PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Single Cell Protein [and Discussion]

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Single cell protein

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The following topics are covered in this paper: the European feedstuff business, size, shape and source; the protein and amino acid requirements of the principal target species; the place of single cell protein (s.c.p.); the raw material options and the technical challenge of large scale s.c.p. manufacture; fermentation of s.c.p., its stoichiometry, mass and heat transfer requirements; static and dynamic optimization and control; the pressure cycle fermenter; the principle of sterility and the engineering design constraints; the nutritional performance of I.C.I.'s 'Pruteen' and the future for s.c.p.

Single cell protein (s.c.p.) technology has had a most extraordinary history during the last 20 years. As a new venture, it has been expensively embraced by many industrial and academic suitors, and yet, as the sceptics remind me from time to time, none of these suitors has gained any satisfaction. This new business venture has had its detractors, who indulge in polemic reminiscent of the arrival of the steam railway, the iron ship and the motor car. The objectors raise issues of practicability, morality, economics, safety and even international politics. However, a number of major industrial organizations not noted for whimsical investment have substantial commitments in this new horizon of industrial microbiology. Perhaps the most satisfying aspect of the development has been the seemingly endless variety of microbiological and engineering approaches which will help in the solution of problems in other areas of what is becoming known as biotechnology.

In this paper I shall describe the progress made by I.C.I. in this field, but the views and opinions expressed are my own. I shall concentrate first on a description of the market in which s.c.ps must compete. The competition makes important demands upon the technology. Many of the answers to these demands have wider application to other developments in large scale industrial microbiology and I shall dwell on two areas in detail: aerobic fermentation and engineering for sterile operation.

S.c.ps have been in use for many years, for both human and animal nutrition. The ruminants rely heavily on the production of bacterial protein, which is absorbed in the hindgut and derived from a well controlled continuous anaerobic chemostat. The organisms in the rumen consume cellulose as the major carbon and energy source, and use plant proteins, or urea as substitute, for nitrogen. These animals have been around for thousands of years, but it has taken man until the middle of the twentieth century successfully to design, construct and operate similar installations for the production of an animal feedstuff. The reasons for the delay are not difficult to find. To enter the s.c.p. business, the complete technological armoury of the large scale chemical industry is essential. To the skills index that includes chemical, mechanical, civil and electrical engineering, chemistry, mathematics and computer science, physics and material

science must be added microbiology, biochemistry, toxicology and nutrition. This is not to deny a place in this technology to the small operator using specialized raw materials and serving a local need for animal feed protein. Operations of this type are already a feature of s.c.p. technology around the world. For the largest scale operation, however, all those technologies are essential, and the technologists must learn to talk a common language.

The production of s.c.p. on the large scale, though, is complex and difficult, so why should it be attempted? What is the driving force? For most serious students of world food production, it is clear. S.c.p. will complement the traditional agricultural system of the advanced western world. It will be used only in the nutrition of intensively reared animals, alongside the traditional protein sources, such as fish and soya. This method of food production in western Europe, for example, is now dominant and has been growing steadily since World War II, as the nutritional requirements and optimum growth conditions of pigs, broilers, laying hens and veal calves have been carefully researched and understood. I believe that s.c.p. will have no place as a direct replacement for traditional human foods during my lifetime. Those countries in the world having the highest *per capita* income consume the most protein per head, mostly as meat.

TABLE 1. 1977 E.E.C. ANIMAL FEED USAGE AND IMPORTS

	usage / Mt	imports / Mt	price / $ t^{-1}
soya meal	10.93	10.88	230
fish meal	0.85	0.39	454
other meals	6.57	4.83	192
total protein meals	18.35	16.10	224
cereals	122	28	105
others	10.7	10.7	150

The converse can also be shown. Countries with the lowest income have the highest carbohydrate incorporation rate in the diet, mostly as root crops, cereal grains, bread and related products. Further, those of us who *can* afford to eat meat are unlikely ever to forego the privilege, and those who *cannot* will find such products of advanced western technology beyond their financial grasp. Our meat production industry is supplied with its raw materials by a feed formulation business of immense proportions. The traditional source of protein for the Western European animal feed industry is the soya bean, with major contributions from fish meal, cotton seed, ground nut and rape seed meal, as well as from other by-products of the vegetable oil and food industries.

The data in table 1 show that soya bean dominates and that nearly all the soya meal, nearly half the fish meal and most of the other protein meals are imported. This import proportion is unlikely to change in the next decade. Indeed, the E.E.C. import bill for animal feed in 1977 was $\$8.1 \times 10^9$, of which protein meals cost $\$3.6 \times 10^9$. At the political level, the substitution of these imports is highly desirable. The destination of these ingredients by target species in the E.E.C. is shown in table 2, which covers the major sectors: pigs, poultry and cattle concentrates. Significantly, the poultry industry, which is the most intensive, uses the highest proportion of factory-formulated feed and the pig industry relies most heavily on farmer-made rations. This increased emphasis on intensive rearing and the greater understanding of animal nutritional requirements has been accompanied by the use of sophisticated techniques to formulate animal diets at least cost to the user. The first commercial application

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of linear programming was the formulation of least cost diets from the bewildering array of raw materials available to the compounder. Table 3 shows a typical list available to a large scale broiler feed compounder, which is influenced by where the mill is situated and the availability of stock materials on the day of formulation. This latter is particularly significant, since working capital tied up in this way is extremely small for such a vast industry. The closeness of the mill to sources of the minor constituents such as wheat middlings, offals and feather meal, frequently governs their use. A typical broiler diet analysis is given in table 4 and the purpose of the linear programme is to formulate a diet to these optimum requirements.

