoset was needed for reassembly. Alternative methods of sealing the electrodes are being investigated.

In line calibration of the electrodes was done with oxygen free nitrogen and air (See section 6, part D). It was noted that when sulphite was used as a zero oxygen check about 2 hours was required before a stable output was achieved. This was thought to be due to the outward transfer of oxygen dissolved in the electrolyte to the sulphite solution.

Air (containing 21% V/V Oxygen) was considered suitable for span tests because the gas phase concentration was never greater than 14.5% V/V. Thus 0% - 21% covered the operating range adequately.

Perhaps the most serious defficiency of the dissolved oxygen electrodes was their high temperature coefficient (Fig.34). This was of no consequence as long as good temperature control was maintained but fluctuations in the temperature made the response of the electrodes unreliable (Fig.34 point X).

It seemed that 40°C was close to the limit at which the electrodes gave a stable response. At 50°C the steady decrease in output seemed to indicate that the reaction at the silver electrode was impaired or that some kind of reverse reaction was taking place at the same time.

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The individual characteristics of the performance of each electrode were a function of the small variations in structure of each electrode. Differences in the volume of electrolyte and area of membrane contributed to a large extent to the different characteristics displayed.

The response time of a dissolved oxygen electrode has significance if the probe is a component in a control loop. In the project described this was not the case so that the response times were not the most important feature. Stability of calibration was considered to be the factor of importance in long term fermentations and both at the zero oxygen level and at saturation concentration the stability of both electrodes was remarkably good.

(v) Control of gas phase oxygen concentration; It is doubtful if control of gas phase oxygen could have been improved upon. The almost straight line control achieved was remarkable. (See fig.43). The oxygen control loop was considered one of the most successful parts of the design of the fermentation plant. With the exception of the contamination of the minim control valve with oil, trouble free service was given throughout the commission run.

(vi) Control of gas phase carbon dioxide concentration; Control of CO_2 was simple and effective (Fig.46). Although only ON/OFF control was employed, carbon dioxide analyser appeared to be sensitive enough to enable good control via the carbasorb scrubbers. Thus the decision to disregard proportional and/or integral control was vindicated.

(vii) Control of gas phase ethane concentration; Again the effectiveness of the ethane control loop was remarkable (Fig.45). The straight line appearance of the control loop may have been partially due to the fact that consumption of the ethane by micro-organisms caused a pressure drop which was controlled by the pressure controller. Thus an additional amount of north sea gas entered the system (containing ethane) and had a buffering effect on the rate of change of the ethane concentration.

(viii) Control of gas phase methane concentration by means of pressure change; The effect of system leaks on the control of pressure was not great enough to significantly upset the methane concentration in the gas phase (Fig.46) and it was decided that although straight line control of pressure could not be attained (Fig.44) the control of methane concentration was considered as acceptable. It was thought at the design stage of the project that control of methane concentration by pressure regulation would have been rendered difficult by the mode of operation of the other control loops involved in the maintenance of a constant gaseous environment. Naturally the consumption of oxygen, ethane and the production of carbon dioxide affected system pressure in the same way that the consumption of methane did. During the commissioning run the influence of these control functions did not appear to have a debilitating effect on methane concentration control. It may be expected that the rate of consumption and production of gases during microbial growth will determine the point at which control of methane concentration by pressure becomes impossible. It is unlikely that in batch culture with high biomass levels, where continual change in the rate of gas production and consumption occurs, that control of the gaseous environment could be effected successfully by the method used in this project. Continuous culture, during steady state conditions is ideally suited to control of the gaseous environment in the manner designed for this project.

(3) THE STERILISATION OF MEDIUM BY MEMBRANE FILTRATION:

The use of medium sterilisation by filtration in preference to any other method has already been noted (Section 46 Part B). The manual filtration provided sterile medium at adequate flow rates for dilution rates up to but not exceeding $1.72^{hr^{-1}}$ (See section 19 part D) for 2 x 5l working volumes. These flow rates were not appropriate for larger fermenter/

fermenter volumes where many gallons of medium per hour are required.

A wider time margin of safety between filtration cycles would have been preferable. This hinged on the maximum flow rates obtainable from the rig, and in this context flowrates in the order of gallons per minute would have been preferable.

The automatic version of the filtration rig was considered as a success in principle. The doubtful thermistors originally fitted have been replaced.

