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PRODUCTION OF SINGLE CELL PROTEIN FROM ALCOHOLS1/

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SUMMARY

PRODUCTION OF SINGLE CELL PROTEIN FROM ALCOHOLS1/

Y. Masuda** and
K. Yoshikawa**

An age of food shortage or an age of protein shortage in particular, will be upon the world in the near future. Therefore the hasty development of synthetic protein is imperative to cope with the rapid increase of population.

Inspection terms extending over 22 points had been designated and it was concluded that mass-production should not be permitted, unless the safety of each step had been confirmed.

The enterprise using the suitable raw materials will overtake the competition and reap the profits. No matter what the present state is the greatest question facing the industry is how to supply in stable quantities a reasonable cost high quality product.

This paper will describe how we decided to pick up a combination of a few specific strains and an alcohol as well as will describe its various features and advantages in terms of economic study of process, qualities of products, etc.

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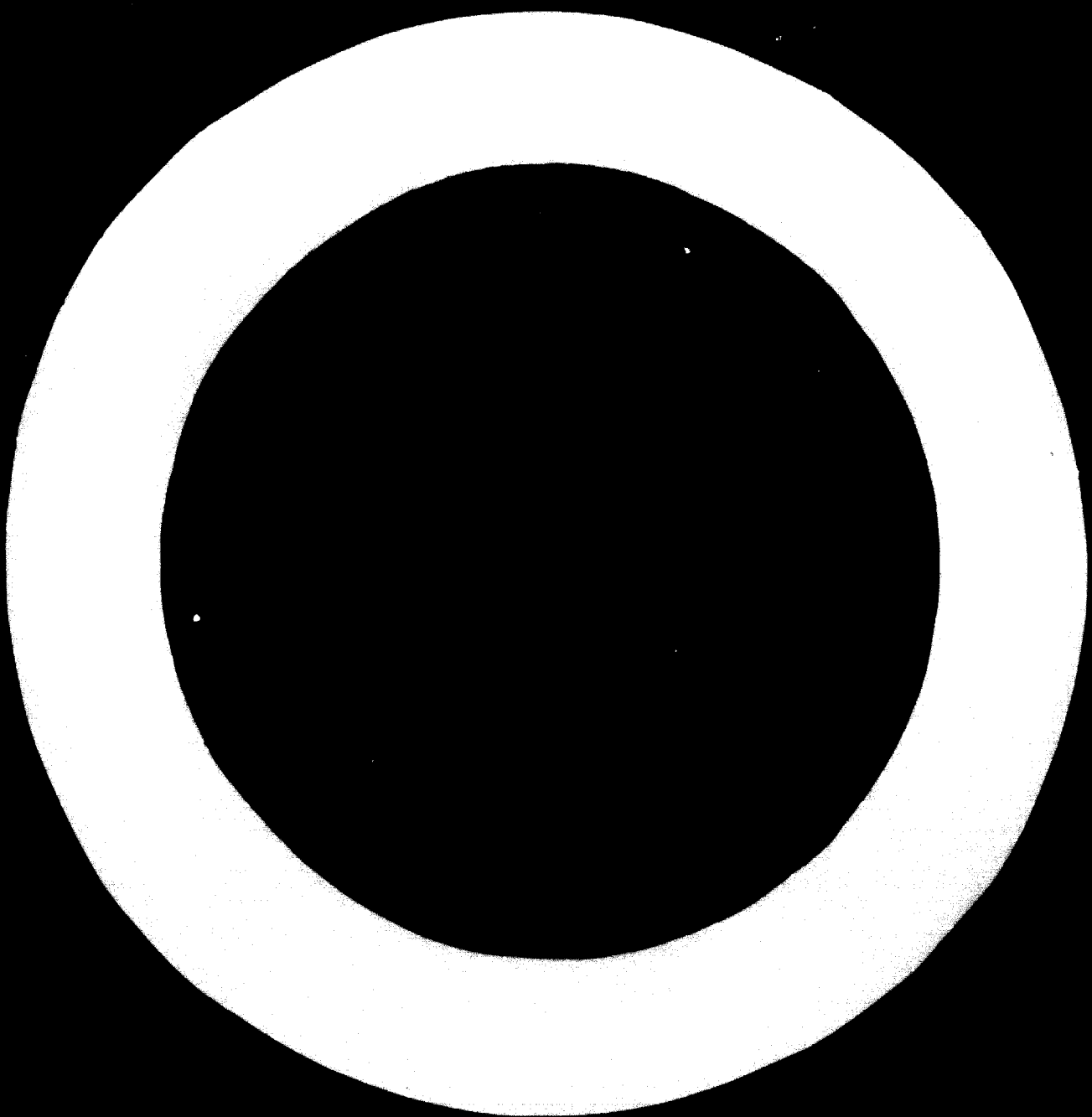