TABLE 2. 1977 E.E.C. COMPOUND FEED PRODUCTION AND PRICES

	feed production/Mt		price
	manufactured	farm-produced	$/t
poultry	18.4	3.9	315
pigs	23.5	23.1	290
cattle	22.9	5.6	275
	2.8	3.4	290
total	67.6	36.2	290

TABLE 3. CONTENTS AVAILABLE FOR TYPICAL BROILER RATION

soya meal	wheat	molasses
herring meal	barley	salt
white fish meal	maize	lime
'Pruteen'	wheat middlings	dicalcium phosphate
meat and bone meal	sorghum	
ground nut meal	tallow	
offals	corn/maize gluten feed	
feather meal	amino acids	

TABLE 4. TYPICAL BROILER DIET ANALYSIS

crude protein	20 %
metabolizable energy	12.5 MJ/kg
methionine	0.5 %
methionine + cystine	0.9 %
lysine	1.2 %
calcium	1.0 %
available phosphorus	0.5 %
salt	0.35 %

TABLE 5. 'AVERAGE' U.K. BROILER DIET COMPOSITION (%)

wheat	70
soya meal	15
fish meal	3
animal meal	5
tallow	5
minerals	2

There are other qualitative constraints that limit the inclusion rate of some raw materials. Many of these are concerned with physical qualities of the compound product such as tallow and wheat, some with problems of flesh colour of taint, e.g. in maize and fish meal, and yet others, because of known or supposed toxicological or nutritional effects. The least cost formulation programme is designed to deal with all these constraints. Having taken into account

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the variables of analysis, availability, price, quality, required composition and inclusion constraints, the U.K. feed compounder will probably end up with the typical analysis shown in table 5.

The place of s.c.p. is clear: it is another source of high quality protein, which has a high concentration of the essential amino acids and is rich in minerals and vitamins, and also has high digestibility and metabolizable energy. Table 6 shows the comparative analysis for I.C.I.'s 'Pruteen' and for competing protein sources. It is just these data that the compounder uses in formulation. There is one unique advantage to all s.c.ps. They are made to a single and constant analysis. Modern instrumentation and control techniques, coupled with a thorough understanding of the effect of processing conditions, guarantee that product quality is maintained within very narrow limits. All the other constituents of a diet will vary in analysis from load to load, field to field and season to season.

TABLE 6. PROTEIN FEEDS FOR POULTRY

	Pruteen	soya	fish meal	herring
crude protein (%)	74	45	66	71
methionine + cystine (%)	2.3	1.3	2.4	2.8
lysine (%)	4.6	2.9	4.8	5.4
Ca (%)	0.07	0.3	7.0	2.5
P (available) (%)	2.4	0.3	3.5	1.8
metabolizable energy/(MJ kg^{-1})	13.8	9.7	11.3	13.4

We can see that the E.E.C. market is immense and sophisticated and, for the purposes of market penetration with a new product, well regulated and relatively easy to understand. It is, therefore, ideal for the product of high technology. Today, I.C.I.'s 60 kt/a facility in Billingham is in the final stages of construction. If this project is successful, consideration will be given to the building of other large plants over the next decade.

Worldwide research into the production of s.c.p. has investigated the use of a wide variety of microorganisms on many carbon substrates. Thus, the growth of yeasts, algae and bacteria on methane, methanol, ethanol, *n*-alkanes and a range of cellulosic and other wastes is the subject of ongoing research. A recent survey carried out by a consultant for I.C.I. and other industrial interests indicated a world s.c.p. capability of over 2 Mt/a, of which 1.2 Mt/a is based upon carbohydrate waste. None of the latter group of facilities has an output larger than 10 kt/a. Needless to say, many of the facilities are no longer operational and a large proportion of the worldwide capability is accounted for by the Soviet Union and the Communist Bloc countries.

The choice between the available substrates, assuming a comparable yield and product quality, depends upon geography, cost, alternative uses for the raw material and, perhaps most important of all, ease of processing. In practice the choice made by most industrial organizations seems to have been determined by their existing business. Thus, it would have been strange to find I.C.I. developing processes based on sulphite liquor from the pulp processing industry; similarly, I would have been surprised if Tate and Lyle had declared an interest in alkane technology.

I.C.I.'s initial thinking was directed towards the use of methane as substrate. While, in part, this was because of the ready availability of methane from North Sea gas, it also seemed, at first sight, to provide an elegant biochemical route from one of the simplest of organic molecules directly to protein. Very rapidly, however, I.C.I. moved away from methane as substrate,

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partly because of the potential hazards associated with attempting an aerobic process based on the gas, but, more importantly, because of mass transfer problems and the problems of process control associated with the need to use mixed cultures. These problems were perceived to be major stumbling blocks resulting from the very sparing solubility of methane and the impossibility of finding a single microorganism likely to give economic levels of productivity and yield from methane.

In the microorganisms that metabolize methane, a key step is the initial oxidation to methanol. Since the bacterium has no way of recovering the energy from this process, it was clear that this substrate could be more effectively predigested for nature in an efficient, modern methanol plant. I.C.I. is a world leader in conventional technology for the production of methanol; some 80 % of world methanol capacity uses I.C.I. technology and catalysts. It was judged that the use of methanol as substrate preserved the elegance of the technical approach and substantially improved the prospects of identifying a single microorganism capable of the rapid and efficient growth essential for the economic survival of an s.c.p. project.

After substrate selection, the microorganism must be found. The characteristics necessary for successful development are that the microorganism must be stable and capable of high productivity and yield on methanol as sole carbon source, that it is safe in use, and that it produces a nutritious end product. A bacterium was seen as likely to be capable of matching these characteristics, since, generally, bacterial growth rates and yields are high and the final crude protein content of the end product of bacterial fermentation is generally higher than that of other organisms.