(9) THE GROWTH OF MICRO-ORGANISMS IN THE FERMENTERS DURING THE COMMISSION RUN:

The mixed culture which was isolated in the commissioning run was not characterised completely but certain interesting features of the culture were noted. There was an initial lag period of four days which may have been due to the presence of toxic materials in the isolation material. As growth of the culture progressed one or more of strains present may have metabolised the toxic materials and thus allowed growth of the north sea gas oxidisers. Alternatively it may have been that there was an unusually long time required for the induction of enzymes necessary for growth on the medium supplied.

It was evident that the culture did not consume either ethane or methane in great quantities and this was reflected in the relatively low cell densities which developed. The fact that ethane did appear to be oxidised was interesting in its own right. Few cases of true ethane oxidation have been reported. The account of ethane oxidation by Graphium species (Volesky and Zajic, 1971) involved a fermentation system which used a simple gas recycle system.

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The poor growth rate of the culture may have been due to the presence of non-metabolisable toxic products or merely the normal growth rate for the culture under the conditions of operation. The total growth period only lasted about 29 hours before the stationary phase set in. The extremely insoluble nature of both natural gas and oxygen (Table 15) and therefore low dissolved concentrations, could not have been responsible for the short growth period because the supply of dissolved gases was continuously and automatically renewed by the control loops. The presence of toxic materials was therefore strongly suggested. The concentration of cells was not great enough to have exhausted the supply of nitrogenous medium. Nor was it likely that a trace element had become limiting, although this was not completely ruled out.

Obviously much extensive and rigorous experiment was required to unequivocably establish the explanation of the above observations, in particular to establish the identity of the component organisms in the mixed culture. The investigation of the interrelationships between the component species would constitute a complete thesis in itself.

(10) PROJECTED EXPERIMENTS:

Subsequent to the commission experiment, the plant must be thoroughly tested so that its potential as an isolation tool can be fulfilled. In this respect its application to continuous culture should be carefully evaluated, both as a single stage chemostat and as multistage equipment. The effect of different dilution rates and gas transfer rates on the nature of culture which is isolated together with experiments at different pH and temperature should be explored fully.

Biochemical investigations into the nature of toxic materials (if any) and the nature of the interdependencies of mixed cultures able to oxidise natural/

natural gas should be carried out. Mixed culture work offers a challenging and potentially rewarding field of study and with the means to rapidly isolate cultures and to establish them in continuous culture and without the tedious and laborious flask culture enrichments procedures, progress should be unusually fast.

As cultures suitable for biomass products are isolated, thorough testing to establish their nutritional status must be carried out. This will involve estimation of the amino acid content, total protein and general biochemical composition. It should be established that toxic materials are absent and that the biomass has sufficient biological value by extensive feeding trials.

P A R T (G): C O N C L U S I O N S

P A G E S (203) - (208)

The need for a source of protein which supplements that obtained from conventional agriculture has been established beyond doubt. It has become increasingly apparent that every additional avenue of protein production should be explored and developed as soon as possible. In this context biomass can make a significant contribution to world protein supply, especially in the industrialised countries in the short term of about 50 years. Those methods of biomass production which conserve energy and materials to the largest extent could be important as a source of protein in the longer term.

The chief impact of the present energy crisis on the developed countries will be on methods of food production. The agriculture on which these countries depend is too energy consumptive to be regarded as a long term means of providing food.

Methane was shown to be an attractive substrate for biomass production. Methane can be obtained from natural gas and from anaerobic sewage digestion, and it is the latter source which offers a long term use for methane as a substrate for biomass. It could be argued that the conversion of fossil fuels to food via conventional agriculture is blatant malpractice and that it would be more sensible to directly convert such compounds to protein directly. A complete reassessment of fuel policy throughout the World would be necessary to achieve this.

Many fermentations systems which have been designed to produce biomass are energy intensive. The plant described was in this category but an accurate assessment of its energy consumption was difficult. The figures quoted in Appendix (IV) did not include energy figures for the manufacture of the plant nor for the distribution and transport of the raw materials.

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To assess the significance of the energy requirement of a given fermentation system requires an overview which cannot usually be given.

The design of the plant described was centered around the fermenters and it has been stated that these along with the ancilliary equipment supplied by Chemap were not tested before despatch. Despite intensive efforts from several technical experts the problems associated with the fermenters were never solved to complete satisfaction. After several months of examination it was concluded that the deficiencies of the hydraulic variators could only be rectified by removal and replacement of the variators.

