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Vienna, Austria, 8 - 12 October 1973

THE PRODUCTION OF SINGLE-CELL-PROTEIN FROM N-PARAFFIN 1/

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SUMMARY

THE PRODUCTION OF SINGLE-CELL-PROTEIN FROM N-PARAFFIN

Y. Sazuki*

Since several years ago, the world-wide shortage of proteinous foods for human consumption and various animal feed is a definetely foreseeable fact. As the never-ceasing shortage exists in the supply of proteins, the necessity is assuming greater importance for developing new protein sources such as S.C.P. that can be produced through industrial processes.

The studies of technical developments of S.C.P. from n-paraffin have been started in 1962. Following elaborated basic studies, industrialization studies have been proceeded and finally established the technology good enough to cover all the aspects on the production of S.C.P. from n-paraffin including its safety, quality and mass production technique.

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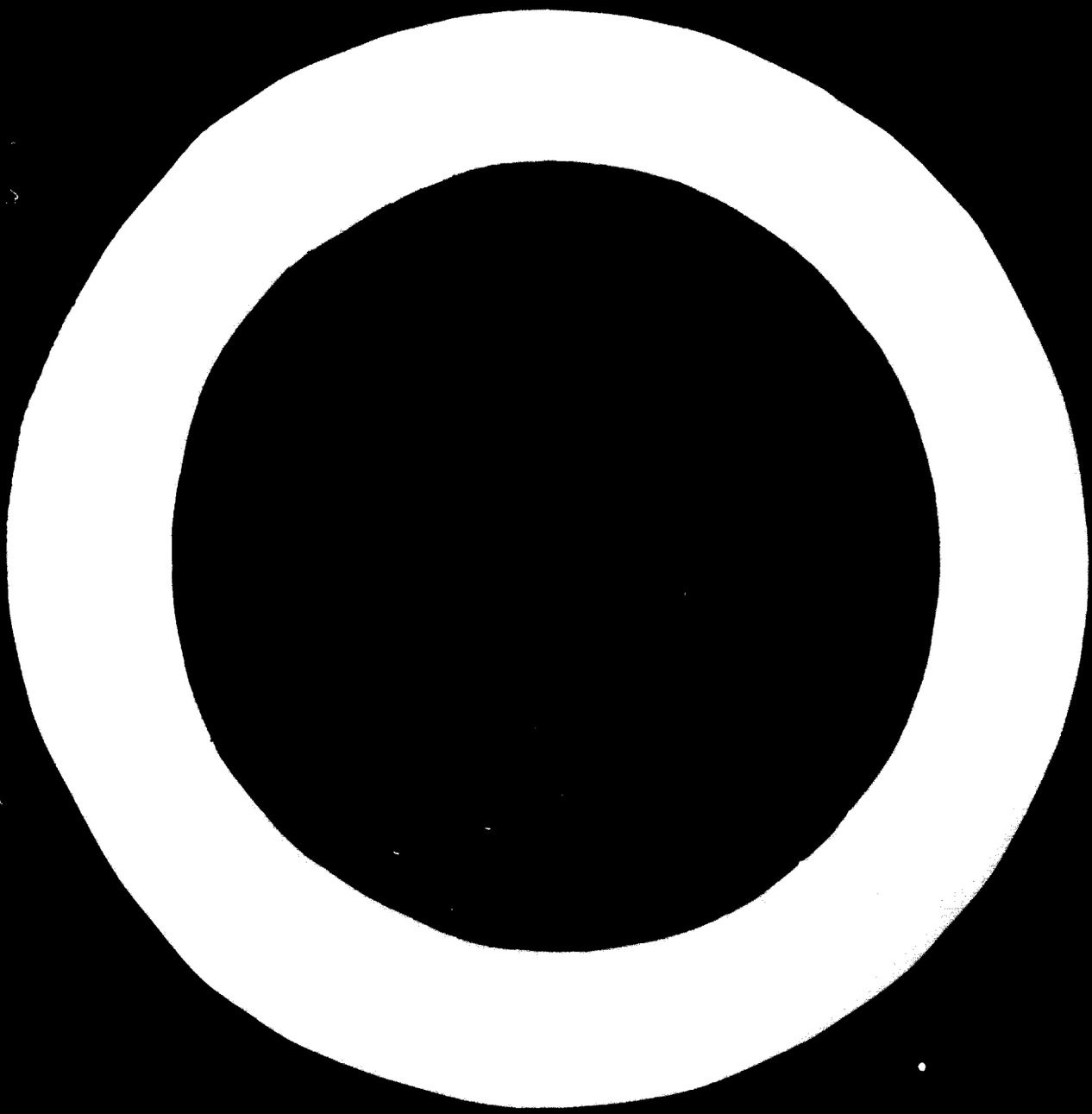
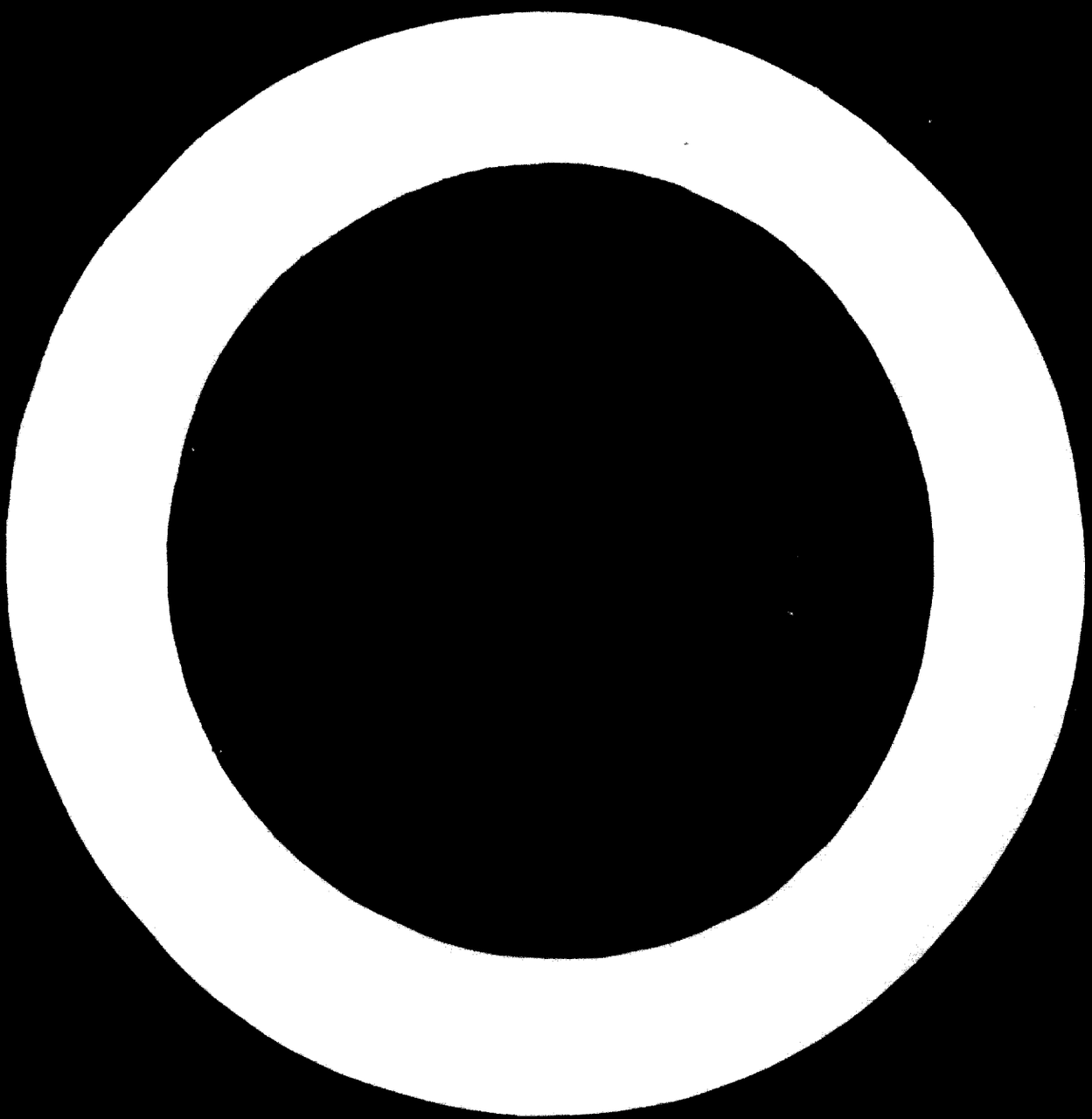