The importance of pathogenicity testing in any microorganism used for large scale production is well known. I.C.I.'s process organism has been tested for conventional pathogenicity in mice. It is not pathogenic and bears no relation to known pathogenic species of bacteria (Stone 1973).

In its initial search, I.C.I. examined a large number of methylotrophs. Those that appeared promising after first evaluation were subjected to preliminary screening for pathogenicity and toxicity. The outcome of these investigations was the selection of a single methanol obligate, now known as *Methylophilus methylotrophus*, for further experimental work.

Simultaneously with this early programme of biological search, consideration was given to the development of process engineering principles to permit the eventual commercialization of the project. From the description of the market place in which any s.c.p. must operate, it is clear that protein is plentiful and cheap. To survive in this business environment, skilled fermentation research is essential to exploit the full potential of the bacterium. This alone is insufficient. From the early days, when capital and operating costs were calculated on the back of an envelope, it was clear that only the exploitation of the economies of scale and the successful development of the single stream plant would allow large-scale s.c.p. manufacture to prosper. This may not be necessary for small-scale producers using cellulosic substrates having a near waste value.

Sadly, these economies of scale could not be realized by the technology available at the end of the last decade. In the three main capital cost centres, fermentation, bacterial separation and dewatering, and product drying, it seemed that multi-stream processing was inevitable. Multiple fermenters and driers and an aircraft hanger full of the largest centrifuges in the world presented problems, which, if unsolved, meant the end of all methanol–bacteria processes and, in my view, of all other large scale s.c.p. processes. I should like to deal with I.C.I.'s solution to these problems in a little more detail.

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Chemical engineers faced with the problem of scale-up, approach fermentation, at least in part, at the level of the first law of thermodynamics, mass transfer and stoichiometry. The complex metabolic process can be reduced to a stoichiometry that defines the targets in terms of heat evolution and oxygen uptake rates. Of course, this is a simplification, but it shows the basic information (table 7). These numbers may then be converted to gross fermentation requirements, heat and mass transfer data per unit volume of fermenter (table 8).

TABLE 7. STOICHIOMETRY OF ALKANE AND CARBOHYDRATE FERMENTATION

n-alkane/yeast

$$0.143\ C_{10}H_{22} + 0.19\ NH_3 + 1.19\ O_2 \rightarrow 1.0\ [CH_{1.7}O_{0.5}N_{0.19}\ \text{ash}]\ \text{cells} + \text{essential nutrients} + 0.43\ CO_2 + 1.01\ H_2O + 4.41\ \text{MJ}$$

carbohydrate/yeast

$$1.43\ CH_2O + 0.19\ NH_3 + 0.40\ O_2 \rightarrow 1.0\ [CH_{1.7}O_{0.5}N_{0.19}\ \text{ash}]\ \text{cells} + 0.43\ CO_2 + 0.87\ H_2O + 210\ \text{MJ}$$

TABLE 8. FERMENTATION HEAT AND MASS TRANSFER REQUIREMENTS

	oxygen requirement / kg m^{-3} h^{-1}	CO_2 removal / kg m^{-3} h^{-1}	heat removal / MJ m^{-3} h^{-1}
n-alkane/yeast	20.2	10.0	234
carbohydrate/yeast	6.8	10.0	111

The reason for approaching the fermentation requirements in this way is just a practical one. Small-scale fermentation does not lend itself readily to the extraction of thermal data. Even oxygen uptake tends to be an inaccurate measurement involving the difference of two large numbers. It is this type of problem that makes pilot plant scale operation essential.

This high rate activity is unusual for conventional fermentation and it was clear to us that the conventional fermenters of the day, while being able to cover this range on a small scale, could not be scaled up simply without considerably more experience of 100–1000 m^3 fermenters. It must be remembered that at the turn of this decade the fermenters of this size that *did* exist were principally used in the antibiotic industry which generally does not have to deal with gross heat and mass transfer problems of this magnitude. There was a further problem: all fermentation research had been carried out in vessels of 1–3 l capacity with a mixing time of less than $\frac{1}{2}$ s. Not only is the gross performance of a large stirred tank hard to predict, but, at the macro level, mixing patterns are uncertain. This leads inevitably to the fear that certain fractions of the volume in a mechanically agitated tank will remain outside the range of physiological condition required for optimum microbiological performance. These conditions include pH, temperature, substrate and other nutrients concentration and liquid phase oxygen and carbon dioxide concentration. Indeed, a natural consequence of the high rate of fermentation is the increased difficulty of controlling the dynamics.

The fermentation research then switched from the search for the optimum condition, which, in real life, can only be approximately achieved, to an examination of the margins of the allowable physiological conditions, and the ability of the microorganism to withstand random or constant cycling within these boundaries. It may well be that a study of change and rate

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of change of physiological conditions during a fermentation, coupled with an understanding of mixing patterns, would explain some scale-up mysteries.

The chemical engineering solution to these difficulties was as predictable as it was successful. The random fluid mechanic process was replaced by the ordered. Only plug flow of the fermenting mass would guarantee the control of the fermentation within the prescribed limits and enable the rate of change of physiological conditions experienced by the microorganisms to be held within calculable limits. Of course, even so-called plug flow has a velocity distribution in the most turbulent condition, but the residence time distribution so obtained is at least subject to simple calculation, no matter how crude. Figure 1 shows a diagrammatic representation of I.C.I.'s pressure cycle fermenter, which is used in the 'Pruteen' process. A variant on this has also been designed and operated for use in the sewage and effluent treatment business, currently sold under licence with the trade name 'Deep Shaft'.