The initial technical difficulties encountered served to emphasise the fact that a prerequisite of complex fermentation systems was good technical backup. Without the inordinate advice and practical help from the engineering faculty of this University, the plant could not have been commissioned and maintained in running order. It is suggested that complex plant of the type described should not be contemplated without first ensuring that this technical support is available.

In addition to the technical advice which was needed, it became apparent that a serious limitation on the progress of the project was workshop facilities. In a research microbiology laboratory, the need for adequate workshop skills is not always appreciated, but fermentation work cannot succeed without it.

The complexity of the plant was partly due to control loops which were incorporated, and it was an advantage to understand some of the basic control theory, particularly during commissioning experiments and for startup conditions.

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It is recommended that a separate air compressor is installed as an integral part of fermentation plant, both to act as a source of air purge and to supply pneumatic instruments. If a separate compressor had been installed at the start of the project commission of plant could have been completed 6 months earlier than it was. The plant also suffered from power cuts from various causes and an independent power generator would have been a distinct advantage.

Once commissioned, the plant functioned in a satisfactory manner except for the variator on fermenter (1). The full potential of the fermentation system could not be fulfilled until the volume control difficulties in fermenter (1) were resolved, but in principle volume control based on changes in current drawn by the stirrer motors according to changes in the density of the gas/liquid emulsion was excellent. Above 2000 r.p.m. control of fermenter volume was acceptably smooth before the malfunction of the variators. The current measurement units were sufficiently sensitive to allow control of volume to within 2%. For this reason it is proposed that volume control based on current consumption could be applied to fermentation work more widely. Certainly it affords more accurate control than weir devices are capable of.

Although the complexity of the plant made maintenance difficult, it was felt that as a means of isolating cultures able to grow on natural gas at 40°C in conditions of high shear rate and gas transfer and under closely controlled environmental parameters it was far superior to most laboratory constructions in terms of reliability and versatility. The range of selective conditions which could be applied to a given inoculum was impressive. The usual industrial practice of screening hundreds of isolates in shake flasks under different conditions was short circuited

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in a fermentation system which was deliberately used as a selective agent. The potential time and labour saving associated with such a technique has yet to be exploited fully in both industry and basic research.

The performance of all of the control loops was satisfactory. Temperature and pH were controlled to a fine degree in batch culture even though a continuous and sustained load change occurred.

Gas phase oxygen and ethane were both maintained at the set points with only minor deviations from the set point, and it is unlikely that better control could have been attained.

Carbon dioxide was controlled well even though simple ON/OFF device was employed. Thus the view that an elaborate control loop with a two term control station was unnecessary, was confirmed.

Methane concentration was indirectly controlled by detecting pressure changes, and although it was not possible to obtain straight line control of pressure, the control of methane concentration was not significantly affected. Fluctuations in the concentrations of gases in the circulating gas mixture caused fluctuations in the concentrations in solution. To what extent such fluctuations affected the growth of the culture was not clear but it was unlikely that the small fluctuations observed had a major effect in batch culture. In continuous culture where a gas is limiting growth, then fluctuations in dissolved concentrations or tension could be significant.

The dissolved oxygen electrodes were extremely stable over long periods, and it was felt that the response times of each electrode to changes in dissolved oxygen were rapid enough to allow their use in a control function.

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The influence of temperature changes on the outputs or the electrodes was insignificant because of the excellent temperature control obtained in the fermenters. Compared with other DOT electrodes used in these laboratories the temperature coefficient was not considered to be unusually high.

Fortunately the safety aspects of the plant were never tested under real alarm conditions but all of the fail safe devices worked perfectly when an alarm condition was artificially imposed. Only the pressure pad was not tested because this was an irreversible action.

The pressure fail safe controls including the purge pressure control worked in a satisfactory manner as did the over ride system which was used in startup conditions. The apparent danger from increased pressure at start up when the fail safes were overridden was not great because the delivery of gases to the recycle lines was under manual control.

Manual sterile medium filtration and delivery of medium to the fermenters was perfectly satisfactory, although the flow rate of medium through the membrane filters did not allow a large contingency time factor when automatic filtration was switched in. The poor performance of the thermistor probes limited the effectiveness of the automatic filtration rig, but in principle the performance of the automatic rig was acceptably good.