S.C.P. from n-paraffin serves as the source of proteins for feed use due to its abundant calory, excellent digestibility, and well balanced components of various amino acids, minerals and a variety of vitamins in substantial amounts.

S.C.P. had been carried out extensive animal feeding trials to confirm the nutritive value and also tested on the safety according to the standards, which are advocated by the Food Hygiene Research Committee under the Ministry of Health and Welfare in Japan.



CONTENTS

EXPLANATORY NOTES

SCP n-P : Single cell protein produced from n-paraffin.

TDN : Total digestible nutrients.

INTRODUCTION

It is a definitely foreseeable fact that the sources of proteins, one of the essential nutriments for mankind will suffer from deficit in the long run if the world's population grows rapidly as it does now.

Unless new sources of proteins are somehow developed, shortage in the supply of proteins for future human consumption would be inevitable, since the expanded output of conventional animal and botanical proteins would not be able to catch up with the increasing consumption.

As the means for solving the deficit in the supply of proteinous foods for mankind, ever-increasing efforts have so far been made for development of new proteinous foods, including the further accelerated development of livestock industry and of oceanic farms for fish aqua-culture to reinforce the output of conventional animal proteins.

The main sources of proteins for the animal feed have so far been used fishmeal, fish soluble, soybean meal and etc., but nevertheless, shortage in the supply of proteins for animal feed also remains a global problem as with the deficit of protein for human consumption.

This is simultaneously one of the controversial problems Japan is facing and also world's problem.

On the other hand, it is mandatory to secure natural requirements such as agricultural land, favourable climatic conditions and efficient use of solar energy in order to expand production of botanical proteins. Hence, this is again associated with a number

of difficulties before being adopted as one of the countermeasures for solution of deficit in the supply of proteins at the present time.

As the never-ceasing shortage exists in the supply of proteins, the necessity is assuming greater importance for developing new protein sources such as SCP n-P that can be produced through industrial processes, unlike existing proteins of animal and botanical sources of which output greatly relying upon natural conditions.

As the carbon sources on the production of SCP, carbohydrate such as starch, molasses, sulfite pulp liquor etc. used so far, but these carbohydrate substances could be also used for the human consumption. So, the production of SCP from petroleum is the greatly important problem.

Protein from hydrocarbons has several other distinct advantages. Unlike the growing of soybean etc., its production does not require agricultural land, and unlike marine resources, there are no limits that must be set in the interest of conservation of species. It is independent of sun, weather, soil and water and indeed largely independent of human labour. Moreover, SCP could be produced by the industrial scale relatively cheap and stable in price. The cell can be grown in large scale continuous conditions. The growth rate of high quality protein cells is two thousand times faster than the rate at which beef cattle convert feed into proteins. The SCP derived from petroleum could, therefore, a solution in bridging the protein gap.

I . THE CHARACTERISTICS ON THE PRODUCTION OF SCP N-P.

A. The characteristics of fermentation.

The production of protein from petroleum means the growth of a micro-organisms on a petroleum fraction and the recovery or harvesting of the micro-organisms which usually comprise of 50 - 70 per cent of protein.