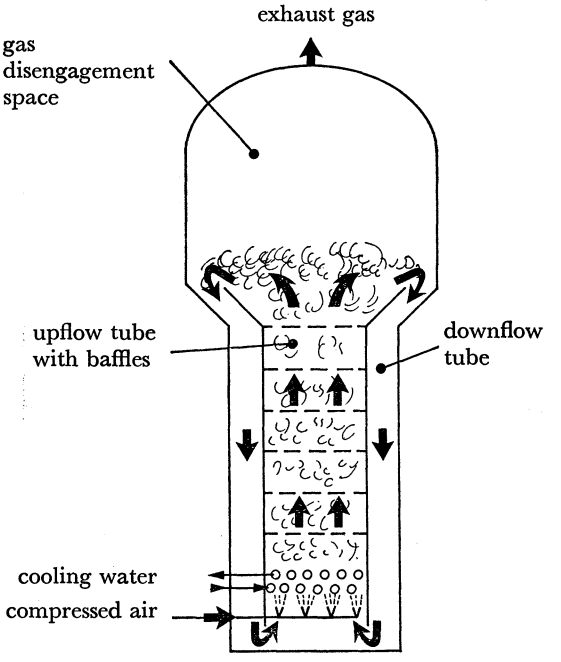

FIGURE 1. I.C.I. pressure cycle fermenter.

The basic principle that this device uses to create mass transfer of oxygen from the gas stream to the liquid medium and of respiratory CO_2 in the opposite direction is the utilization of the turbulent energy dissipation occasioned by the flow of the gas–liquid mixture up the ascending side of a baffled gas-tight tube. In this way, adequate values of $K_L A$, the product of oxygen mass transfer coefficient and the interfacial area per unit volume, are maintained. The fermenter is thus essentially a large diameter ascension tube together with a smaller return tube, or tubes, for the substantially unaerated liquid. Circulation is obtained by the static head difference obtained from two liquid columns of equal overall height but different gas voidage fractions.

The top of the fermenter is shown widened to enable the gas bubbles to separate from the ascending liquid stream before the liquid returns down the descending tube. Baffles are placed at intervals across the ascension tube, which generate turbulent mixing in the liquid–gas mixture,

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thereby creating new gas–liquid surface and a high mass transfer coefficient. The fermenter riser tube can be compared with a series of conventional, sparged, baffled and turbine agitated tanks. It differs, however, in two ways: the liquid flow pattern is plug flow through and not mainly back-mixed, and the agitation is imparted by the pressure cycle pump energy and not by a turbine.

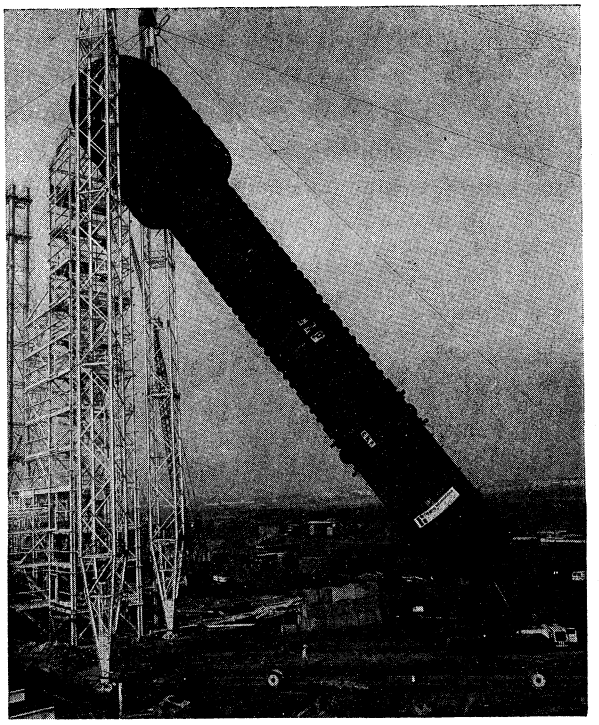

FIGURE 2

In this device, the fermenting mass is circulated between areas of high and low pressure, which favour oxygen uptake and carbon dioxide dissolution, respectively. Having established the circulation rate and hence cycle time, and knowing the gross activity of the fermentation, the rate of change of physiological condition may be calculated. Back in the laboratory, it is possible to model this dynamic condition and check the ability of the microorganism to withstand it. This complex interaction between the microorganism and fermenter design must be properly understood. The designs developed for 'Pruteen' and for effluent treatment are quite distinct, and are unlikely to be suitable, without further research and design, for other fermentations.

The fermenter shown in figure 2 was built in France and brought to the Tees on a powered barge. The barge had not been built when the fermenter was being designed, and some modification to the barge during its construction was necessary to take this load. A special road was built for its journey from dock to site including traversing rail tracks and public roads. It weighs 600 t and is over 60 m high. It was workshop-built in one piece to minimize the number of site welds. This, I believe, is a major contribution to the principles of sterile engineering and operation. The fermenter is self-supporting, but eventually will be clad within its own sheeted housing. This is principally a heat loss reduction exercise for sterilization.

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I referred earlier to separation and drying as areas requiring some invention if the economies of scale were to be exploited. The problem was double-edged. Large numbers of centrifuges were needed to provide a protein-rich stream suitable for drying, and, even then, the drying load was too large for single stream operation. This problem has effectively been solved by a flocculation process that permits very high solids concentrations of bacteria to be formed, and thereby makes a valuable reduction in drying load.