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PRODUCTION OF SINGLE CELL PROTEIN FROM ALCOHOLS

I. NEEDS FOR SINGLE CELL PROTEIN

I-A. CRISIS OF FOOD, ESPECIALLY OF ANIMAL PROTEIN

The world's population is now on steady increase, 2% or more per year, and according to various kinds of statistics, it is expected to reach a figure of about seven billions by the end of this century. It is a generally accepted opinion that the food supply will fall short around the end of this century, if the agricultural production merely keeps its current growth rate by that time. The limitation of natural resources including food stuffs has become a matter of great concern and has also become almost an everyday's topic.

According to the statistics of FAO, human diet has been remarkably improved as a result of better human life in the world, showing a distinctive increase of the intake of animal protein. As a result, it has become difficult to maintain the demand-supply balance of meats, and many countries has been faced upon high prices of meats.

It is also an inauspicious occurrence that hauls of anchovy in Peru coasts has become extremely small due to an extraordinary change in the sea current. It will be a matter of worldwide concern, because anchovy in Peru coasts is one of the major sources for a worldwide fish-meal supply.

To make the matter worse, growth conditions of crops turned out unfavorable in various parts of the world due to unusual changes in weather occurring on a worldwide scale. It has caused an unbalance between the demand and supply of the crops and, accordingly, skyrocketing of crop price this year. Among all, the price of soy bean which is one of the most important protein sources in feedingstuffs marked a jumping price. Particularly in the United States, soy bean price marked a figure three times or more higher than the ordinary price, presumably under influence of speculative demands.

I-B. NEEDS FOR SINGLE CELL PROTEIN

In order to solve such an absolute shortage of food, particularly the shortage of animal protein, the industrial production of microorganism protein, so-called single cell protein, has become to stand in the spotlight in the feedingstuffs industry from several years ago.

Since old days, people know by experience that microorganisms can be utilized in the production of foods, as in the production of alcoholic drinks, bread, vineger, cheese and the like. In Japan, moreover, microorganisms has been used in the production of seasonings such as soy bean paste and soy sauce since very old days.

However, there are two main differences between the single cell protein production and the conventional fermentation.

Firstly, in the single cell protein production, microorganisms themselves can be utilized as food or feedingstuffs.

Secondly, in the single cell protein production, raw materials are often non-food materials.

Thus, the production of single cell protein may be considered as a revolution in the history of food production in view of the fact that protein can be industrially produced without any influence of natural environment.

I-C. REPLACEMENT OF FISH MEAL WITH SINGLE CELL PROTEIN

Although our final target is to utilize single cell protein as our direct food, our first plan is to utilize the materials as a replacement of fish meal which is compounded in feedingstuffs.

Therefore, here is some description on the advantages of single cell protein over fish meal.

Fishes which are one of main protein sources in feedingstuffs are caught from seas in almost the same manner from the old times except that catching techniques have been gradually improved.

Therefore, a haul of fishes are sometimes badly affected by the change of an ocean current and others. Moreover, the fish resources are limited to and depend on the amount resulting from the natural reproduction.

On the contrary, the single cell protein production is hardly affected by natural conditions, and therefore have the following advantages:

(1) Constant supply

(2) Constant quality

(3) Less fluctuations in price

(4) There is no problems of polluted products such as the case of fishes.

Single cell protein has the following other features to be noted

(1) It contains digestable protein in almost the same amount as usual protein sources do.

(2) It also contains various vitamins and mineral components and is also useful as a resource of them.

(3) It contains fat in high proportion and is also rich of total digestable nutrients.

I-D. SAFETY OF S. C. P

It may be accentuated that single cell protein manufacturing technologies are not fundamentally different from the existing fermentation technologies as they both use natural and edible microorganisms, and therefore presents no new problems with regard to safety of the products. The only difference between the single cell protein production and the existing fermentation is that the former uses raw materials which are not generally provided as food. However, even the mono sodium glutamate which is now world-widely employed as a food-additive is prepared by fermentation of synthetic acetic acid made from petroleum.

