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FERMENTATION PROTEIN FROM METHANOL1/

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FERMENTATION PROTEIN FROM METHANOL

Introduction

Rising standards of living in all countries of the world have led to an increased demand for protein in human diets. Although the total amount of protein available to the population is, in the case of nearly every country in the world, adequate to meet the protein requirements of an average individual in that country, in several countries not enough protein is made available to those that need it most - babies, growing children and nursing mothers. An increased supply of dietary protein in these countries is necessary, not only to meet increasing demand, but also the ensure the health of each sector of the population.

In the last two or three decades, increasing supplies of protein have come from intensively reared pigs, poultry and veal calves in so-called factory farms. The economics of the production of these animals demand that the maximum rate of production shall be obtained from the minimum investment by arranging that the animals are grown quickly during their early life and then slaughtered for food at a comparatively early age. The achievement of high early growth rate depends upon the supply of a food containing the appropriate amount of protein of the right kind. Most animal foods are based on grain which by itself does not contain the amount of protein needed in a balanced diet. A protein supplement is therefore used, and this is chosen so as to lead to the correct distribution of amino acids required by the growing animal. Such supplements have, during the recent development period of factory farming, been formulated from vegetable protein such as soya bean meal and animal protein such as fishmeal.

Although the production of soya bean has increased dramatically in recent years, there is currently a shortage. Production of fishmeal has also been greatly increased but this too is in short supply and is unlikely in the future to be available in the quantities required to meet the growing demand for high-grade protein food supplement.

Attention is now directed to the production of single cell protein by fermentation processes in order to meet the foreseeable deficit in protein supplies. The carbon in the produced protein is obtained from a fuel, which also supplies the considerable quantities of energy required by the fermentation process. Nitrogen is added in inorganic form, such as ammonia, and phosphorus and other mineral components are supplied from inorganic sources.

A. Choice of Feedstock

In known processes, the carbon and energy source may be either a hydrocarbon, an alcohol or a carbohydrate. The choice of substrate depends on the circumstances in the country in which the protein is to be produced, and may also soon depend upon global considerations of energy supply. In a few countries carbohydrate wastes (for instance, molasses) may be chosen. Their use does not deplete the world's stocks of fossil fuels: instead, the carbohydrates are produced continuously using the sun's energy. In other countries in which a large demand for protein is likely to arise, there are indigenous sources of hydrocarbons or coal. Attention has until recently been concentrated mainly upon the production of protein from liquid petroleum fractions, and the only fully developed processes currently available are of this type. The developing energy crisis will, however, lead to increased prices for liquid hydrocarbon products and there will be increasing concern at the depletion of resources. Known carcinogens are present in petroleum and an elaborate purification process is needed in order to reduce the level of these carcinogens in the feedstock to the protein plant in order to ensure that none can find their way into the product.

Most oil-producing countries also have abundant supplies of natural gas. Because of the readier transportability of liquid hydrocarbons to consumer countries, much of this natural gas is not used. Ideally, then, in such countries a protein process should be based upon natural gas. Unfortunately, despite intensive work in many laboratories throughout the world, no economic process for producing protein directly from

natural gas has emerged, and it must be now concluded that it is unlikely that one will be found.

Although the first generation of protein process has been based on liquid hydrocarbon substrates, attention is now being increasingly directed to the use of alcohols, and in particular methanol. Methanol can be produced by well-known and established processes from any liquid, gaseous or solid fossil fuel, including coal. It is now recognised that the world's coal resources greatly exceed the petroleum reserves so that increasing demands for energy will have, in future, to be met by the use of coal rather than hydrocarbons. In some countries, such as India, where there is clearly a case for the local manufacture of protein, there are inadequate supplies of hydrocarbons but much easily-won coal and the labour to win it. Although the gasification of coal to produce methanol synthesis gas requires higher capital investment than does the gasification of hydrocarbons, the coal-rich, hydrocarbon-poor countries are likely to accept the extra expenditure in order to render themselves independent of imports of increasingly more expensive and scarce hydrocarbons.