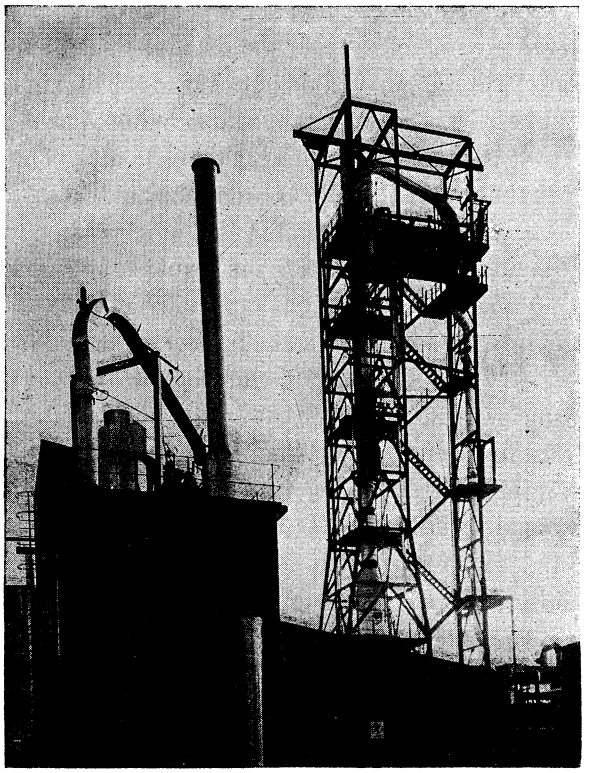

FIGURE 3

All these advances were incorporated in a 1 kt pilot plant constructed in the Research & Development Department of I.C.I. Agricultural Division, which was commissioned in 1972, just 4 years after the first laboratory fermenter had been inoculated (figure 3). Needless to say, the plant has been modified many times and is just now completing an extensive refit to test the latest additions to the technology for the next generation of plants.

The last major area in which major advances were required was sterile engineering. This new collection of disciplines is aimed at the maintenance of high on-line time for continuous fermentation plants. The major target is the exclusion of all foreign microorganisms at the fermentation stage. Any foreign aerobic microorganism introduced accidentally could, and usually does, stabilize to form a large and unacceptable proportion of the fermenting mass. At worst, the contaminating microorganisms are of unknown toxicity and pathogenicity and are likely to have a deleterious effect on product quality as well as failing to satisfy the justifiably high standards required by national registration authorities. Also, it has been our experience that contaminating bacteria reduce substantially the yield of *Methylophilus methylotrophus* on methanol.

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Much has been written over the last decade about a microbiological solution to the problem. The search for thermophilic organisms and those that grow at the extremes of pH will, it is believed, if successful, solve the problem of contamination by avoiding the need to maintain high standards of engineering. This approach is a trap for the unwary, and, as far as I know, no major organization has used it seriously. This route could lead to low levels of contamination but an almost 100% chance of getting one. It is an appalling admission of failure to solve a major problem in industrial microbiology. The problem has been solved by the application of the conventional techniques of science and technology.

The problems of the maintenance of sterility divide into three main areas. The continuous sterilization of feed streams, whether gaseous or liquid, the sterilization of the plant at start-up and the operation of the plant in a way that minimizes the chance of loss of sterility. I put them in that order since that is the order of increasing difficulty. The continuous sterilization problem has, of course, a trivial solution. Heat sterilization is well known and the absolute filtration of gases has been commercially available for some years. Sterile filtration of liquids, however, poses some problems. Absolute filters for liquids have a life based upon the dirt loading, which, even for potable waters, is high, especially in the sub-micrometre range. The heat sterilization or depth filtration solutions, though, pose an interesting paradox. The solution required is absolute, that is to say, during the working run of the plant, no foreign micro-organisms will enter the sterile area. However, the means of calculation for design is relative. A failure rate has to be assumed before design can start, since the calculation is based on the fraction surviving or escaping the sterilizing process. An assumption of a zero failure rate leads to an infinite numerical solution, either infinite residence time in heat sterilization or infinite depth in filtration.

The second area is sterilization of plant and equipment. The first observation is that the sterile area of plant, with associated pipework and equipment, is complex, and considerable attention to design detail is required to avoid the inclusion of areas that may fail to achieve sterilizing temperature. Valve design and pipework isometrics need special attention. Here, I am referring to steam sterilization. There is little experience of the chemical sterilization of large process plant, and I suspect that this will always be so. There is no substitute for therms. Interestingly, the selection of appropriate steam pressure for this initial sterilization owes nothing to the microbiological process but rather depends on the engineering design of the equipment. The maximum sterilizing steam pressure is partly determined by the allowable working pressure, which is considerably higher than the usual 15–25 lbf/in²† used in microbiological laboratories, since the primary design needs of the fermenter are its hydraulic and supporting loads. The other determinant is the maximum operating condition of the non-metallics used in fermenter design. Fixing this temperature as high as possible is important. Vessels of the size of the 'Pruteen' fermenter present large heat sinks to the sterilizing steam, especially around flanges, pipe supports and the many non-steam-carrying appendages that are a feature of large fermenter design. In I.C.I.'s pilot plant experience, failure to achieve initial sterilization was the major cause of fermenter down time.

The third major problem area is the maintenance of sterile integrity, especially during the start-up sterilization stage. The number of individual manual operations of valves, instruments and pumps can, even for the pilot plant, run to many hundreds. Failure to observe the precise sequence can lead to incomplete sterilization with consequent failure by contamination within

† 1 lbf/in² ≈ 6895 Pa.