I-E. JAPAN'S SAFETY STANDARDS FOR PETORPROTEIN

Although single cell protein is believed to present no problems with regard to its safety, it is quite reasonable to examine single cell protein from every possible angle in order to confirm its safety, since it is quite a new product in a sense.

In Japan, there were several companies who worked on the development of single cell protein manufacturing technologies since almost ten years ago. The raw materials they used are mainly normal paraffins which are introduced from petroleum, which is the reason why single cell protein is usually called as petroprotein in Japan as well as in some other countries.

In order to guide these companies' new development toward commercialization, the Ministry of Agriculture and Forestry and the Ministry of Public Welfare studied and made their public views on the use of the petroprotein as feedingstuffs and on its safety, consulting the authorities in microbiology, toxicology, pharmacology, medical science, dietetics, food hygienics and cancer research.

The report "Safety of petroprotein as feedingstuffs" by the Ministry of Public Welfare, or the so-called "Safety standards for petroprotein", is shown in Table 1 attached herewith. The standards are prepared for single cell protein from normal paraffins.

In accordance with the safety standards, two Japanese companies who were most advanced in the development of petroprotein furthered their plans towards commercialization.

II. SINGLE CELL PROTEIN FROM ALCOHOL DEVELOPED BY OUR COMPANY

Our company is one of the leading companies in the field of petrochemical industry of Japan, and has been producing various kinds of petrochemical products. Table 2 attached herewith shows main products of our company.

We have started research and development of single cell protein several years ago with an end of supplying the market with single cell protein which has high calory, good quality and reasonable price, and is safe.

The background of our decision for that research and development is as follows.

(1) Our good position to select and adopt the most suitable raw materials for production of single cell protein.

(2) Our high potential for developing chemical reaction processes.

(3) Possibility of utilizing highly advanced technologies in Japan's traditional fermentation industries.

(4) Needs for developing single cell protein to get rid of our traditional situation that Japan largely depends upon foreign countries for most foods.

II-A SCREENING CONDITIONS

In the course of the development of single cell protein, it is a key point how to isolate useful microorganisms from the natural sources. Our company mainly worked on the screening of yeasts because of the following reasons:

(1) Various kinds of yeasts have been traditionally employed in the food industries for over 2,000 years, and therefore, yeasts are considered to have a high degree of safety by experience.

(2) There has been found hardly any pathogenic yeast.

(3) Yeasts are eucaryotic cells and have little possibility of mutation.

(4) Yeasts have greater cell size and density than bacteria, and therefore, allow easier cell collection.

Collected soils have reached a number of almost 10 thousand kinds, and collection of soil is still going on covering the whole parts of Japan and almost all kinds of soils thereof.

The collected soils are each subjected to isolation of yeasts by the use of an enrichment technique, and properties of the isolated yeasts are examined. Factors of screening are as follows:

(1) Specific growth rate (μ)
(2) Yi ld on carbon source
(3) Protein content
(4) Cultivation temperature
(5) Cultivation PH

} Factors for batchwise flask and jar tests

(6) Stability of factors (1) through (3) in continuous tests
(7) Productivity
i) Working volume
ii) Cell concentration
iii) Dilution rate

} Factors for continuous tests

(8) Safety of Products
(9) Preference
(10) Digestability

} Factors for feeding tests

Raw Materials used for screening were as follows, although an alcohol is presently applied as it is mentioned afterwards.

1) n-paraffin (C_{13} - C_{20})
2) Aromatic hydrocarbons such as benzene
3) Organic acids such as acetic acid
4) Monohydric alcohols such as methanol
5) Dihydric alcohols such as ethylene glycol

II-B DECISIONS BY SCREENING RESULTS

After screening a number of yeasts with more than twenty of batch type and continuous type fermenters of 2 liter to 2m^3 volume, our company reached a decision to select a combination of a few specific yeasts and an alcohol. As compared with hydrocarbons, the alcohol has following advantages from a viewpoint of fermentation technologies.

(1) Easier contact between raw materials, oxygen and strains because of easy water-solubility of the raw materials. In other words, no special mixing techniques are required.

(2) Less heat emission and less cooling cost during fermentation.

(3) Less requirement of oxygen.

(4) Easier separation of cells from broth.

(5) Easier washing

There are also several advantages in qualities of the products.

(1) Theoretically speaking, Cells are not contaminated by polycyclic aromatics.

(2) Hardly any oddcarbon fatty acids are contained in cells.

(3) There is no possibility of petroleum-like odor of the products.

Our company is now concentrated on obtaining various technical data for materialization as well as field test data of the products based upon a combination of a few specific yeasts and the alcohol.

II-C OUTLINES AND ECONOMIC STUDY OF OUR PROCESS

Our process is shown in Fig. I.

In addition to the several advantages of our process which were described in paragraph II-B, other notable features are as follows.

(1) For better efficiency of oxygen supply, a particular type of fermenter is being developed.

(2) The productivity per unit volume of fermenter is pretty high compared with n-paraffin process because of comparatively high cell concentration and high dilution rate.

(3) The operating temperature of the fermenter is kept comparatively high and therefore, considerable amount of cooling water can be saved. It is because our yeasts are mesothermophillic.

(4) Comparatively low PH is applied during fermentation, which is quite effective to prevent contamination by other microorganisms as well as high operating temperature.

Regarding economic study, our process is believed to be quite competitive as compared with n-paraffin process, as it is shown in Table 3. Several factors for making our process quite competitive are as described below.

(1) The investment cost can be remarkably cut due to the use of compact or simplified several equipments resulting from the high productive strain, the water-soluble substrate, etc..

(2) As a result, other relating costs such as depreciation, maintenance fee and labour, are reduced.

(3) Savings of utilities are possible due to the low heat emission, thermophillic properties of the strain, saving of agitation power etc..

These factors can compensate the raw material cost which is relatively high due to higher unit price and higher consumption of the raw material.

II-D QUALITIES AND FEATURES OF OUR YEASTS

Table 4 shows an example of component analysis of our yeast compared with that of other protein sources. The figure of crude protein content of our yeast is in between those of soy bean meal and fish meal, however, the energy of the yeast is comparatively high as it is well understood from the content of its crude fats and nitrogen free extracts. In addition, the content of poorly digestible fiberes in our yeast is negligible.

Table 5 shows an example of amino acid analysis of our yeast and other protein sources. The content of essential amino acids in our yeast, particularly the content of threonine, valine, isoleucine, leucine, phenylalanin, is larger than in fish meal, and the content of lysine, methionine and tryptophane is larger than in a soy bean meal.

Table 6 shows an example of the composition of fatty acids. Our yeast contains oleic acid, linoleic acid, palmitic acid, and linolenic acid as its main fatty acid components. In addition, oddcarbon fatty acids are contained only in an extremely small amount (less than 3% of the total fatty acids).

Table 7 shows an example of vitamine content. In general, yeasts are rich of vitamines and our yeast is not an exception too showing far greater vitamine content than usual protein sources such as fish meal and soy bean meal.

II-E MARKET DEVELOPMENT OF OUR YEAST

As it is a quite popular idea already, our company is also planning to apply our yeast for feedingstuffs.

Several feeding tests of our yeast on livestocks, poultry, fishes, etc. are being performed as well as safety evaluation using experimental animals, livestocks, poultry and fishes.

Regarding the future application of our yeast, there are several ideas other than its use for human food, such as its use for a substitute of casein and gelatin, artificial leather, protein fiber and surfactant.

However, it will be the most ideal application if the protein can be used as food for human beings.

Our company really hopes the time comes in the near future when single cell protein is well accepted and used in every direction throughout the world.

Reference

(1) Single Cell Protein by MIT press (1968)

(2) Safety Standard for Petroprotein

by The Ministry of Public Welfare's Food Sanitation Investigation Council (1970)

(3) Y. Masuda; Technocrat Vol 4 No. 7 (1971)

Table 1

Safety standards for petroprotein

I. Safety standards for petroprotein

Items to be examined for confirmation of safety of petroprotein when used for feedingstuffs.

1. Strain (which means microorganisms used for the production of petroprotein)

   (1) Micological inspection with respect to morphological and biological properties.

   (2) Inspection with respect to mutagenicity

   (3) Inspection with respect to infection

2. Material (fermentation media including normal paraffin)

   (1) Inspection of polycyclic aromatic hydrocarbons

   (2) Inspection of heavy metals

3. Cells (strains at the maximum growth stage of one production cycle)

   (1) Inspection of polycyclic aromatic hydrocarbons

   (2) Inspection of heavy metals

   (3) Inspection of mycotoxins

   (4) Toxicity inspection

4. Fermentation broth (at the maximum growth stage of one production cycle)

   (1) Inspection of polycyclic aromatic hydrocarbons

   (2) Inspection of heavy metals

(3) Inspection of mycotoxins

(4) Toxicity inspection

5. Final products (obtained after completion of the production process and used for feedingstuffs)

(1) Inspection of survival strain

(2) Inspection of polycyclic aromatic hydrocarbons

(3) Inspection of heavy metals

(4) Inspection of mycotoxins (which is not required when any mycotoxin was found in the cell and fermentation broth).

(5) Toxicity inspection

(6) Inspection of effects on following generations

6. Milk, meats, etc., (edible substances such as meats, eggs, milk, etc., produced from livestocks, poultry and fishes which were fed)

(1) Inspection of polycyclic aromatic hydrocarbons

(2) Inspection of heavy metals

(3) Inspection of mycotoxins (not required when no mycotoxin was recognized in the product).

II. Standards for normal paraffin

The specification of normal paraffin which is used as a raw material in the production of petroprotein is as follows:

1. A purity greater than 98%

2. Regarding contamination by polycyclic aromatic hydrocarbons, n-paraffin should satisfy the specification of "Liquid Paraffin" which is listed in the standards for Food Additives, and should not contain 3.4-benzpyrene, 1,2,5,6,-dibenzanthracene and 20-methylcholanthrene, each in an amount over 1.0 ppb.

Table 2 Main products and their capacities of our company in 1973

Product	Capacity
Ethylene	682,000 T/Y
Low density polyethylene	205,000
High density polyethylene	30,000
Polypropylene	150,000
Expandable polystyrene*	42,000
Ethylene oxide	74,000
Ethylene glycol	86,000
Styrene monomer	285,000
Benzene	326,000
Toruene	47,000
Xylene	49,000
Cumene	110,000
Alkylbenzene	47,000
Epoxyresins	20,000
Acrylic esters	20,500
Butanol*	30,000
Ethanol*	20,000
Polymer dispersion*	3,000
Polymer solution*	
Glycol ether*	6,000
Ammonia*	980 T/D
Urea*	1,400 T/D

* Produced by our affiliated companies

Table 3 Cost comparison of our process with n-paraffin process

(the larger cost is considered as 100)

item	our alcohol process	n-paraffin process
(Variable cost)	64	57
raw material	44	31
chemicals, utilities	20	26
(Fixed cost)	32	43
depreciation	9	15
labours interest, etc.	23	28
total production cost	96	100

* Plant scale is supposed to be 60,000 metric tons of product per year

Table 4 Ex mple of component analy is

component	our yeast	fish meal (anchovy)	soy bean meal	n-paraffin yeast
moisture	4.1 %	10.4	13.0	4.4
crude protein	55.6	62.3	45.7	56.5
fat	7.6 *	4.5 **	1.3 **	1.4 **
fiber	0.3	0.5	5.9	4.2
ash	8.4	16.3	8.6	7.7
nitrogen free extracts	24.0	6.0	31.4	25.8
total	100.0	100.0	100.0	100.0

* acid - decomposition method
** ether - extraction method

Table 5 Example of amino acid content

amino acids	our yeast	fish meal (anchovy)	soy bean meal	n-paraffin yeast
lysine	3.7 %	4.3	2.8	3.8
histidine	1.1	1.5	1.2	1.3
arginine	3.1	3.9	4.7	2.7
aspartic acid	5.8	-	-	5.9
threonine	2.8	2.4	1.6	2.0
serine	2.6	-	-	-
glutamic acid	8.8	-	-	7.2
proline	1.9	-	-	-
glycine	2.7	3.2	1.8	2.5
alanine	2.9	-	-	3.1
cystine	-	0.5	0.8	0.7
valine	3.9	2.8	2.3	2.9
methionine	1.0	1.2	0.5	0.8
isoleucine	3.2	2.4	2.1	2.7
leucine	4.6	3.7	3.3	3.9
tyrosine	1.8	1.8	1.5	1.8
phenylalanine	2.8	2.1	1.9	2.4
tryptophane	0.8	1.3	0.5	0.7

Table 6 Example of fatty acid composition

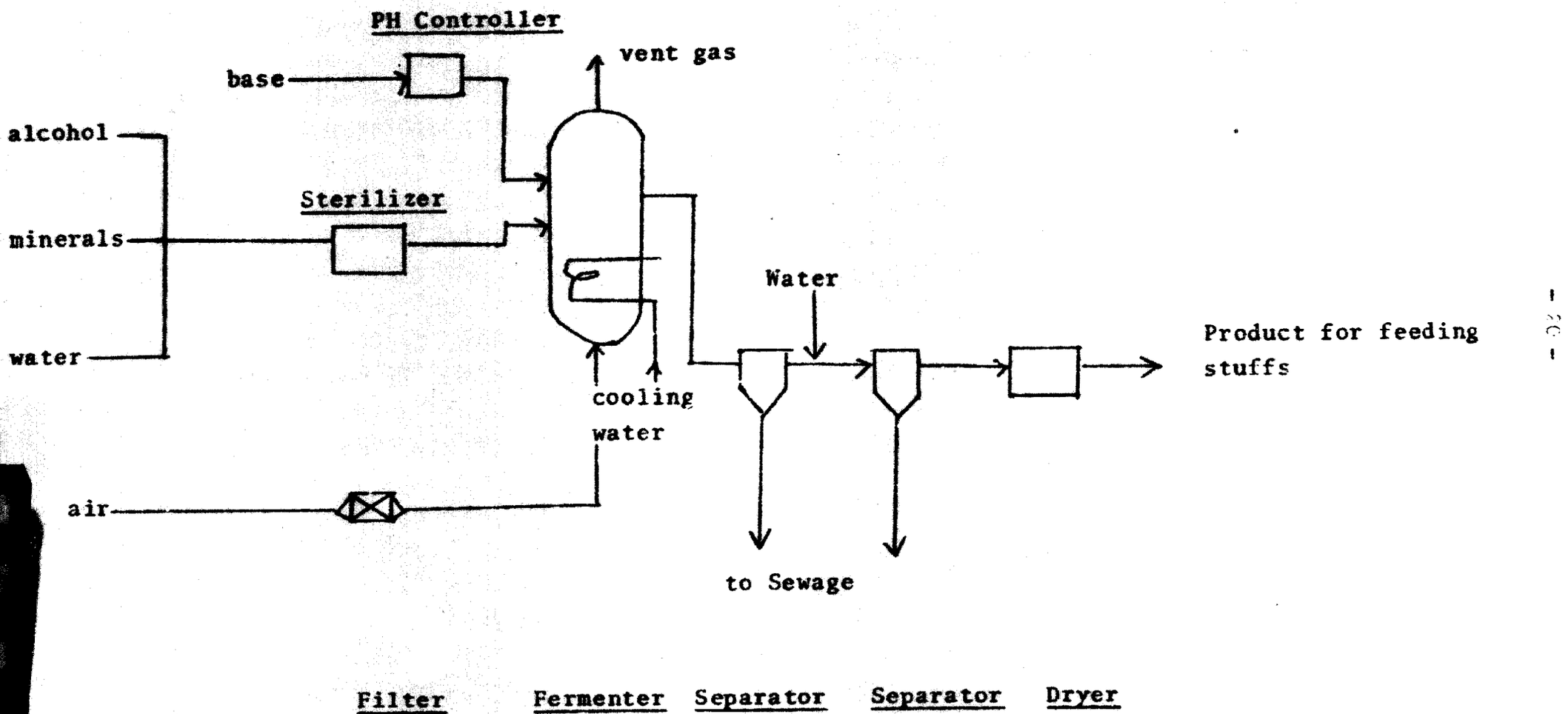
fatty acids	our yeast	n-paraffin yeast
C_{12}	trace %	4.7 %
C_{13}	0.8	
C_{14}	trace	
C_{15-0}	1.0	9.6
C_{15-1}	0.3	2.4
C_{16-0}	16.3	7.3
C_{16-1}	6.9	12.5
C_{17-0}	trace	4.5
C_{17-1}	0.8	33.0
C_{18-0}	2.3	trace
C_{18-1}	33.6	17.6
C_{18-2}	27.0	8.3
C_{18-3}	11.0	—
Total	100.0	100.0

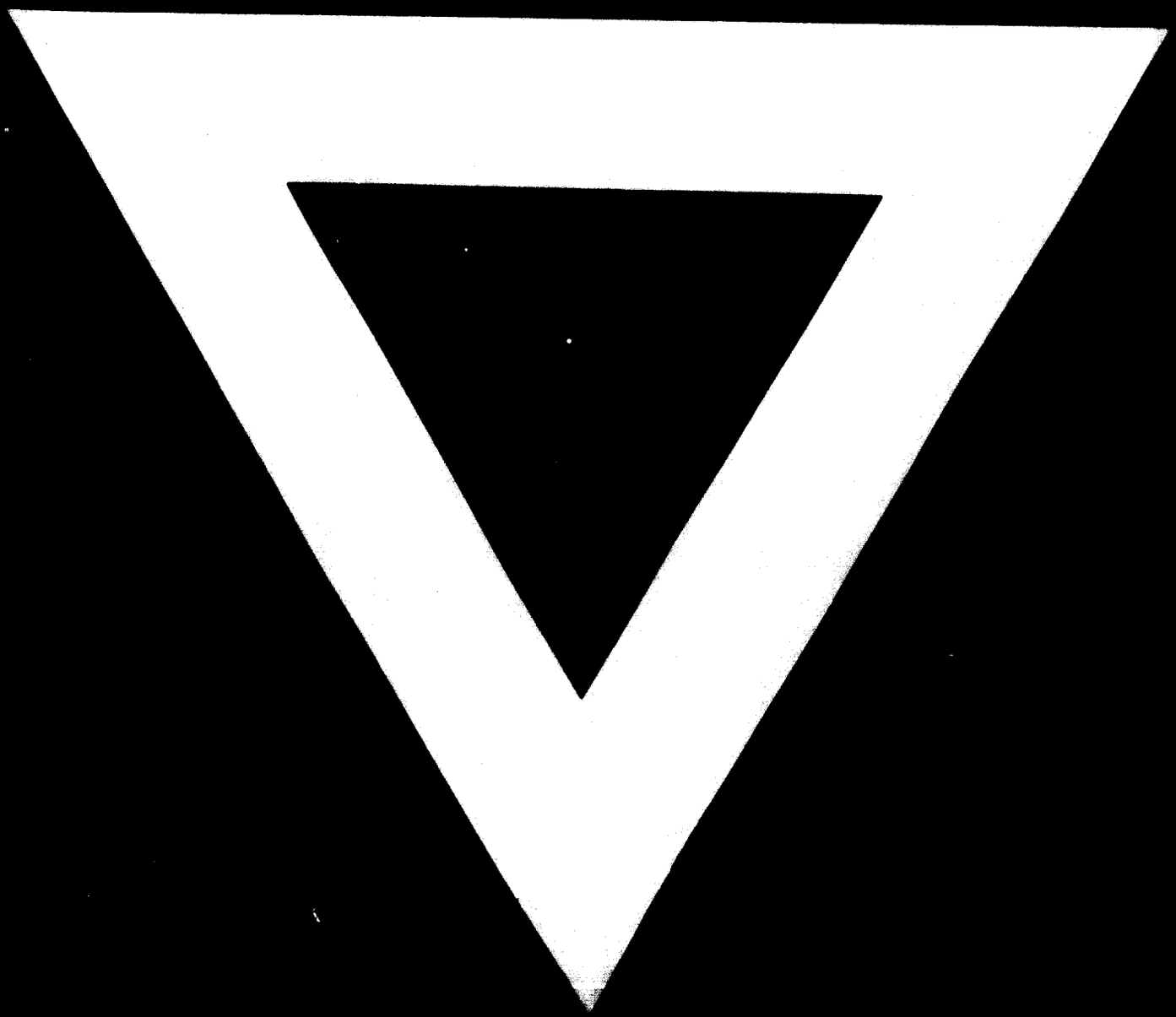
C_{15-1} ; normal fatty acids with 15 carbon numbers with one double bond

Table 7 Example of vitamin content

item	our yeast	fish meal (anchovy)	soy bean meal
Vitamin B_1	0.37 mg%	0.04 mg%	0.66 mg%
" B_2	3.35 "	0.59 "	0.33 "
" B_6	2.01 "	0.17 "	0.70 "
" B_{12}	6.57 μg%	19.4 μg%	0.20 μg%
Pantothenic acid	9.31 mg%	0.92 mg%	1.45 mg%
choline	0.33 %	0.30 %	0.27 %
Nicotinic acid	39.5 mg%	6.20 mg%	2.68 mg%
biotin	0.064 "	—	—
folic acid	0.19 "	—	0.07 "
inositol	1.22 %	—	—
vitamin E	22.1 mg%	20.9 "	0.08 "
" F	2.01 %	—	—
provitamin D	0.104 %	—	—

Figure I Flow scheme of SCP production process





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