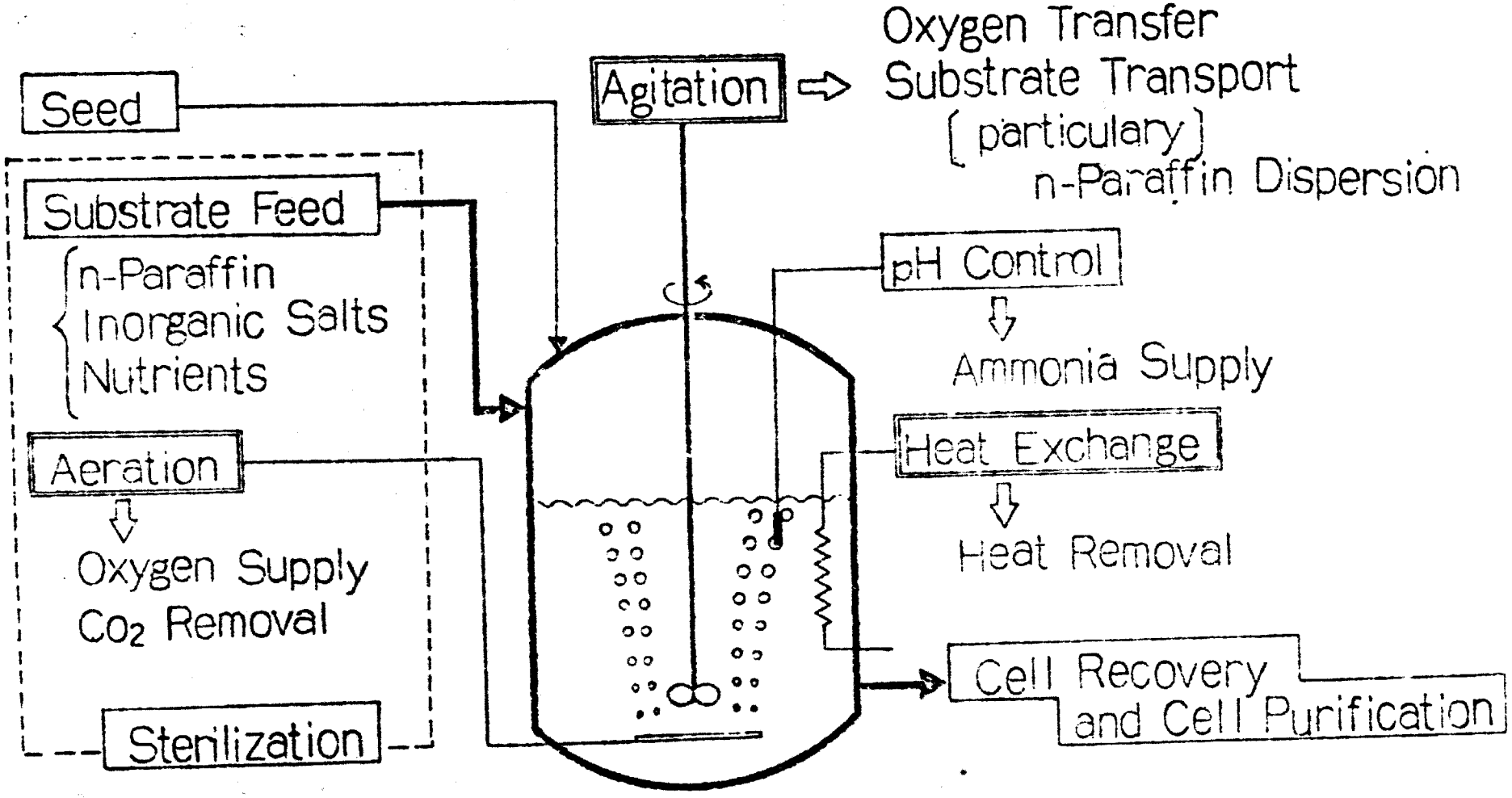
Firstly I would like to schematicaly explain the growth of a micro-organisms on a petroleum fraction, namely n-paraffin. . Culturing work in the fermentor (Figure - I) is the producing cells with n-paraffin, oxygen, auxiliary matters such as nitrogen and magnesium etc., as the substrate and the providing a suitable substrate concentration and optimum culturing condition such as pH, temperature etc. of micro-organism so that this reaction proceeds effectively well.

n-Paraffin are supplied as a carbon-source for the growth of micro-organism, but it does not solve in the water and floats on aqueous-phase. So it is necessary to disperse the n-paraffin as discrete oil droplets in the aqueous-phase of the fermentor by aeration or mechanical agitation.

The mechanisms of transfer of this hydrocarbon to the cell is not properly understood. It is not clear whether the cell obtains the carbon-source of the hydrocarbon directly by contact with the oil droplet or by intermediate solution in the aqueous-phase.

It would be interesting to see whether addition of suitable emulsifying agent would help in this problem without harming the cell growth and without introducing an additional toxicity factor in

Figure- I. The General Aspects of Single-cell Protein Production from n-Paraffin



the final product.

Nitrogen-source, phosphorus, magnesium and other minerals necessary for the growth are supplied in the aqueous-phase as the solution.

In some cases the addition of a growth factor, such as vitamins, amino acids, derivatives of nucleic acid also becomes necessary. It is important to ensure the balance, namely the relative availability to the cell of the various nutrients --- carbon-sources, nitrogen-sources, minerals and growth factors.

The oxygen required for the process is supplied from the atmospheric air. This is bubbled in the aqueous-phase in which the micro-organism grow. In industrial practice, it is important to obtain the high yield factor in fermentation. This very much depends upon the oxygen transfer. If the oxygen transfer is not proper, the cell oxidizes more hydrocarbon away as carbon dioxide and under more favourable conditions directs more of the carbon to its own cell mass.

pH of the medium, and temperature must be kept at optimum condition of the used micro-organisms.

Since the process is an exothermic reaction, the heat output under the various conditions of oxygen transfer becomes important as the fermentor has to operate at 30 - 35 ^{o}C.. Excess heat leads to more demand for cooling water and heat exchanger capacity and consequent increase in the power consumption.

Seed micro-organisms can be obtained from the strain preserved in a slant culture through several steps for their subsequent culture. The process for culture of the seed yeast shall be put under

completely aseptic conditions. The success of the cultivation relies on the prevention of co taminat[illegible]s and hygienic consideration has been severely used throughout the entire equipment for this purpose.

Table - 1 shows the SCP production from various carbon-sources. Each carbon-sources gives the different theoretical yield and experimental results. Hydrocarbon shows the highest experimental yield in the present time. In summary, it may be stated that for gaseous and solid hydrocarbons and others of shorter carbon chains, bacteria would be useful but for longer chain hydrocarbons, yeast strains are must suited.

In the production of SCP n-P , our standard reaction formula is stoichiometricaly shown as Table - 2. As you know in this Table, on the production of SCP from hydrocarbon, supplement of much oxygen and removal of big amount of heat are necessary comparing with from carbohydrate.

B. The selection of strain.

The production of SCP depends upon the selection of micro-organism used. The key to selection of micro-organism for producing SCP n-P shown in Table - 3.

The strains which can be used are bacteria (Pseudomonas, Micrococcus) , fungi (Penicillium) and yeast (Candida, Torulopsis, Pichia) . By far the greatest experience in the manufacture of SCP has been done with the yeast. Yeast is generally preferred because the cells are bigger than bacteria. They have higher amino acid content , particularly lysine, and can grow at a low pH, eliminating the use of elaborate system of sterilization. Many kind of yeast which could assimilate n-paraffin have been developed so far, however,

Table-1. The relationship of carbon-source and cell yield on the SCP production from various carbon source

Carbon source		Yield %		Microbe
		theroretical	result	
Carbohydrate	$C_6H_{12}O_6$	56.7	50	Saccharomyces, Torulopsis, Candida
Hydrocarbon	$C_{15}H_{32}$	136.3	100	Candida, Pichia
Methanol	CH_3OH	59.1	42	Kloeckera, Candida, Torulopsis
Ethanol	C_2H_5OH	82.0	70	Candida, Pichia, Hansenula
Acetate	CH_3COOH	41.9	37	Candida, Pichia, Hansenula, Pseudomonas
Methane	CH_4	157.0	60	Bacillus, Pseudomonas, Bacterium

Table- 2. The oxygen demand and heat evolution on the SCP production..

Carbon source	Yield %	g O_2 required / 100 g cell	kg-cal evolved / 100 g cell
Carbohydrate	50	67.2	383
Hydrocarbon	100	196.3	780
Ethanol	70	152.0	653
Methane	60	520.6	1860

stoichiometry

Carbohydrate

$$6.67\ CH_2O + 2.10\ O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 2.75\ CO_2 + 3.42\ H_2O$$

Hydrocarbon

$$7.14\ CH_2 + 6.135\ O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 3.22\ CO_2 + 3.89\ H_2O$$

Ethanol

$$3.11\ C_2H_5OH + 4.75\ O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 2.30\ CO_2 + 6.08\ H_2O$$

Methane

$$10.42\ CH_4 + 16.265\ O_2 \longrightarrow C_{3.92}H_{6.5}O_{1.94} + 6.50\ CO_2 + 17.59\ H_2O$$

Table -3. The key to selection of microorganisms for producing SCP from n-paraffin.

1) High growth rate in n-paraffin media.

2) High yield of cellular material produced per unit weight of n-paraffin.

3) High content of protein, vitamins and unknown growth factor, and high nutritive value for animal.

4) Growing ability in simple media without additional growth factors (yeast ext., corn steep liqure etc.).

5) High optimum growth temperature.

6) Growing ability at wide range of pH and at low pH, at that pH contaminated bacteria could not grow.

7) Sufficient capacity to emulsify n-paraffin into culture broth without additional surface-active agent.

8) Easier separating capacity of organisms from culture broth at the step of cell separation.

9) High digestibility.

assimilable distribution of carbon-length of n-paraffin, optimum pH, temperature, yield are different by the each yeast strain.

Figure - II shows that the each yeast strains shows the different growth pattern on the carbon number of n-alkanes. Pichia genus shows an availability on the broadly range of carbon number.

The selected yeast strain decide the carbon number of n-paraffin. The commonly used fraction are n-alkanes in the region of C 10 - C 20.

C. Medium.

(a) n-Paraffin.

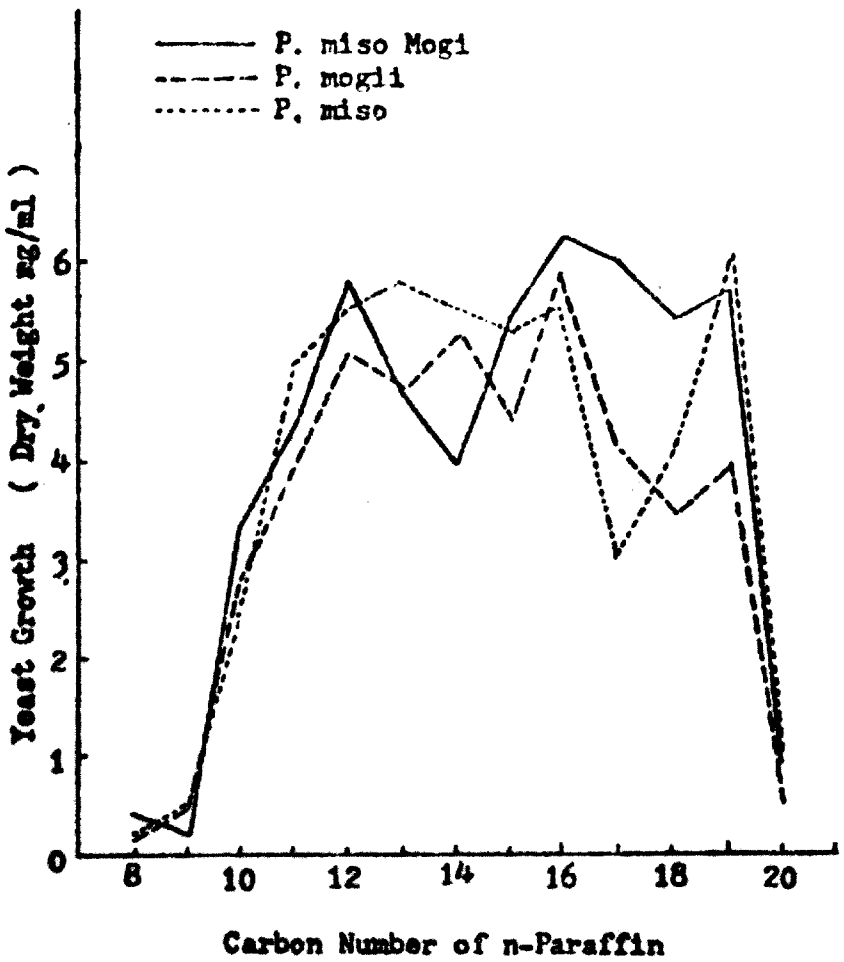
As the main raw material for production of SCP, n-paraffin is employed. Either gas oil or n-paraffin is evaluated as the main material for SCP at the present time, but purified n-paraffin appears to be definitely superior to gas oil in view of its various advantages in its freedom from toxicity, hazard, or untoward smell and safety of the final product.

By n-paraffin is meant a purified n-alkane (98 per cent) substrate. This is obtained by processing refinery distillate rich in n-alkanes either through molecular sieve or urea-adduct procedure. Usually, the mixture of n-alkanes, which have the number of carbon 13 - 20 , are used for the production of SCP n-P.

Some characteristics of a sample of the commonly used n-paraffin are shown in Table - 4.

Table - 5 shows that one kind of yeast strain has the different growing ability depend upon the different distribution of carbon number in n-paraffin..

Figure-II. Accumulation of Yeast Cells Developing on n-Paraffin —(2)



* Y. Suzuki et al. Congress of Nippon Nogeikagakukai p.204 (1966) Kyoto

Table- 4. Normal-paraffin properties.

Item		Result	Test method
Specific gravity	(15/4C)	0.7753	JIS K-2249
n-Paraffin purity	(wt %)	97.6	Gas chromatography
Carbon distribution	(wt %)		
C- 13		trace	
C- 14		16.4	
C- 15		42.9	
C- 16		25.1	
C- 17		9.4	
C- 18		2.7	
C- 19		0.8	
C- 20		0.2	
C- 21		0.1	
C- 22		trace	
Mean molecular weight	(g/m)	218.2(C-15.4)	
Iso-paraffin content	(wt %)	2.4	
Aromatics content	(wt %)	0.0007	ASTM D-2008-65
Bromine number		0.008	ASTM D-1491-60
Sulfur content	(ppm)	0.7	JIS K-2555
Colour (SAYBOLT)		30 higher	JIS K-2267
Distilation range	(C)		JIS K-2254
IBP		255	
10 %		265	
50 %		270	
90 %		281	
95 %		288	
97 %		293	
EP		296	
Recovery content	vol %	98	

Table - 5. The carbon distribution of n-paraffin and cell growth.

n-Paraffin	carbon distribution %									specific growth rate		growth rate $\frac{dx}{dt}$
	carbon 10	11	12	13	14	15	16	17	18	μ max (hr^{-1})	μ^*)	($kg/m^3 hr$)
No.1			19.5	51.6	28.0	0.9				0.198	0.180	1.60
No.2			9.8	26.0	39.3	24.9				0.203	0.190	1.78
No.3				0.8	46.8	52.2	0.2			0.206	0.200	2.00
No.4					23.4	36.6	28.0	11.5		0.239	0.230	2.40
No.5						21.1	55.9	23.0		0.265	0.265	2.65
No.6			7.8	20.6	20.6	19.2	22.4	9.2		0.250	0.250	2.40
No.7	5.3	14.4	14.6	15.0	14.6	13.7	15.9	6.5		0.258	0.250	2.30
A				0.8	46.8	52.2	0.2			0.206	0.206	2.00
B					16.4	42.9	25.1	9.4	2.7	0.232	0.230	2.30
C	2.1	3.6	5.2	7.7	10.3	13.9	16.2	14.9	11.2	0.283	0.280	2.30
				19=74		20=39						

μ^* : mean specific growth rate in the range of 2 - 10 kg/m^3 cell concentration.

(b) Auxiliary materials.

Upon considering characteristics of used strain, the cell composition, and economy, the composition of the medium is decided.

Usually following auxiliary materials are used. The commonly useful kind of nitrogen-sources and auxiliary materials are shown in Table - 6.

D. Fermentor.

At the present time three types of fermentors are used, (i) mechanically agitated fermentors, (ii) air-lift fermentors, (iii) a hybrid of the (i) and (ii). There is ample scope for designing more improved type of fermentors which can ensure greater cell density and productivity. In the selection of the fermentor type, various studies were done on various types of fermentors. It is necessary that following characteristics of fermentor on aerobic cultivation are compared.

Table - 7 shows the necessary characteristics of fermentor. We adopted the bubbling tower type with confidence from the points of economization and largeness of the equipment scale. Morever, in continuous culturing, we studied in details the reaction of each fermentor for determining the number of vessels to use and decided to adopt the two reactors formula, arranged in series under continuous cultivation.

This cultivation is the only and most important reaction field in this process. One line is made from two vessels, and under the two vessel series continuous culture are operated. First fermentor

Table-6. The kind of auxiliary materials for SCP production.

Item	Materials
Nitrogen	NH_3 , $(NH_4)_2SO_4$, $(NH_2)_2CO$
Phosphorus	KH_2PO_4 , H_3PO_4 , $(NH_4)_2HPO_4$
Potassium	KCl , K_2SO_4 , KH_2PO_4
Magnesium	$MgSO_4$, $MgCl_2$
Iron	$FeSO_4$, $FeCl_2$
Natrium	NaCl , Na_2SO_4
Calcium	$CaCl_2$, $Ca(NO_3)_2$
Zinc	$ZnSO_4$
Manganese	$MnSO_4$
Copper	$CuSO_4$
Corn steep liquor	..... if necessary
Yeast extract	..... if necessary

Table - 7. The characteristics of Fermentor on the aerobic cultivation.

1. OXYGEN TRANSFER

..... from bubble to liquid (broth)

2. MASS TRANSFER

dissolved oxygen, substrates and other nutrients

..... from liquid to cell

carbon dioxide and other metabolites

..... from cell to liquid

3. UNIFORMITY OF BROTH

to prevent sedimentation and flocculation.
to keep the uniform concentration of limiting substrates.

..... especially for continuous cultivation

4. HEAT TRANSFER

to maintain the optimal temperature for growth.

..... removal of evolved heat

was mainly for propagation of cells and second fermentor for consumption of residual n-paraffin.

II. THE PROCESS OF PRODUCTION OF SCP N-P.

On the cultivation of SCP from hydrocarbon, the followings are especially required, comparing with from carbohydrate. Supplement of much oxygen, because of lacking oxygen in the molecule of n-paraffin. Removal of big amount of heat, formed during the reaction into SCP, and dispersion of n-paraffin, because of its insolubility in the water, dissolving these problems, we developed unique fermentor.

In the fermentor design, circulation of liquid was achieved by the action of injected air and internal device without any mechanical agitation. Supplement of oxygen from air was satisfied as much as high growth rate by mean of giving pressure.

Power requirements, which were mainly for air compressor, were low.

Cooling system including effective heat-exchangers was equipped out of fermentor.

In the adopted fermentor, continuous cultivation method was carried out in series of two fermentors. First fermentor was mainly for propagation of cells and second fermentors for consumption of residual n-paraffin. Strictly speaking, both first and second fermentors are similar but not same in their internals.

We accomplished entire equipments and techniques of continuous cultivation for long period without any trouble of microbial infection. In regard to scale-up of fermentor, we had already completed its study with the bench plant and the pilot plant as the testing objects.

Growth rate at first fermentor on continuous culture was 3.0 kg dried cell/m^3.hr. or more than and the final cell concentration of second fermentor was decided 20 - 22 kg dried cell/m^3. By this culturing process, 100 kg of dried cells was given from 100 kg of n-paraffin or less amounts.

Regarding to the medium composition of auxiliary raw materials, economical and well balanced combination including trace minerals and others was made. Gaseous ammonia as a nitrogen-source was injected into the process air.

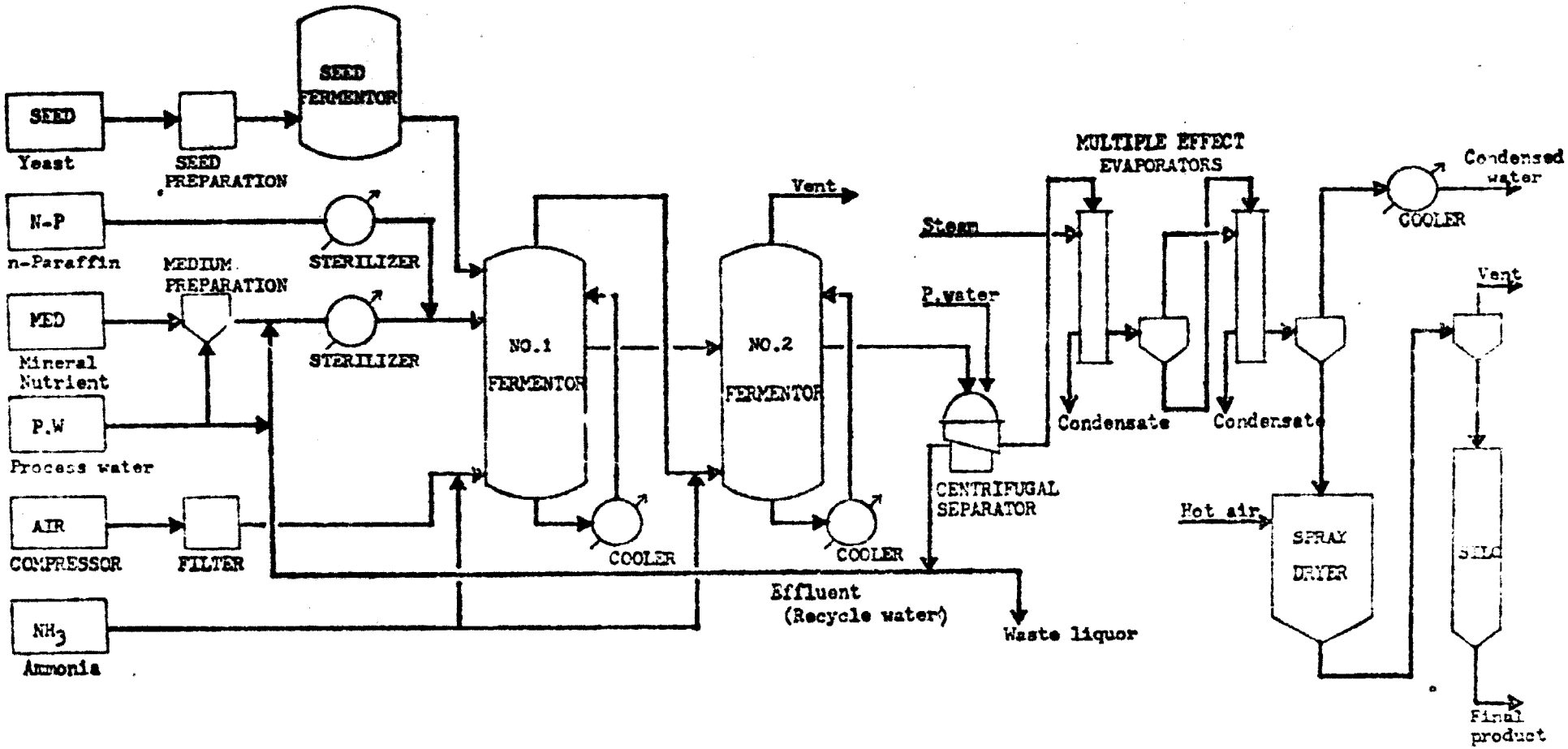
Concerning to the water for cultivation, we had succeeded in reusage of the effluent which had been obtained through the centrifugal separation of cultivated broth, as the process water. The technique of the reusage of 85 per cent or more than of the effluent was accomplished. Even when this effluent was used as process water, there was absolutely no harm to culturing and rather we reached up to the technique of obtaining a better productivity by reusage. This technique was advantageous point from the viewpoint of problem " water pollution ".

After cultivation, the processes of separation, washing , condensation and drying were further followed until getting final products. The solid content after separation and washing was 16 per cent which was raised up to 23 per cent in the evaporator. After condensation, the product was dried either in a spray drier or in a flash drier. The spray dried product gave fine powders and the flash drier gave small granules suitable for use in solid animal feeding stuffs.

Figure - III shows the process of the SCP n-P production.

Figure - III. D I C PROCESS ; S C P FROM N-PARAFFIN

(SCHEMATIC REPRESENTATION)



III. THE CHARACTERISTICS OF SCP n-P.

A. The general constitution of SCP n-P.

SCP n-P , which is used as a protein source for animal feed, is a dried powder or granule with yellowish brown and a slight yeast flavor , and contains approximately 58 - 60 per cent high content of protein, vitamins, minerals and unknown growth factor. Its protein has well balanced components of various amino acids and excellent digestibility.

The SCP n-P can be featured as having outstanding composition and quality in comparison with the conventional feed materials such as soybean meal and fish meal (Table - 8,9,10,11).

In connection with the amino acid composition of the SCP n-P, it should be attended to that its lysine content is very high, and that vitamin B group is contained in it a particularly high level. Thus, it can be claimed that SCP n-P is an ideal material for feed stuff because it contains protein with essential amino acids, high calory, and levels of vitamins and minerals.

The SCP n-P has so far been complained generally of its poor digestibility because of its hard cell-walls, but we have already solved this problem through our elaborated research. A number of protein digestibility tests have been carried out using swines fed with the SCP n-P . It can be realized from the results that the SCP n-P has a very good digestibility a better TDN than fish meal by more than 10 per cent. (Table - 12)

Table - 8. General analysis of SCP N-P

Constitution	Content (%)
Crude protein	60.10
Moisture	2.56
Crude fat	6.83
Crude ash	10.04
Crude fiber	3.97
Nitrogen free extract	16.50
Digestibility (Pepsin)	92.10

Table - 9. Amino acid composition of SCP N-P.

Amino acid	Content (%)
Lysine	11.9
Histidine	3.4
Arginine	6.3
Threonine	5.4
Aspartic acid	10.5
Serine	5.4
Glutamic acid	11.9
Proline	1.9
Glycine	4.7
Alanine	5.7
Cystine	1.1
Methionine	1.7
Valine	5.7
iso-Leucine	5.1
Leucine	9.4
Tyrosine	3.9
Phenylalanine	4.6
Tryptophane	1.0

Table- 10. Contents of vitamins in SCP N-P.

Vitamin	Content (mg %)
Vitamin B_1	1.26
Vitamin B_2	9.61
Vitamin B_6	1.73
Vitamin B_{12}	0.02
Pantothenic acid	30.90
Choline chloride	690.00
Nicotinic acid	100.00
Biotin	0.13
Folic acid	0.22
Inositol	580.00

Table - 11. Contents of Minerals in SCP N-P.

Mineral	Content(mg %)
P	1990
K	1630
Ca	104.63
Mg	248.96
Fe	68.47
Zn	21.91
Cu	0.44
Mn	4.74

Table - 12. Digestible nutrients of various proteinous materials for swine.

Protein sources	Digestibility				Digestible Crude proteins	Total Digestible nutrients
	Crude proteins	Crude fats	Nitrogen free ext.	Crude fibers		
SCP N-P	90	76	97	14	54.1	84.2
Defatted soybean	91	80	92	60	41.7	73.9
Peruvian fish meal	92	81	-	--	57.3	65.4
Torula yeast	82	50	85	-	38.0	71.7

B. Feeding tests.

Upon termination of feeding tests, milking cows, beef cattle, swines, layers, broilers, eels, young yellowtails, sea broams, prawns, carps. rainbow trouts, and Ayu fishes employed for the tests on the nutritive values of the SCP n-P were subjected to outopsy for histopathological examination.

Feeding tests with SCP n-P were conducted by partly or perfectly replacing with SCP n-P fish meal, soybean meal or skim milk.

As a result, the tested groups shows better results than the control groups with no substitution, no significant difference could be seen between the control and tested groups in terms of body weight and feed consumption. After feeding test, we tried histopathological examinations of specimens for microscopic examination of meat, liver, kidneys and pancreas of the animals used for the SCP n-P feeding.

C. Safety tests.

The Mininstry of Health and Welfare of Japanese Goverrment set forth the strictest ever standards of all the other ones so far stipulated for confirmation of safety of animal feeds.

The standards covered 6 chapters containing 22 items as illustrated in the Table - 13.

Therefore, we have carried out elaborated tests on every item of the standards in addition to our detailed studies previously conducted on its safety. The results of these studies were submitted to the Food Sanitation Investigation Council which is an

Table - 13. Summary of safety standard regarding SCP N-P in Japan.

1. Microbial strains (Microbes employed for production of SCP N-P)

   (1) Microbiological tests involving morphological and biological tests.
   (2) Presence of variability.
   (3) Infectivity.

2. Materials (Materials for media containing n-paraffin)

   (1) Polycyclic aromatic hydrocarbon.
   (2) Heavy metals.

3. Living cells (Microbial strain at the peak of its propagation in any cycle of the production process)

   (1) Polycyclic aromatic hydrocarbons.
   (2) Heavy metals.
   (3) Mycotoxins.
   (4) Toxicity tests.

4. Broth (Liquid broth at the peak of microbial propagation in any cycle of the production process)

   (1) Polycyclic aromatic hydrocarbons.
   (2) Heavy metals.
   (3) Mycotoxins.
   (4) Toxicity tests.

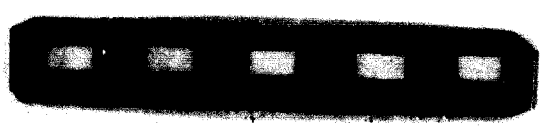
5. Product (The product to be used as animal feed after the process of production.)

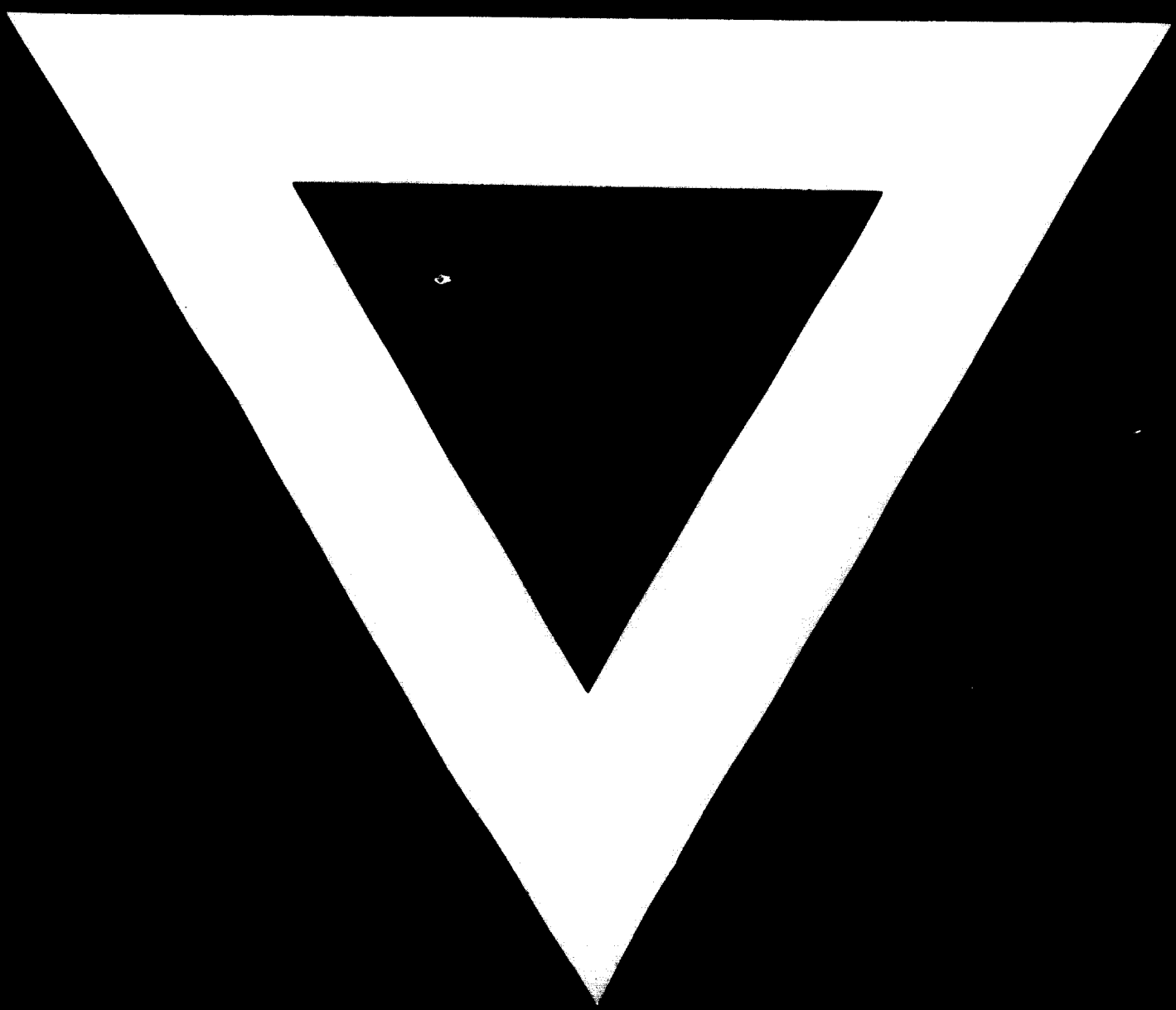
   (1) Presence of living cells in the product.
   (2) Polycyclic aromatic hydrocarbons.
   (3) Heavy metals .
   (4) Mycotoxins. (This can be skipped if mycotoxins are detected in the tests on living cell or broth)
   (5) Toxicity tests.
   (6) Multiple generation tests.

6. Meat, Milk and Others. (Edible products such as meat, egg, milk derived from livestocks, poultry and fishes reared by feeding of the above final products)

   (1) Polycyclic aromatic hydrocarbons.
   (2) Heavy metals.
   (3) Mycotoxins. (If no mycotoxins can be detected in the tests on final products, this can be skipped)

advisory organization to the Ministry. The council investigated these data for a long period and finally drew a conclusion on December 15th, 1972, that the products of our company are safe enough for use as feedstuff.





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