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a few days. The solution to this problem is the computerization of the start-up sequence and all subsequent process operations within the sterile sections of the plant. The sequence may not be broken easily, only by high level intervention, and not, for example, by *ad hoc* manual operation of valves. Any such intervention is noted by the computer and recorded on hard copy for later examination. In effect, the sequencing programme prevents the next set of valve movements or operations before the complete execution of the current set, which includes the achievement of the time and temperature constraints where necessary.

TABLE 9. PRINCIPLES OF STERILE AUDIT

independent auditors
freedom of examination
report to senior management
content – physical state of plant
sequence of operations
training of operators
plant records
engineering integrity

The final element of sterile operation concerns the mechanical integrity of the plant. This lesson has been learnt the hard way during the development of the 'Pruteen' project. The pressure cycle fermenter provides a major step forward by eliminating the mechanical drive through the sterile boundary. After mechanical seals, all plant closures, whether welds, non-metallic joints, valve closures, probe insertions or sample ports, are the major source of leak paths between the sterile plant and the outside world. Our procedure has been to replace mechanical closures by welds wherever possible, even if subsequent maintenance operations are made more difficult by replacing simple spanner operations by cutting and rewelding. There is little novelty in that however, since it has been a procedure adopted in acid plants for many years. Considerable design effort has been put into the improvement of closures and into the supervision of assembly. For particularly difficult or complex areas, the closure is surrounded by a further sterile barrier, usually a steam chest. The obsessive attention to detail in this area is justified, since any continuously operating plant, whether microbiological or otherwise, is dependent upon high on-line time for its profitable operation. Over the last few years, the pilot plant at Billingham has regularly achieved uncontaminated runs in excess of a hundred days, which is just reward for this obsession.

With such lengthy runs I suppose that complacency is the biggest threat, and, recently, a new phrase has entered the vocabulary of fermentation plant operators. We are all familiar with the financial audit, the safety audity, the 'haz.-op.' study, and method and operability studies. The 'sterile audit' is now a regular feature of plant operations and, as table 9 shows, follows similar principles to the financial and safety audits. Independent auditors, not usually directly concerned with plant operations, and having complete freedom of examination, carry out, at regular intervals, an exhaustive study of the plant. The report is available to senior management, who are held accountable for carrying out such recommendations as are made. The examination covers the physical state of the plant, which includes such aspects as house-keeping and state of lagging. The operational sequence and working practices, which include the training levels achieved by the plant management, and all process and maintenance records, are looked at. Engineering integrity is usually the most fruitful area of search. Table 10 shows some features of this section of the audit and covers minute details of the history of

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plant operations as well as questions concerning the design of the plant with respect to most recent practice.

I have spent some time outlining the needs of s.c.p. plant design and operation; it is instructive to examine how all these features of fermentation, raw material efficiency and capital cost influence the capital and operating costs. From table 11 two facts emerge. Within the capital group, fermentation plant itself is by no means dominant. It is a fact of industrial life that costs of services, which include cooling water, gas and electricity supply off-site and roads, offices, laboratories, workshops and amenities on site, are always high on the list. Notice also that, even in this modern technology, the cost of packing, storage, materials handling and export facilities are depressingly high. The dominant position of the carbon source, methanol, in operating costs is only to be expected and is the driving force behind the microbiological research.

TABLE 10. STERILE AUDIT – ENGINEERING INTEGRITY

number of, and need for, sterile closures
balance between sterile hazard and essential maintenance
piperun isometrics
line diagram integrity
temperature history of non-metallics
evidence of leaks at closures, valves and welds
sterile risk reports, records and actions
temperature record integrity
steam trap operation

TABLE 11. SINGLE CELL PROTEIN COSTS

capital (%)		operating (%)	
dewatering	19	methanol	59
offsite services	16	energy	23
fermentation	14	medium	17
drying	12	water	1
storage packing	12		
onsite services	11		
compression	9		
effluent treatment	4		
raw materials	3		

I.C.I.'s s.c.p. has been available in development quantities from the pilot plant since 1972, and nutritional performance data has been obtained from over 250 carefully designed experiments, involving over 500000 animals. These evaluations have been carried out by I.C.I. and by independent research organizations, as well as potential customers in Europe, the Middle East and Japan. The main evaluation has been against fish and soya meal at equal dietary amino acid level.

To illustrate the high performance of 'Pruteen', I have selected a major new horizon in larger scale biology. A few years ago fish farming, the growth of fish, particularly trout and salmon, in contained environments, was virtually unknown. Today 60000 t/a of fish for human consumption is so produced. There is active research and development by Government agencies and industry; the business is growing at a rate of between 5 and 10% per year. Figure 4 shows performance improvement in terms of live mass gain from the inclusion of 'Pruteen' in the diets of salmon, trout and carp. All data obtained to date, from some twenty trials, lie between these lines, which shows live mass gain improvement from the inclusion

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of 'Pruteen' in traditional fish diet. The scatter of results implied by the figure is typical for commercial nutrition experiments. Perhaps it is appropriate that such exciting results should be reserved for the combination of two novel projects.

The *future* of s.c.p. depends almost entirely on technological improvements. If s.c.p. is to replace traditional foodstuffs in increasing quantities, substantial progress, with contributions from all the scientific and engineering disciplines, is required to continue to reduce production costs. In this sense it is no different from the development of fibres, plastics and commodity chemicals, which have been driven hard down the learning curve by technological change.