The still ready availability of natural gas in certain areas of the world, coupled with an increasing shortage of natural gas in the industrialised developed nations, has led to studies into methods of transporting the energy of the natural gas to distant users[1][2]. Certain countries, increasingly concerned about pollution, are insisting that in future fuels of much lower sulphur content are burned instead of the high-sulphur heavy oils now used. Natural gas has at most only a low sulphur content and therefore is now in demand as a premium fuel to replace heavy oil. The transport of natural gas energy can be carried out either by liquefying the natural gas and carrying it in specially constructed tank ships, or by converting the natural gas to methanol which is then transported in conventional tank ships. For distances over about 5,000 miles, it is more economic to make and ship methanol rather than use the LNG route. There are now many proposals for building the so-called fuel methanol plants in the Middle East at sources of natural gas. The cheapness of the natural gas feedstock and economies of scale will result in the methanol being made at prices only about half that of methanol made in

conventional plants today. Fuel methanol will be then much cheaper than liquid hydrocarbons as feedstock for the production of protein. By diverting some of the fuel methanol output into a protein plant, surplus natural gas will in effect be converted into protein for consumption by the developing local community.

Methanol has certain technical advantages in a protein process. It is soluble in water, which simplifies the design of the fermenter in which an organism is grown in an aqueous medium. Methanol, being more volatile than water, is easily and completely removed from the product during the drying stage. Methanol is readily produced by known techniques in a very pure form, thus ensuring reproducibility of performance and the absence of health hazards. Pure methanol is a transportable world commodity and can therefore be bought commercially and used as feedstock to a protein plant in any location.

B. Choice of Organism

The various processes that are available or are under development use yeast, bacteria or fungi. Bacteria have a clear advantage over the other two types of micro-organism in that they have a more rapid growth rate and a higher protein content. Compared with yeast or fungi, the distribution of amino acids in bacterial protein is nearer to that in animal protein, and therefore has a greater value in an animal feeding-stuff. Bacteria are, however, smaller than yeast or fungi and pose additional separation problems. It can be said that the successful development of a bacterial process depends in large measure upon the development of an efficient and cheap product recovery system. Perhaps because of the recovery problem, most workers with methanol substrate have chosen yeast as the organism to be produced.

C. Continuous Fermentation

A novel process, using methanol substrate and a pseudomonad bacterium[3] is now at an advanced state of development in the UK. It is to be used in installations each of at least 100,000 te/yr capacity. In order to obtain the economies possible from large-scale operation, these large plants will so far as possible use continuous single stream operation.

Besides the advantages to be gained in any chemical process through the use of continuous plant, there are additional advantages to be obtained in the case of a fermentation process. In order to obtain maximum productivity, the dilution rate employed in the process is chosen so as to be close to the maximum dilution rate possible for the selected organism. Foreign organisms that grow more slowly than the selected organism are swept out of the system before reproduction can occur. So, if the selected organism is a fast-growing one, the chance of accidental infection is reduced.

It has also been shown that the use of a continuous fermenter minimises the risk to the process that might result from phage infection. In a model experiment, a phage-sensitive organism (not the pseudomonad selected for the process described below) was grown in a continuous fermenter operated at near the maximum dilution rate. After having deliberately introduced infective phage, the population of the organism rapidly diminished to a very low level. After a few hours, however, the phage-infected organisms had been swept out of the system and the phage-resistant organisms had reproduced so as to restore the original population.

It has proved impossible, in many attempts using a variety of phage sources, to infect the organism chosen for the methanol-pseudomonad process. It seems likely that phage-resistance has been created in the organism's previous history.

During the research and development period, the chosen pseudomonad has, by natural selection, evolved improved properties. For instance, crude protein content has increased from 83% to 85%; carbon conversion in the process has increased from 54% to 60%; and the maximum dilution rate has increased by 30%.

D. The Pressure Cycle Fermenter

Because conventional mechanically stirred fermenters cannot readily be extrapolated to large unit sizes, and in any case have a large energy requirement, the new process will use a novel design[4] of continuous fermenter in which circulation is effected by the air that is injected to meet the requirements of the fermentation process.

The 'pressure cycle fermenter' is shown in Figure I. It consists of two vertical columns of substantial height (for instance, 30 m or more) connected at top and bottom by horizontal members. The lower horizontal section and the vertical columns are full of the fermentation broth, while the upper horizontal vessel is partly full, thus providing a gas-liquid interface.

Air is injected into the base of one of the vertical columns to form bubbles. The bulk density of the column contents is reduced in comparison with that in the other vertical column, and the liquid is thus caused to circulate round the system by an air lift effect.

The air in the bubbles at the base of the riser is under pressure because of the hydrostatic head of liquid at this point. Oxygen transfer to the liquid is therefore rapid. The organism in the broth absorbes the dissolved oxygen and gives off carbon dioxide, which dissolves in the broth as fermentation proceeds. As the broth approaches the top of the riser, the hydrostatic pressure diminishes and the carbon dioxide comes out of solution. The pressure cycle described leads to high oxygen utilisation and efficient carbon dioxide removal, necessary to maintain the health of the organism.

The carbon dioxide, nitrogen and unused oxygen are disengaged from the broth at the gas-liquid interface in the upper horizontal section of the fermenter. The liquid returns to the base of the plant through the downcomer column, which includes a heat exchanger to remove the heat of fermentation.

Methanol, water and inorganic nutrients are introduced at the bottom of the plant. No organic nutrients are used. Broth is removed from

the circulating system or the top of the plant from which it passes to the product recovery section.

E. Product Recovery

The pseudomonad organism has a size of about one micron and a density very close to that of water. Recovery by a conventional centrifuging operation would require a very large installation and would be prohibitively expensive. The commercial success of a process using a pseudomonad, or indeed any other bacterium, depends therefore on the development of an economic recovery system. In the case of the process being described, the recovery problem has been solved by the introduction of a novel step in which the organisms are caused to adhere to one another in large agglomerates. The agglomerate suspension is treated by a simple centrifuge installation in which 99% of the ingoing solids are recovered as a stream containing 25% concentration of solids. This stream is then dried to produce either a fine powder (suitable for dispersal as a milk replacer for calves), or a coarse powder or pellets (for solid animal feed supplements).

F. Research and Development

The research into and development of the process comprised the following steps:

1. Selection of the micro-organism using as criteria:
   - (a) Ability to use methanol efficiently.
   - (b) Protein content.
   - (c) Reproduction rate.
   - (d) Absence of toxicity and pathogenicity.
   - (e) Ability to work at high temperature (to simplify the cooling problem).
   - (f) Suitability for recovery in the novel system proposed.
   - (g) Nutritional value.

(h) Ability to grow using ammonia as the only nitrogen source.

(j) Ability to withstand the varying conditions of pressure, oxygen concentration, carbon dioxide concentration and temperature experienced in the pressure cycle fermenter.

2 Operation of semi-technical fermenters in order to:

(a) Establish design criteria (for instance, the cell concentration to be used in the design of the pilot scale and full-scale plants).

(b) Provide material for the development of the product recovery system.

(c) Provide material for animal feeding trials.

3 Design, construction and operation of pilot pressure cycle fermenter. This plant, which as been operating since 1972, has a capacity of 1000 te/yr. A recovery section of the same capacity has just been completed. The fermenter has been designed so as to have the same height as the full-scale plant in order that hydrostatic, hydrodynamic and gas-liquid relationships will be the same, and that the only major extrapolation required will be in the diameter of the vessels.

G. Product Analysis

The analysis of the product is given in Table 1.

H. Nutritional Evaluation

The product analysis shown in Table 1 indicates a protein source of high potential value, and this has been confirmed in nutritional trials. Nutritional and toxicological data have been displayed and discussed in detail elsewhere[5].

In broiler tests, all diets were formulated to a total of 21.6% crude protein. The fermentation protein was included at various levels from 0 to 10% of the total feed. Details of the diets, and the results obtained from them, are given in Table 2. No significant

difference in liveweight gain and the food conversion ratios were found between the groups fed different amounts of fermentation protein.

In 84-day pig trials, no significant differences were found between the weight gains of different groups of animals fed up to 10% fermentation protein during the first 50 days. After this time, animals fed with a diet containing 16.0% crude protein showed a significant 7% increase in weight gain when the diet included fermentation protein. Numerical results are given in Table 3. There were no significant changes in blood urea or uric acid levels, despite the increase in dietary nuclaic acid.

Short term toxicological studies in rats have been used for further investigation of the effects of high dietary nucleic acid levels. Again, blood uric acid levels were not elevated; allantoin levels, significantly above controls, indicated that purines derived from nucleic acid had been completely degraded to their final metabolic excretary product.

These results show that, in pigs and poultry, there are no undesirable effects of the high nucleic acid content of the protein. Substitution of traditional protein sources by the protein, on an iso-nitrogenous basis, gave equivalent weight gain performance.

J. Capital and Operating Costs

The capital cost (per tonne-year of product) of a fermentation protein plant using the methanol-pseudomonad process will be low because of:

(a) The use of very large unit size fermenters.

(b) High productivity of the fermenter (about 7 kg dry product per hour from each cubic metre of liquid space).

(c) High oxygen efficiency, with a consequent reduction in the size of the compression installation.

(d) The use of a novel compact product recovery system.

The operating costs will be low because of:

(a) The high carbon efficiency (about 60%) and the consequent low usage of methanol (about 2 te methanol per tonne of dry product).

(b) The competitive cost of the methanol feedstock, which is expected to become much lower with the construction of very large methanol plants using natural gas in the Middle East.

(c) Low energy requirements arising from the high oxygen efficiency and the absence of mechanical stirring.

The works cost of product (not including return on capital) may be broken down as follows:

	%
Raw Materials (mainly methanol, charged at current price)	63
Services (energy, water, etc.)	11
Operating labour and maintenance	11
Overheads and depreciation	15
	100

K. Commercial Development

The 1000 te/yr pilot plant is being operated to provide information to be used in the design of a commercial plant, of at least 100,000 te/yr capacity, which will be built to be operating in 1977 on a site in Europe. Product from the pilot plant is being used for continuing animal feeding trials.

It is intended that the process technology be used in other plants throughout the world. The number of plants to be constructed will depend on the extent to which the fermentation protein can compete on price with existing protein food supplements such as soya bean meal and fishmeal. If cheap fuel methanol became available for use as feedstock, then the fermentation protein is likely to be competitive not only with the more expensive supplements, such as fishmeal, but also with the cheaper vegetable protein.

Table 1. Product Analysis

Analysis	As fed %	Dry Weight Basis %
Moisture	5.0	-
Crude Protein (N x 6.25)	78.9	83.0
Ash	8.2	8.6
Extractable Lipid	7.0	7.4
Carbohydrate (Anthrone positive, as glucose)	3.2	3.4
Crude Fibre	<0.05	<0.05
ME K cals/Kg (poultry)	2870	3020

Nitrogen Analysis	%
Nitrogen	13.3
Nucleic Acid	15.9
Ammonia (NH_3)	0.5

Amino Acid Analysis
(g amino acid per 100 g dry material)

Aspartic Acid	7.08	Methionine	2.00
Threonine	3.81	Isoleucine	3.57
Serine	2.82	Leucine	5.62
Glutamic Acid	7.97	Tyrosine	2.55
Proline	2.50	Phenylalanine	2.85
Glycine	4.18		
Alanine	5.66	Histidine	1.53
Cystine	0.51	Lysine	4.88
Valine	4.34	Arginine	3.71
		Tryptophan	0.74
		Total	65.0

Ash Analysis	%
Calcium (Ca)	0.14
Phosphorus (P)	2.90
Sodium (Na)	0.17
Potassium (K)	1.4
Sulphur (S)	0.9 (inorganic <0.4)
Iron (Fe)	150 ppm
Fluorine(F)	<100 ppm

Fatty Acid Distribution	% of Total Fatty Acids
C 14 : 0	1
C 16 : 0	44
C 16 : 1	49.8
C 18 : 0	0.7
C 18 : 1	1.5

Table 2. Broiler Growth Study

	Group A	Group B	Group C	Group D
% Dietary Crude Protein from:				
Cereal	38	38	38	38
Soya	18	0	18	18
Fish	44	44	26	8
Fermentation protein		18	18	36
% Fermentation Protein in diet:	0	4.9	4.9	9.8
Liveweight gain (kg) in 56-day trial	1.902	1.932	1.958	1.895
Food conversion ratio	2.33	2.27	2.30	2.33

The observed differences in liveweight gain and food conversion ratio are statistically not significant.

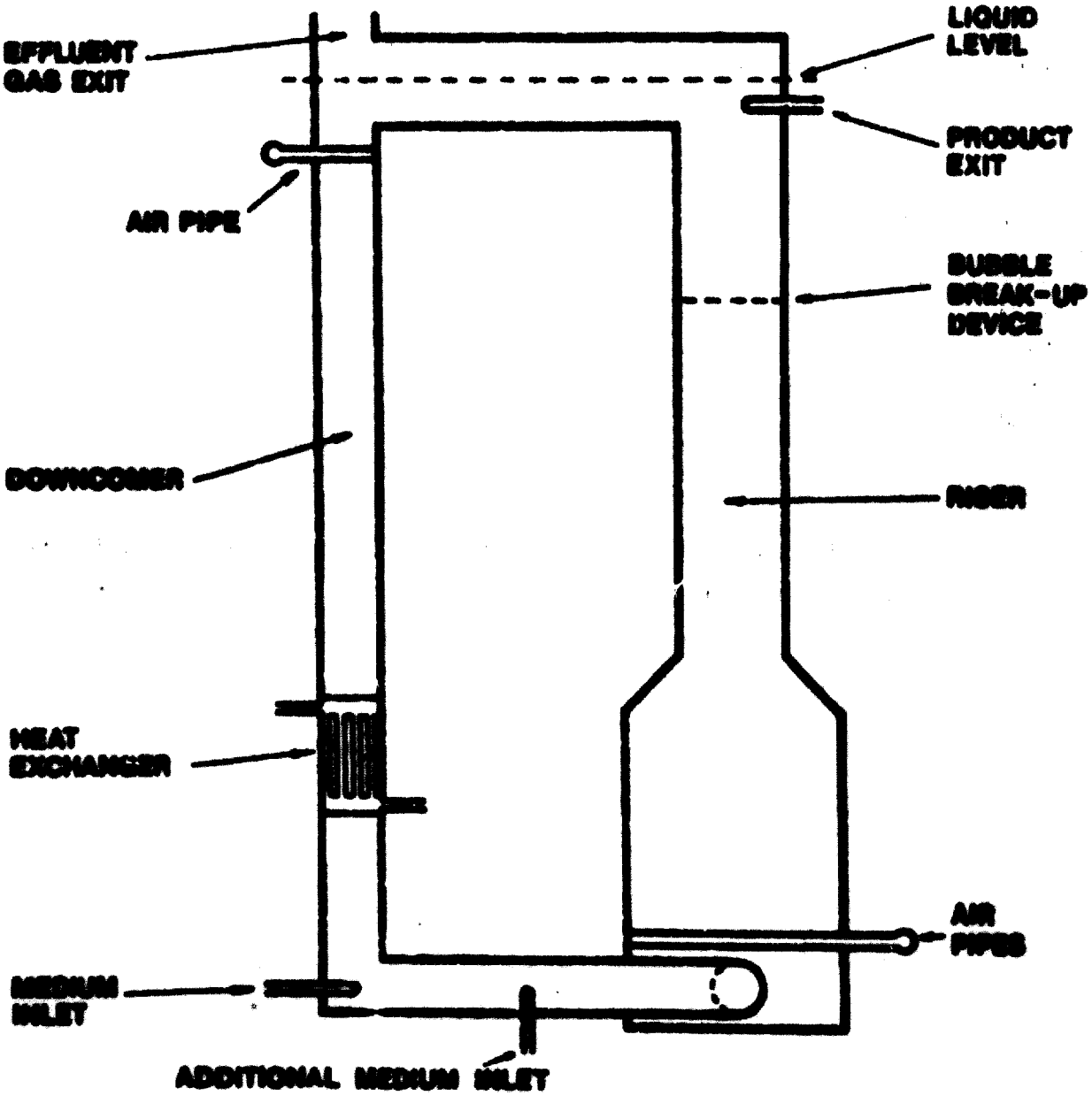
Table 3. Pig Growth Study

	Group A	Group B
% Dietary Crude Protein from:		
Cereal	67	67
Soya	33	0
Fermentation protein	0	33
% Fermentation protein in diet	0	6.7
Liveweight gain (kg/day) in period 50-84 days	0.598	0.638
Food conversion ratio	3.34	3.13

The observed differences in liveweight gain and food conversion ratio are statistically significant.

Figure 1.

The Pressure Cycle Fermenter



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SUMMARY

FERMENTATION PROTEIN FROM METHANOL 1/

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Fermentation protein may be produced from hydrocarbon, alcohol or carbohydrate substrates, and the organism used may be a yeast, bacterium or fungus. A novel process using methanol substrate and a pseudomonad bacterium is at an advanced stage of development in the UK. Methanol, which has no carcinogenic properties, can be produced in pure form by known processes from any fuel (including coal). At current prices methanol is competitive with purified hydrocarbon substrate and is likely to become much cheaper following the construction of very large fuel methanol plants using natural gas in the Middle East. The pseudomonad organism has a higher growth rate and a higher protein content than has yeast. A higher optimum growth temperature simplifies the cooling problem. The process employs a continuous fermenter of novel design.

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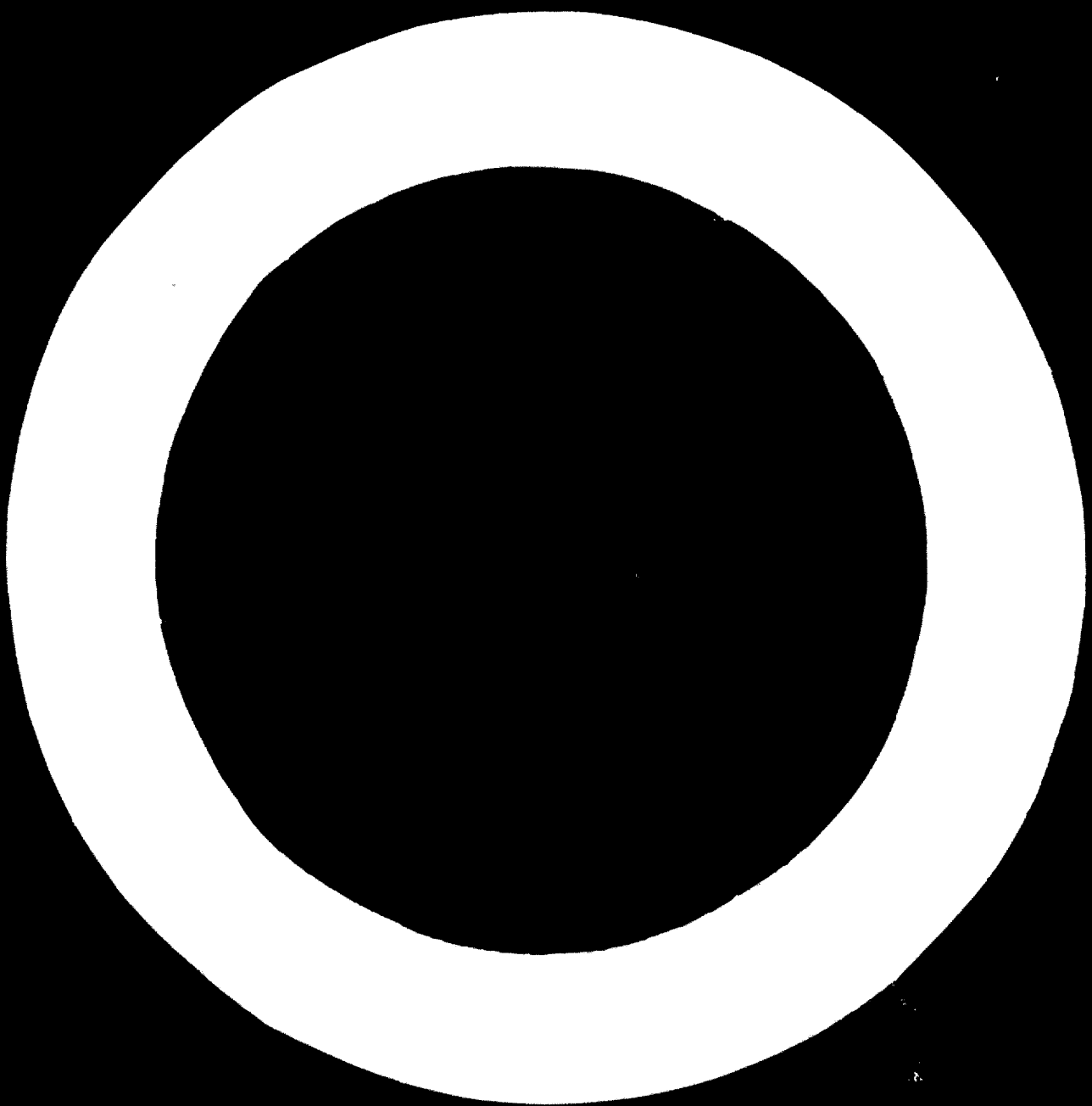
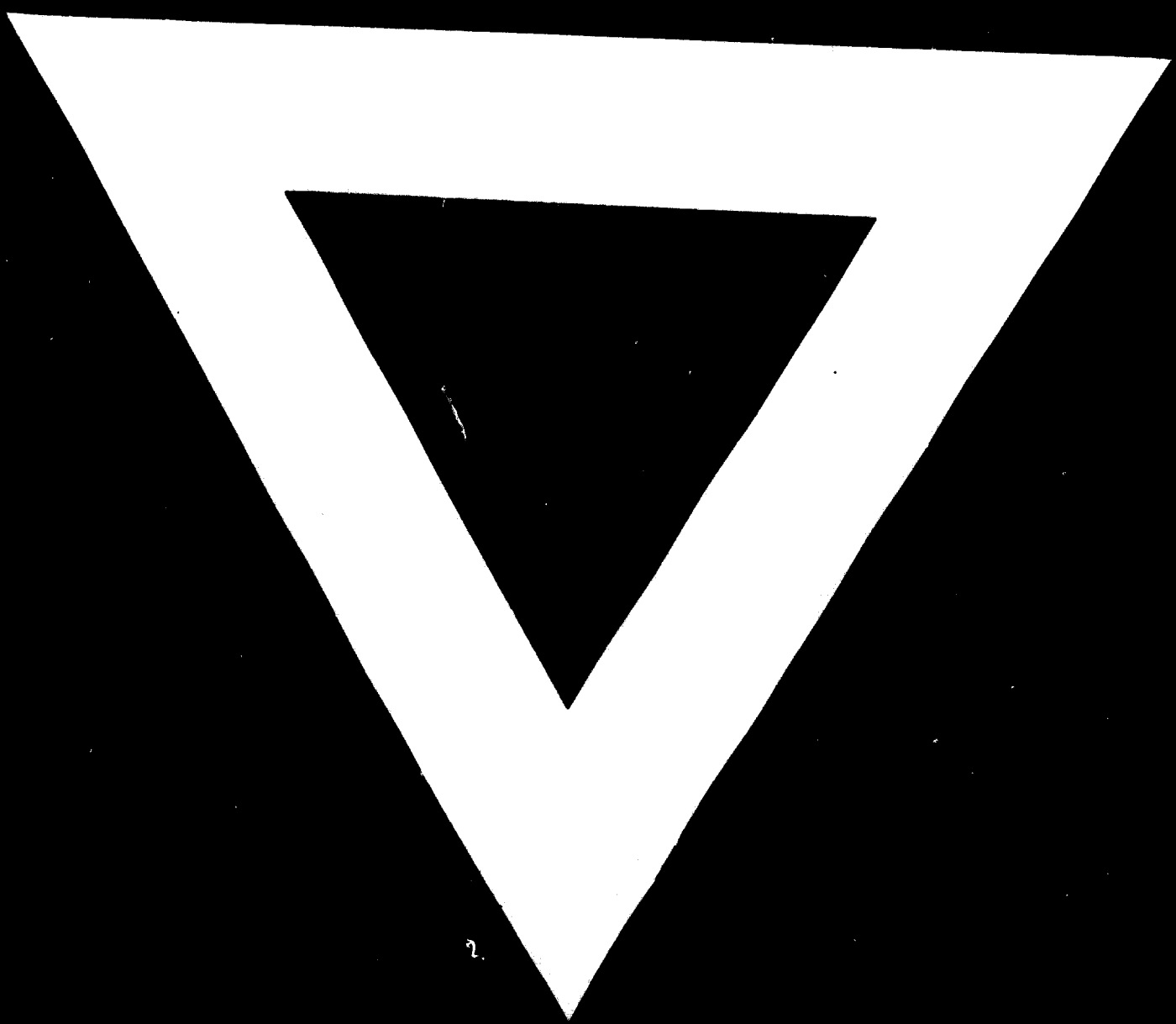


Process operating costs are low because of the low cost of the substrate, the high carbon efficiency (60%), and the low energy requirement arising from the absence of mechanical stirring and the relatively good oxygen efficiency. Capital cost is low because of the high productivity ($7 kg/M^3.hr$), the use of very large unit size fermenters, and the compact nature of the novel harvesting system.

Extensive animal feeding trials have shown the product to have no toxic properties and a high nutritional value.

It is planned that the first commercial plant, with a capacity of at least 100,000 tonnes per year, shall be operating in Europe in 1977.



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