It was possible to isolate a mixed culture able to oxidise both methane and ethane in the fermentation plant described. The culture was probably affected by the presence of an unidentified inhibitor. This may have been a product of one or more of the component species in the culture or was present in the inoculation material initially. It

was/

was not unequivocably established that the organisms in the greatest number as seen in the microscope were responsible for the consumption of ethane and methane observed.

The potential of the plant as an isolation tool was thus established and further experiments involving different inoculum sources and applying different selective conditions will show its value in this context.

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APPENDICES:

(I) AUTOCLAVING PROCEDURE

(II) DETAILS OF ASSEMBLY OF THE FERMENTERS

(III) AUTOMATIC STERILISATION OF MEDIUM BY FILTRATION: LOGIC SEQUENCE

(IV) POWER CONSUMPTION OF THE PLANT

(V) ADDRESSES OF THE MANUFACTURERS OF THE EQUIPMENT USED IN THE PROJECT

APPENDIX (I)

PROCEDURE FOR STERILISATION OF THE FERMENTERS BY AUTOCLAVING:

Both of the fermenters were sterilised in situ by means of high pressure steam. After assembly (See appendix II) blind plugs were fitted to those ports with no inserts at the time of autoclaving. The pH electrode, oxygen electrode, thermometer housing, condenser, thermocouple and branched socket were left in place and sterilised with the fermenter. The gas exit and inlet filters were removed and autoclaved in a portable autoclave prior to the sterilisation of the fermenters. Similarly, the liquid lines were sterilised separately and connected to the fermenters at start up. Stainless steel protection hoods were always placed around each fermenter for the duration of the autoclaving run.

(1) The fermenters were filled to the 5 litre mark with medium.

(2) The temperature control flow regulating valve under each fermenter table was closed.

(3) The steam outlet valve on the vertical pipe in cubicle (II) was opened and the valve on the horizontal outlet pipe closed.

(4) The fermenters were switched on and the mixer run at about 500 r.p.m.

(5) Each of the steam supply valves under the fermenter tables were opened to allow steam to pass into the network of tubing in each fermenter.

(6) The pressure release valve on the oxygen electrodes were loosened.

(7) A 20 p.s.i. compressed air supply was applied to each of the pH electrodes to prevent the electrolyte from boiling off.

(8) The temperature was allowed to reach 96°C in both fermenters. (Read on the thermemeters in the thermemeter housings).

(9) The blind plugs in the plug holders on the condensers were loosened so/

so that steam issued out slowly. Similarly the blind plugs at the ends of each of the branched sockets were loosened. This ensured that pressurised steam reached all points of the fermenters.

(10) The temperature was allowed to reach 121°C and was maintained at that temperature for 30 minutes.

(11) After 30 minutes the steam valves were closed, the steam outlet valves in cubicle (11) were reversed. i.e. the vertical valve was closed and the horizontal one opened.

(12) The cooling water valves used for temperature control were re-opened so that the fermenters began to cool.

(13) At about 105°C, the loosened blind plugs were tightened up to prevent ingress of non-sterile air.

(14) When the temperature of the fermenters reached aroun 102°C, the previously sterilised exit gas filters were aseptically connected to the condensers in place of the blind plugs.

(15) The fermenters were then allowed to cool to around 45°C at which time the temperature control for each fermenter was switched on (See Start up procedure, part E section 1).

(16) The gas inlet filters were connected aseptically to the internal sparge pipes.

(17) The fermenters were ready for Start up when the temperature reached 40°C.

DETAILS OF ASSEMBLY OF THE FERMENTERS:

At various times it became necessary to dismantle and reassemble the fermenters, and since it was easy to damage the sealing block by careless assembly, the following procedure was rigorously adhered to.

(1) All new membranes and 'O' rings were lightly greased with a silicone grease. The membranes were pierced before insertion into the ports on the head plate and the sockets. Only one side of the membranes was greased - so that the danger of contamination of the fermenter contents by silicone grease was minimised.

(2) No metal to metal contacts were made without the presence of an 'O' ring or a membrane between the metal surfaces.

(3) All new or feshly cleaned stainless steel threads were coated with molybdenum grease to give a better seal.

(4) The disc turbines were assembled onto the shaft with the connecting screw facing upwards.

(5) Each of the discs was connected to the shaft so that it was opposite small holes of the emulgator.

(6) The baffle assembly and tubing network were attached to the base plate by 4 feet which fitted into holes in the base plate. It was essential that care was taken in inserting these feet so as not to damage the 'O' rings on the feet.

(7) The discs of the fundafoam foam breaker were connected to the shaft by means of grub screws. It was important that these were attached tightly to prevent them slipping during operation.

(8) The spacing of the carbon seal relative to the shaft was critical. To ensure correct spacing, a small red metal spacer was used. This spacer/

spacer was placed on the shaft above the top fundaform disc, and the carbon seal in its split ring retainer screwed down onto it. As soon as contact was made with the spacer, the retainer was tightened up thus holding the seal in the correct position on the shaft. The retainer plus seal was withdrawn, the spacer removed and the seal reinserted onto the shaft. The seal in its split ring retainer was screwed down so that it regained its previous position.

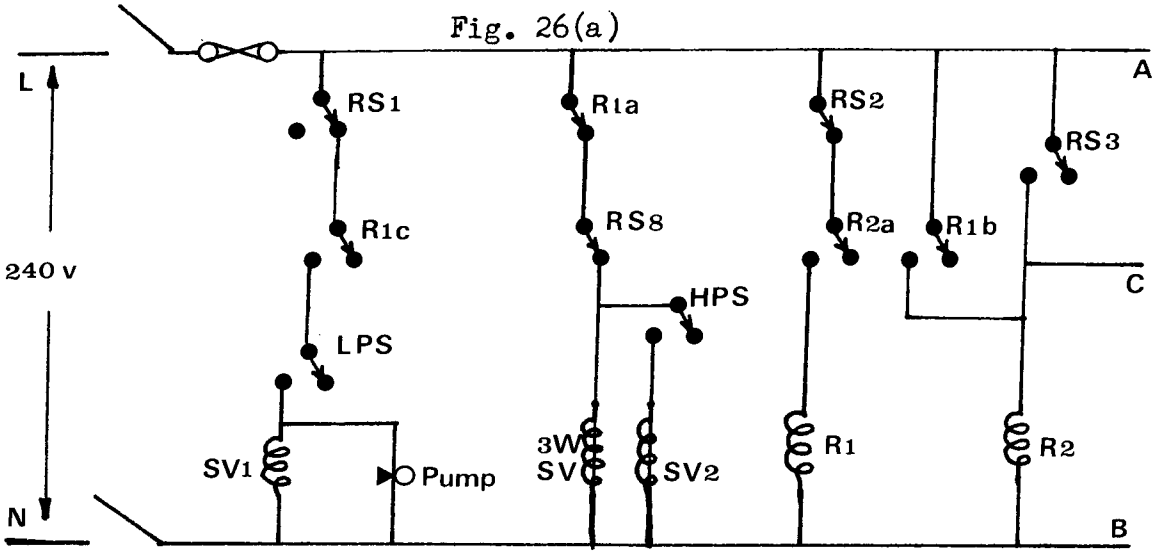
(9) A locking nut was then screwed down over the retainer on to a PTFE gasket so that the head plate was securely fixed in position.

(10) The fermenters were then placed in position in the wells on the fermenter tables, and sterilised.

(1) Automatic Sterile Filtration of Medium;

(a) Filling, Pressurising and Filtration

Fig. 26(a)



FUNCTION: CONDITION OF LOGIC CIRCUIT

	RS1	RS2	RS3	R1	R2	SV1	P	T	SV2	EPSL	PSH
PVS empty	E	E	E	E	E	E	E	D.E.	D.E.	C	O
PVS filling	E	E	D.E.	E	E	E	E	D.E	D.E.	C	O
PVS full	E	D.E.	D.E.	D.E.	D.E.	D.E.	DE.	E	D.E.	C	O
PVS full at 25 p.s.i.	E	D.E.	D.E.	D.E.	D.E.	D.E.	DE.	E	E	O	C
Filtration begins	E	E	D.E.	D.E.	D.E.	D.E.	D.E.	E	E	O	C
Bulk Storage Vessel and PVS empty	D.E.	E	E	E	E	D.E.	D.E.	D.E.	D.E.	C	O

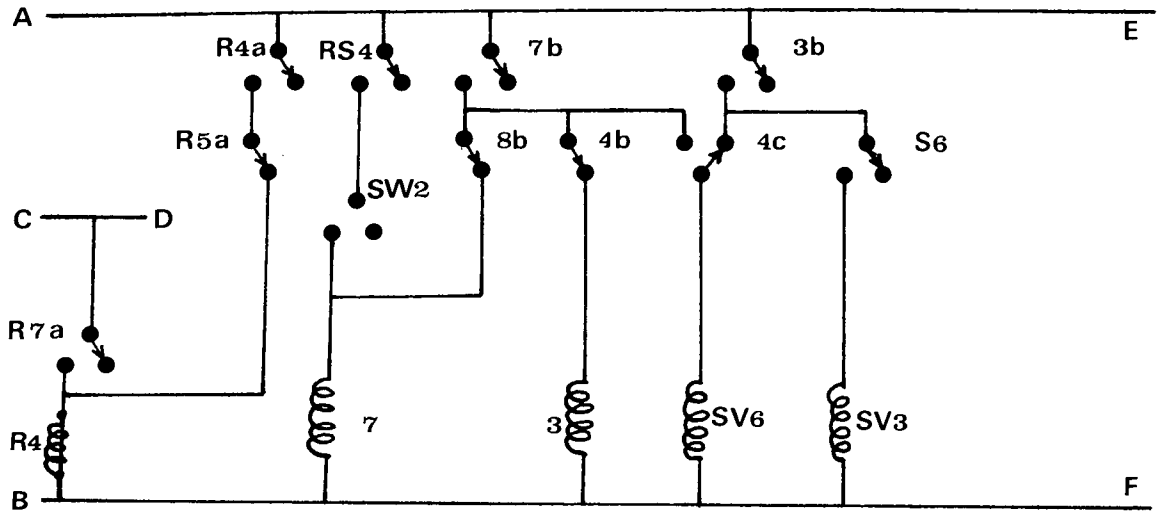
KEY:

D.E.	De energised
E	energised
O	open contacts
C	contacts closed
R_1	relay contact
R_2	reed switch relay
T	Three way solenoid Volume
PSL	Low pressure switch
PSH	High pressure switch

Automatic Sterile Filtration of Medium

(b) Filtration to receiver jar 2
Fermenter feed from receiver jar 3

Fig. 26(b)

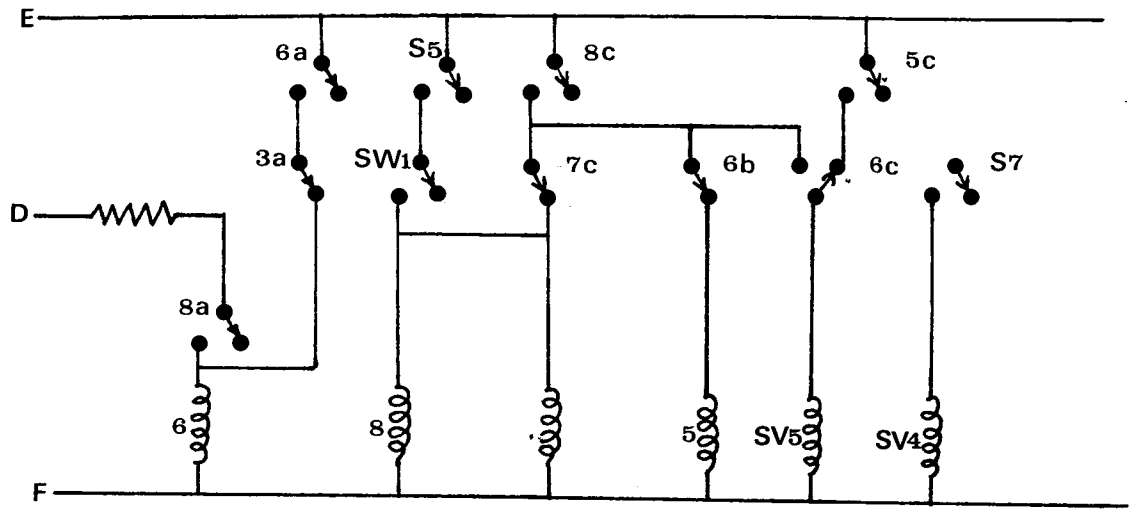


FUNCTION:	CONDITION OF LOGIC CIRCUIT						
	RS4	RS6	R3	R4	R7	SV3	SV6
Receiver Jar 2 empty	E	E	E	D.E.	E	E	E
Receiver Jar 2 filling	D.E.	E	E	D.E.	E	E	E
PVS empty: stop filtration	D.E.	E	D.E.	E	E	D.E.	E
Receiver jar overfilling Fail safe		D.E.					

Automatic Sterile Filtration of Medium

(c) Filtration to receiver Jar 3
Fermenter feed from receiver Jar 2

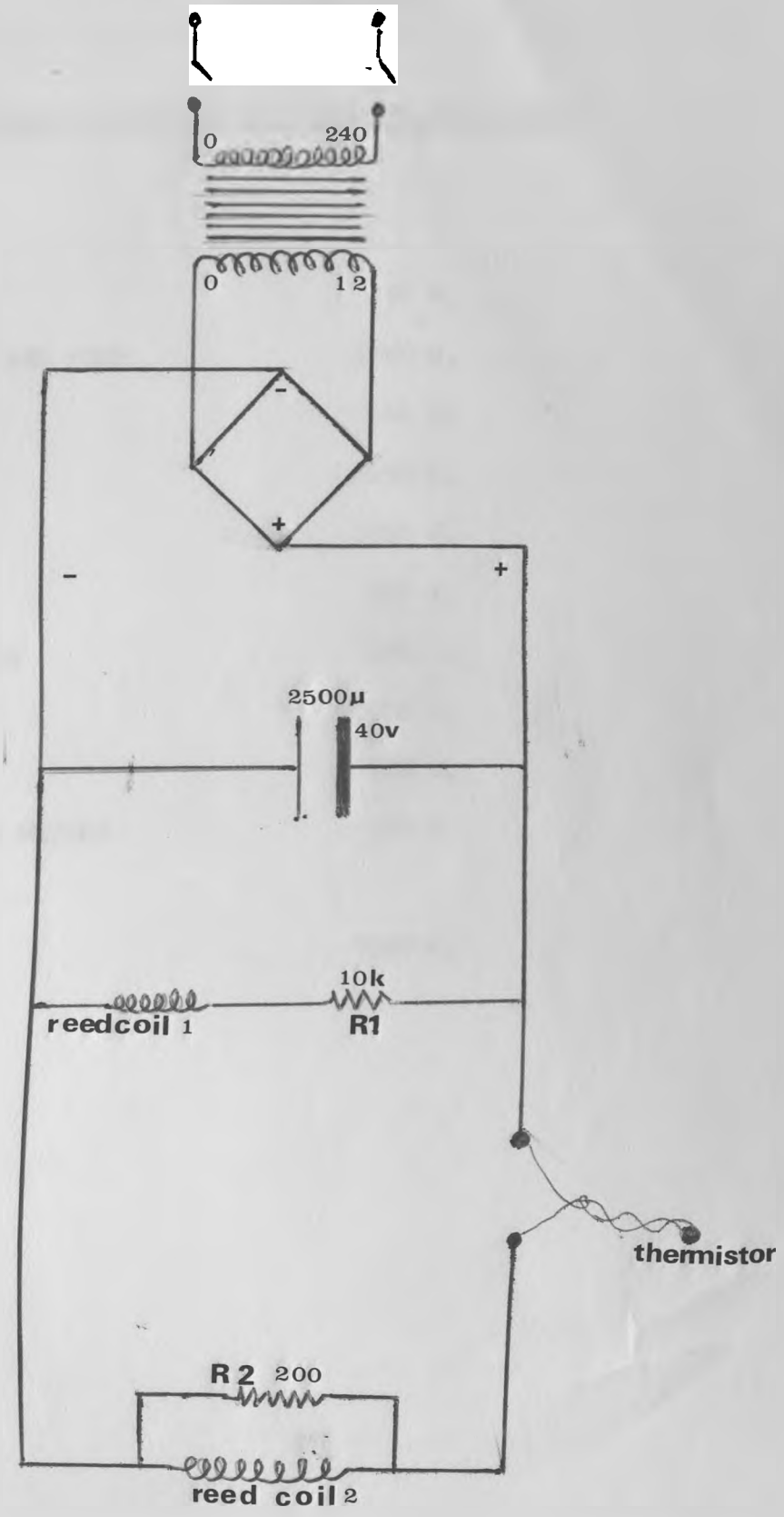
Fig. 26 (c)



FUNCTION:	CONDITION OF LOGIC CIRCUIT						
	RS5	RS7	R5	R6	R8	SV4	SV5
Receiver Jar 3 empty	E	E	E	D.E.	E	E	E
Receiver Jar 3 filling	D.E.	E	E	D.E.	E	E	E
PVS empty: filtration stops	D.E.	E	D.E.	E	E	D.E.	E
Receiver Jar overfull: fail safe		D.E.					D.E.

Fig. 26 (d)

THE POWER SUPPLY CIRCUIT FOR EACH THERMISTOR



APPENDIX (IV)

THE APPROXIMATE CONSUMPTION OF POWER BY THE FERMENTATION PLANT:

FILTRATION UNIT	50 W.
REFLUX CONDENSER COOLERS AND PUMP	1000 W.
ANALYSERS	400 W.
RECORDERS	250 W.
AIR COMPRESSORS	2300 W.
AIR DRIER	600 W.
F.Z.7 AND ASSOCIATED PUMPS	350 W.
STIRRER MOTORS	2100 W.
GAS RECYCLE PUMP	400 W.
SOLENOID VALVES AND PURGE METERS	100 W.
TOTAL	7550 W.

APPENDIX (V)

PRICE LIST AND SUPPLIERS OF THE EQUIPMENT USED IN THE FERMENTATION PLANT:

ITEM/MODEL No.	FIGURE IN TEXT	NOTATION	ADDRESS	PRICE (£) each
GF007 Fermenters	4	F1, F2	Chemap AGalte Landstre.415 8708 Mannendorf ZH Schweiz.	3,100
F.Z.7 Units	17	F.Z.7	"	400 included in fermenter.
90JF pneumatic recorder	4/2	4	Taylor Instr.Co. Gunnels Wood Rd., Stevenage, Herts.	122
86K Pressure Indicator/ Controller	4/2	6	"	152.90
70 1TE 11 Current to pneumatic transducer	4/2	12	"	94.65
72 ITD 153 Millivolts to pneumatic transducer	4/2	20A	"	287
9IJ/402R Pneumatic recorder	4/2	20B	"	235.40
10255 Transcope controller (X 2)	4/2	with 20/A20B	"	95.60
Pneumatic differential transmitter 10B3494	4	5	"	120.40
10L 3460S Shunt tube D.P. Cell (X 2)	4	3	Fischer-Porter Co. Warminster Pennsylvania,U.S.A.	137.60
Glass tube rotameters	4	17/24	"	17.00
Solenoid Valves	4/13/24	23/32/33/ SV1-6/Mx/N S1/S2/R1/R2	Dewrance Controls Grimrod Place, Skelmersdale,Lancs.	9.10

Speedomax H/recorder controller	4/2	13	Leeds and Northrup	370.00
Speedomax H/recorder	4/2	41/22	"	415.00
Speedomax W recorder	4/2	36	"	472.00
Flowstat constant flow valve	4	18	G.A.Platon Wella Road, Basingstoke,Hants.	24.00
Minim control valves	4	7/14/25	"	85.00
Gas cylinder regulators (X 3)	4	2/9/6	British Oxygen Co. Welging Products Div. North Circular Rd. London, N.W.2.	22.00
300 LIRA Gas analysers (X 3)	4	19/20/21	Mine Safety Appliances, Queenslie Inds. Estate,Glasgow	670.00
OA137 Oxygen analyser	4	11	Servomex Controls Crowborough,Sussex	551.00
E11 91B pH meter/controller (X 2)	4	38	Electronic Instr. Ltd. Richmond, Surrey.	190.00
Glass tube rotameters (X 2)	4	29/30	G.E.C. - Elliott Process Ints. 330 Purley Way, Croyden	18.20
Filtration Rig	24	Various	Millipore (UK)Ltd. Millipore House, Abbey Rd. London N.W.10 75P	321.92
Self Priming Pump for the Filtration Rig 11810-200	24	P	ITT Fluid Handling Ltd. Belcar Indus. Estate,Essex Rd., Hoddesham, Herts.	36.86

Filtration Rig control unit	26	Various	Dept.App.Microbiology Strathclyde University Glasgow G.1.	50.00
Ethane Purge Line pulse counter/integrator	14/15	"	" "	40.00
Swagelok Quick release couplings	4/24	Q_1Q_2	Techmation Ltd.,58 Edgewar Way, Edgeware Middlesex HA 88 JP	15.00
Wet Tyre Rotary Gas meters	4	26	Alex.Wright 77 Gloucester Rd., Croydon, CR 925 A	100.00
Siko Pressure Switches	4		Siko Controls Ltd. Romford,Essex	12.00
Pipework and Couplings		General	Brownall Ltd. Milton St.Royton Lancs.	200
pH Electrode	16		Activation Glass Kinglassie,Fife	16.00
O_2 Electrode	23		App. Microbiology Strathclyde University Glasgow	10.00