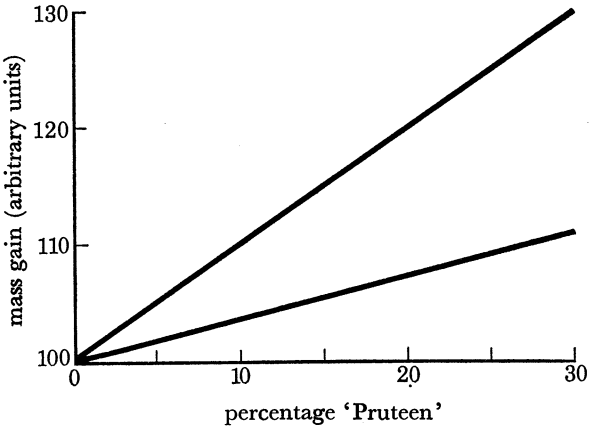

FIGURE 4. 'Pruteen' in fish diets. The gain in mass is calculated relative to a control with a mass gain set to 100 units.

A certainty, however, is a substantial gain in know how and technology from the operation of plants of this type. Such gains must be exploited if the manufacture of high volume, low value products is to be a sensible target for biotechnology.

REFERENCES (Smith)

Stone, H. 1973 In *Single cell protein*. Rome: C.T.I.P.
Stringer, D. A. & Wilson, A. B. 1973 Toxicological studies with Pruteen. *Protein advisory Group Bull.* **6** (3), 45.

Discussion

E. G. BEVERIDGE (*School of Pharmacy, Sunderland Polytechnic, Sunderland SR*1 3*SD, U.K.*). First, at what point in the process does strict sterility control stop, and, secondly, is 'Pruteen' crude biomass?

S. R. L. SMITH. There is a sharp cut-off between strict sterility control and hygenic conditions, but I am unwilling to specify precisely where. In answer to the other question, 'Pruteen', in common with all single cell protein products, is essentially crude biomass.

C. ELTON (*Beecham*). There seems to be a slight paradox here. This morning we heard of the efforts to convert biomass (sugar) into ethanol and now we are told of a process to convert methanol into biomass. Why not keep the methanol as a liquid fuel and convert the biomass directly into feedstuff?

S. R. L. Smith. The economics depend on location; there is no single best course.

Anon. Are mutational problems expected?

S. R. L. Smith. No; and, in fact, *Methylomonas methylotrophus* is difficult to mutate deliberately.

J. F. Danielli, F.R.S. (*Worcester Polytechnic Institute, Worcester, Massachusetts* 01609, *U.S.A.*). Does the I.C.I. production plant at Teesside consist of a single fermenter?

S. R. L. Smith. Yes, it does.

I. R. Booth (*Department of Microbiology, University of Aberdeen, Aberdeen AB*9 1*AS, U.K.*). Since methanol is produced from a non-renewable resource, surely the cost of the production of 'Pruteen' will rise faster than that of competitive products?

S. R. L. Smith. I do not think that this will be a problem in the long term.

Anon. Does I.C.I. intend to use 'Pruteen' for human consumption?

S. R. L. Smith. No.

Anon. Is effluent treatment a problem?

S. R. L. Smith. No, but I.C.I. has taken the precaution of installing sufficient waste treatment capacity to cope with an entire fermenter load of liquor, should this prove necessary.

H. B. Herbert (*Imperial College, London, U.K.*). Has I.C.I. examined the toxicology of the constituents of 'Pruteen' that are not normally found in eukaryotic protein sources? I am thinking particularly of the components of bacterial cell walls.

S. R. L. Smith. Yes, toxicological tests have been performed on 'Pruteen' that would probably be sufficient to permit its use as a direct human food source.

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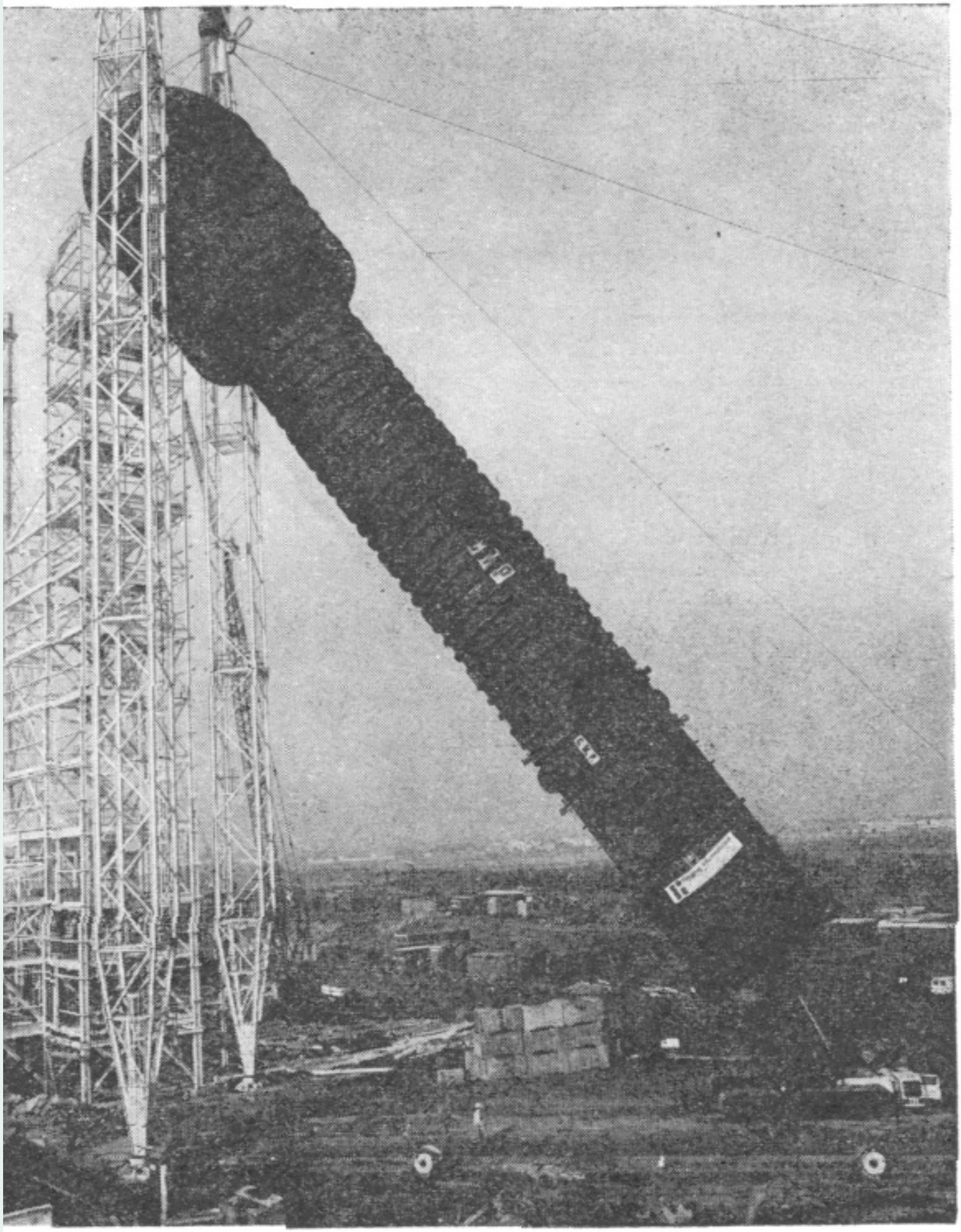

FIGURE 2

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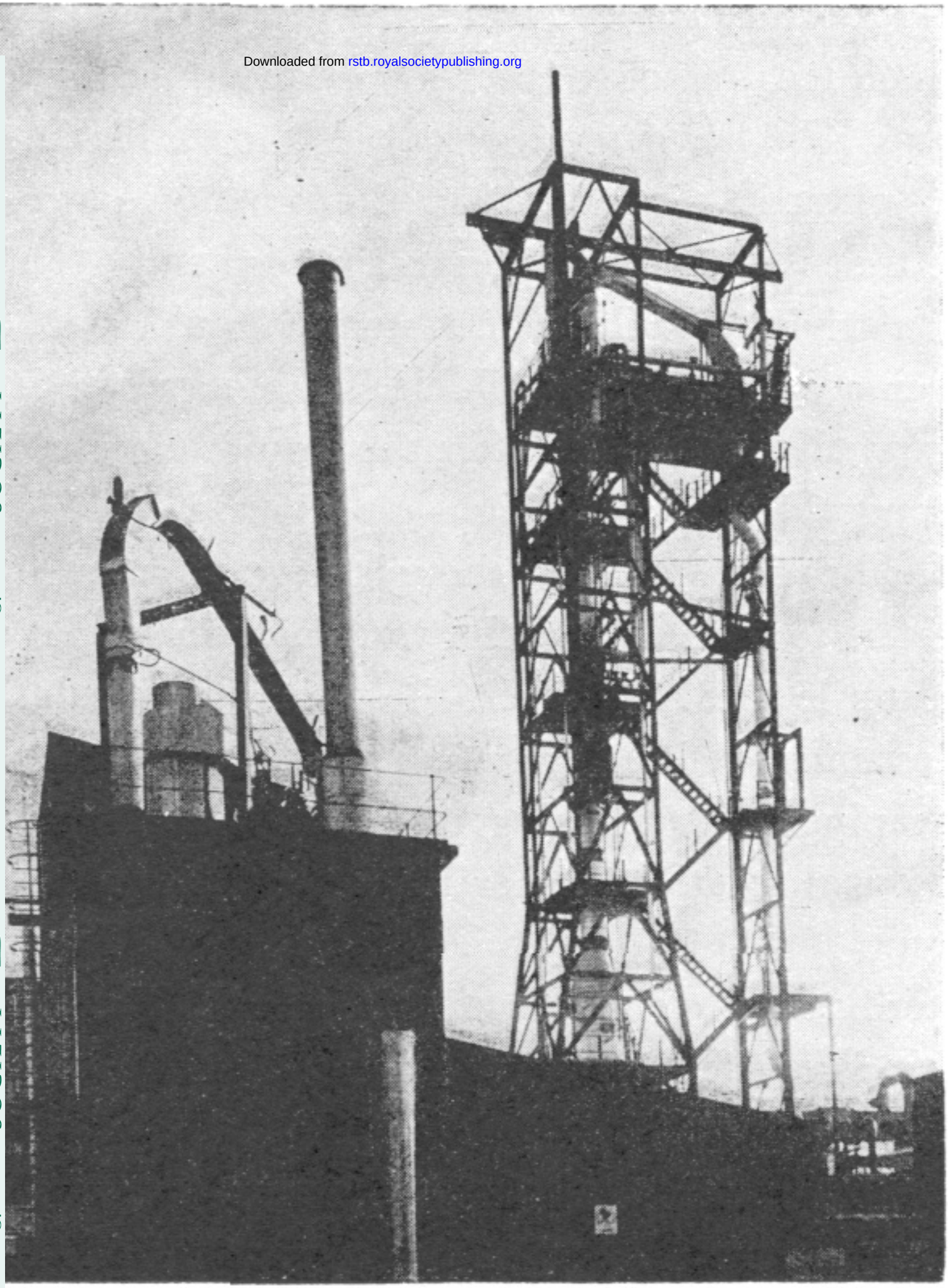

FIGURE 3

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES