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Vienna, Austria, 9 - 12 October 1973

**PRODUCTION OF SCP FROM DIFFERENT
RAW MATERIALS OF PETROCHEMICAL
INDUSTRY IN CZECHOSLOVAKIA ^{1/}**

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SUMMARY

PRODUCTION OF SOLUBLE MICROBIAL RAW MATERIALS OF PETROCHEMICAL INDUSTRY IN CZECHOSLOVAKIA ¹

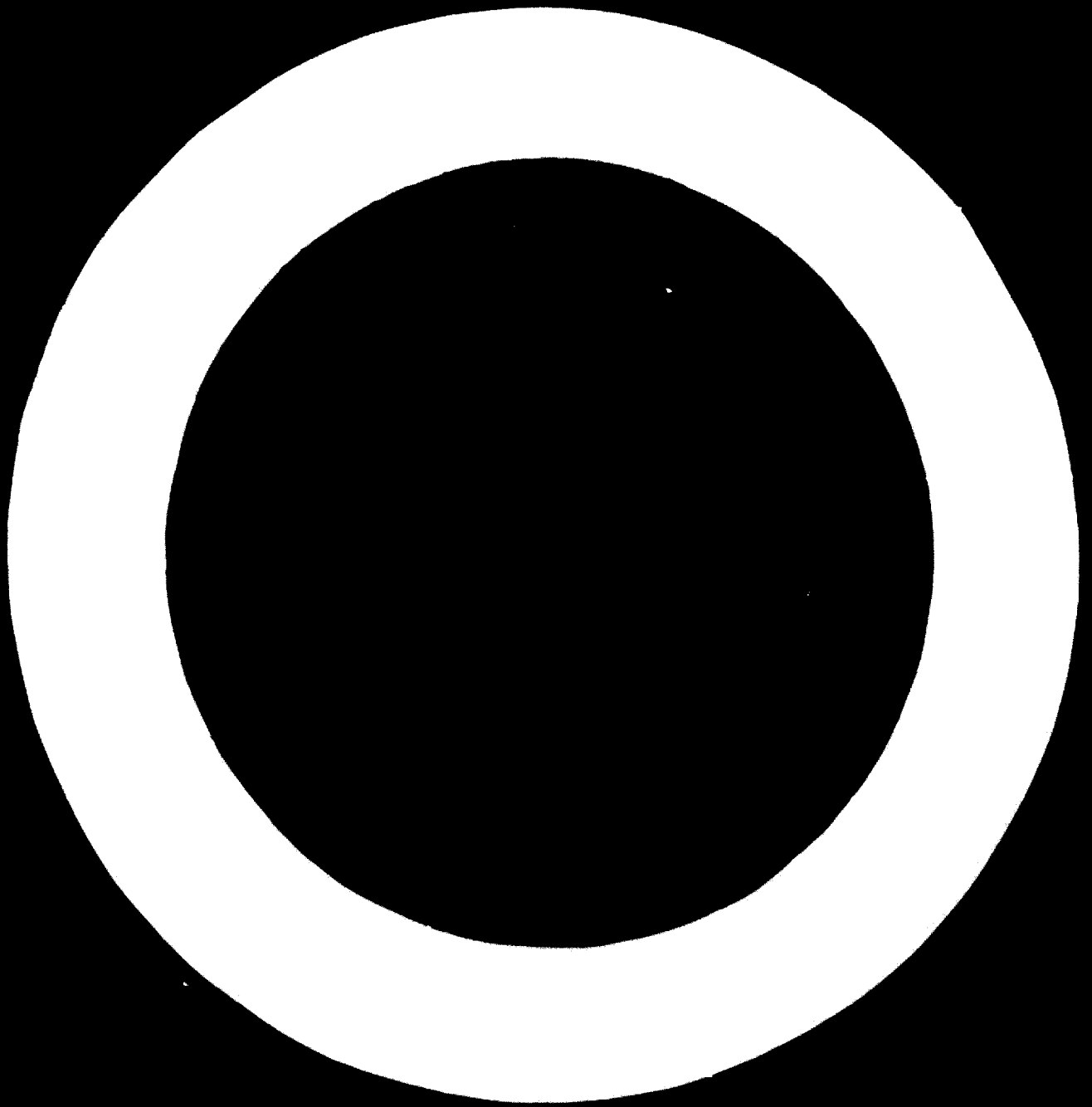
J. Vlastný and M. Řešík*

Czechoslovakia is a country which does not possess sufficient classical agricultural sources for the production of protein for feeding purposes. Therefore considerable attention has been paid to protein production by microbial methods. As a basic raw material, different carbon sources produced by petrochemical industry, have been used such as gas oil and other oils, n-alkanes and synthetic ethanol. The aim of this research was not only to produce SCP, but also to obtain gas oil fractions having a lower freezing point. The formation of biomass and the depth of deparaffination can be controlled with the dilution rate and the concentration of oil.

Three different technological processes for the production of SCP using carbon sources mentioned above have been developed on a laboratory scale and scaled-up to pilot-plant unit with the total capacity of 20 cubic meters.

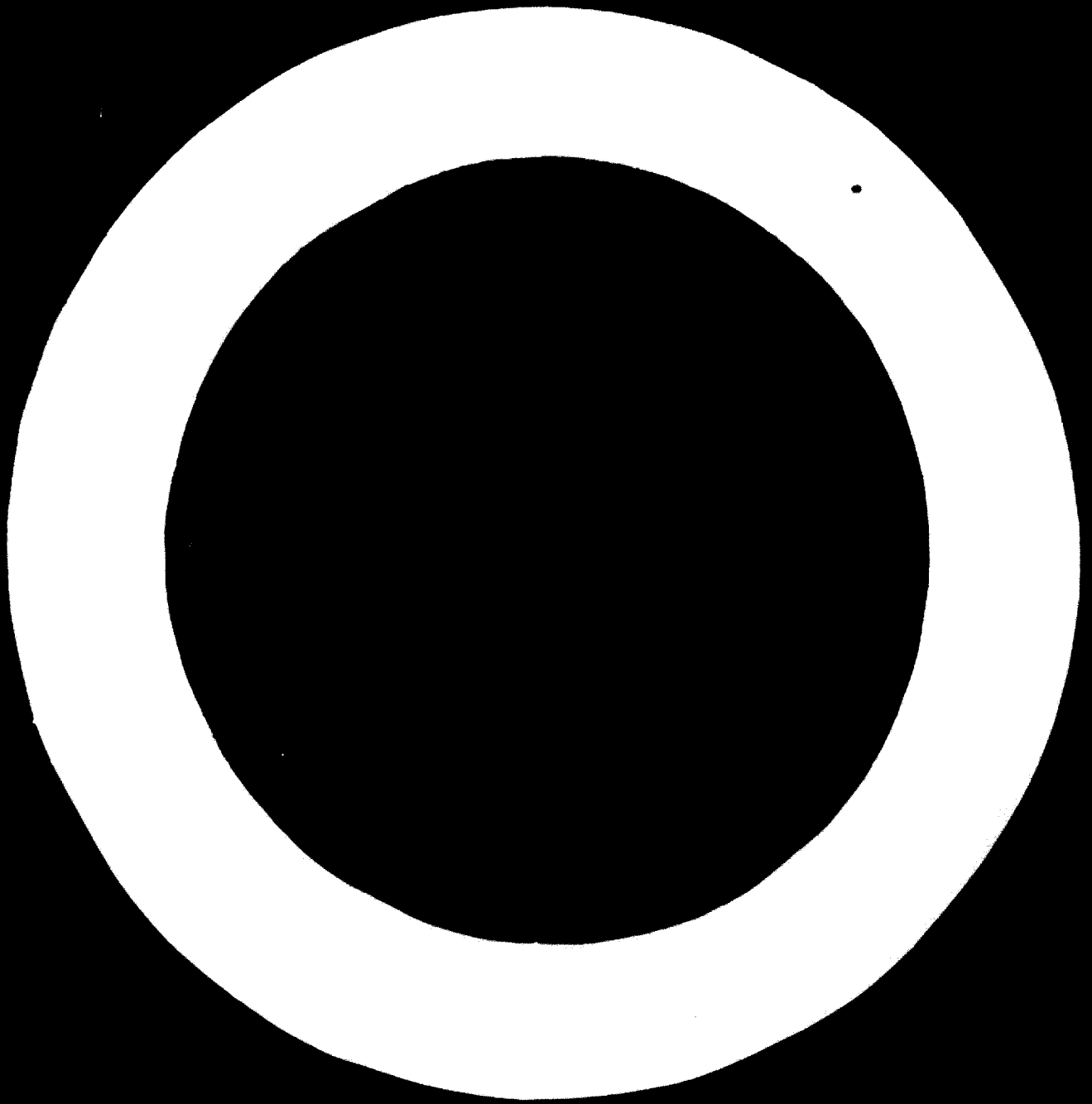
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The nutritive value of the yeast was tested on chickens, piglings and pigs. As a comparative material, yeasts grown on stillage from distilleries were used. The values for digestibility, metabolizable energy and biological value were almost identical.

The yeasts grown on ethanol were used for isolation of pure proteins and were added to different type of food (meat, biscuits) up to 10% for direct human nutrition.



In Czechoslovakia the problem of producing the microbial protein as a supplement to classical protein sources for human and animal nutrition has been studied for more than two decades. This effort exerted both in theoretical and in practical sphere, resulted in the verified technological processes of SCP production from various traditional (molasses, sulphite liquors, alcohol stillage and waste water from citric acid production) and non-traditional petrochemical raw materials.

Many times Czechoslovak scientists reported on their results achieved in this field and presented many papers at various international congresses and symposia which were included in the proceedings. Some basic papers are given in references (1-4).

Results of the experimental work have been realized and at present SCP from all sorts of traditional raw materials is produced in several smaller plants in Czechoslovakia. Economically, the most advantageous process is the production of SCP from sulphite liquors and from waste waters from citric acid production. In our conditions the production of SCP from molasses is too expensive and therefore synthetic ethanol is employed. This production was patented in Czechoslovakia in 1964 and was experimentally realized several years ago. The advantage of this process is obtaining of an absolutely harmless product suitable for the preparation of edible protein for human nutrition and the minimum amount of waste water because during the fermentation process the water recirculates and is only filled up. Compared with the production from molasses, the consumption of germ-free water decreased to about 1/10. Therefore the production based on petrochemical raw materials is advantageous for the areas without a sufficient supply of biologically harmless water for fermentation purposes.

Another production based on petrochemical raw materials which was verified experimentally is the SCP production from n-alkanes isolated from gas oil. The realization of this process has been postponed because in Czechoslovakia no sufficient amount of isolated n-alkanes which would cover the production of at least 20 000 tons of SCP is produced.

On a pilot-plant scale the production of SCP from gas oil was verified in one plant equipped with two 100 hl fermentors and for several years the product was tested from the point of view of biological harmlessness in medical and agricultural laboratories in Czechoslovakia and in German Democratic Republic. This SCP was found fully suitable for feeding purposes. The elaborated process of extraction of the lipidic fraction decreases the content of total lipids to 2 % and the content of hydrocarbons under 0.3 %. Until now the Czechoslovak authorities have not given the approval for the use of SCP from gas oil as fodder.

For production of SCP from gas oil no growth substances are necessary, as the yeasts are capable to grow only on mineral substances and n-alkanes. This production, similarly as that of ethanol, is based on the recirculation of water.

Czechoslovakia produces most of the equipment for production of SCP. The volume of the agitated fermentors amounts to 200 cbm.

The new research problems are coordinated by the state plan and are solved by many institutes, the most significant of them are: Institute of Microbiology of the Czechoslovak Academy of Sciences, Prague, Research Institute of Fodder Industry, Pečky, Institute of Chemical Technology - Technical University, Prague, Research Institute of Chemical Equipment, Chepos, Brno, Medical Faculty of Charles University, Prague, Institute of Human Nutrition of the Ministry of Health, Prague and the UKSUZ, Prague.

In the following part of this report we would like to inform you about some Czechoslovak scientific results in this field. In the theoretical sphere much attention is devoted to the question of yeast growth on hydrocarbons of various chain length, on their mixtures and derivatives, starting with methanol, ethanol and ending with oils and waxes containing prevaillingly paraffins of the chain length $C_{20} - C_{26}$. Metabolism of these substances, influence and mechanism of the effect of ensuing intermediates on the yeasts are studied. Considerable attention is paid to mesophilic kinds of yeasts and to analytic methods which are important from the viewpoint of following the course of fermentation and isolation processes, as well as from the viewpoint of evaluation of the end products. With respect to the scale up and to the process of growth optimization several bioengineering problems are followed. To this sphere belongs also the study of isolation of proteins and yeasts.

As we pointed out earlier, different yields are reached on hydrocarbons of various chain length. We followed the kinetics of intermetabolite formation during fermentation and we found out that in dependence on the aeration conditions and on the medium composition various amounts of fatty acids with the chain length approaching that of hydrocarbons (5-7) accumulate in the cells as well as in the environment. We succeeded in proving that fatty acids with the chain length shorter than C_{12} inhibit preferentially the anabolic processes and only at higher concentrations also respiration and catabolism. Simultaneously, they inhibit the CO_2 fixation. The result of this effect is a decrease in the yield and in the growth rate (θ). These findings explain to a great extent the above mentioned findings concerning the yield decrease during cultivation of yeasts on hydrocarbons of the chain length of $C_{10} - C_{12}$. By controlling the aeration, the cultivation conditions and by selection of the strain these losses can be decreased and the process optimized.

In contrast to paraffins with a shorter chain length from lower distillation fractions which influence negatively the fermentation, the paraffins from higher fractions with the chain length over C_{20} are by many yeasts worse utilized. By means of selection we succeeded in obtaining strains of *Candida lipolytica* which do not only preferentially utilize these hydrocarbons, but do also multiply optimally at the temperature of about $35^{\circ}C$ which is very advantageous from the viewpoint of solubility of hydrocarbons as well as from the viewpoint of consumption of cooling water (9, 10). The aim of cultivation of the yeasts on mineral oils, similarly as earlier on gas oil, was not only to obtain the biomass with a high protein content but also to deparaffinate the oil. By this process freezing point was decreased by $50-60^{\circ}C$ and that up to $-15 - -12^{\circ}C$.

In this field of bioengineering, the problem of insoluble substrate and of oxygen transport during hydrocarbon fermentation is studied. Some models were elaborated describing the role of a continuous and of a dispersion phase during the growth of the culture on n-alkanes and the optimization of the process was studied from the viewpoint of the yield and oxygen consumption. The optimum size of the oil droplets can be controlled by the fermentor. A detailed description of these results is given in the paper by Prokop and Sobotka (11) presented at the II. Conference on SCP at MIT in Boston.

The questions of optimization and of increasing the volume of fermentors are studied intensively. The new prototype of the fermentor is able to produce 1.8 kg of yeast from 1 cbm of gas oil per hr (and 2.5 kg from n-alkanes), and its design enables to increase the volume in order to guarantee the minimum production of about 500 kg of dry weight of the yeast per hour.

The realizability of the SCP production from various non-traditional raw materials is determined by the applicability of protein in animal and human nutrition. Therefore all experimentally produced SCP is studied from the viewpoint of biological value and biological harmlessness and the chemical analyses on residual hydrocarbons and their derivatives are performed. This systematic analytical work guarantees the introduction into production only of processes which produce harmless products. Determination of the biological value of the yeast cultivated on n-alkanes and on synthetic ethanol are in accordance with the published results. When using synthetic ethanol as a starting raw material there is no need to refine it. No admixtures contained in ethanol were found in the yeast. 150 kg of ethanol is required to produce 100 kg of the yeast dry weight. The yeast contains about 60 % of nitrogen substances and only 5 % of fats and it does not contain any odd fatty acids and hydrocarbons. Synthetic ethanol therefore seems to be the most advantageous starting raw material for the production of the isolated protein for human nutrition.

In many cases the production of SCP is disadvantageous because the production costs are much higher than those of classical protein fodders. An essential change in the economical aspects would occur if a part of the SCP produced were used directly in human nutrition. This would prevent the losses of protein occurring during feeding and save the manpower necessary for breeding of animals and the price of the protein for human nutrition can be about 3 times higher than that of the protein in fodder. Therefore in our institute the isolation of protein from yeasts during which both fodder and edible protein is obtained is studied. The results of this work were presented at the symposium on "Advances in Microbial Engineering", held in Mariánaké Lázně in 1972 (12) and therefore we mention only some

essential data. For this production the yeast cultivated either on sugar substrates or on alcohols is employed. When using the yeast from n-alkanes an efficient extraction is to be included into the process to remove all residual hydrocarbons, fatty acids and fats. This treatment is necessary because fatty acids and fats contain also molecules with odd number of carbons.

After desintegration of cells the level of nucleic acids is decreased and the protein is precipitated. The final concentrate contains over 90 % of protein. As far as the physical properties are concerned the protein concentrate is equal to or even better than the soya protein. It binds well the water and emulgates the fats. The NPU is 70 and the chemical score 72, which is caused by the lack of sulphur amino acids. In other amino acids the chemical score approaches 100 or is even higher. Excellent physical properties of the isolated protein enable its use in some meat products as well as in the baking and dairy industry. The content of nucleic acids is below 1 % of dry weight and is lower than in meat.

From 3 kg of the yeast 1 kg of isolated protein and 2 kg of fodder are obtained. According to preliminary calculations 1 ton of isolated dry protein should in European countries cost maximally 800 US \$ per 1 ton. With respect to the fact that during the preparation of foodstuffs the protein is diluted with water in the minimum ratio 1:1 then the price of the end product should amount to less than 400 US \$ per 1 ton.

Conclusion. In Czechoslovakia, several processes of the SCP production from traditional as well as from non-traditional, i.e. petrochemical raw materials, were elaborated.

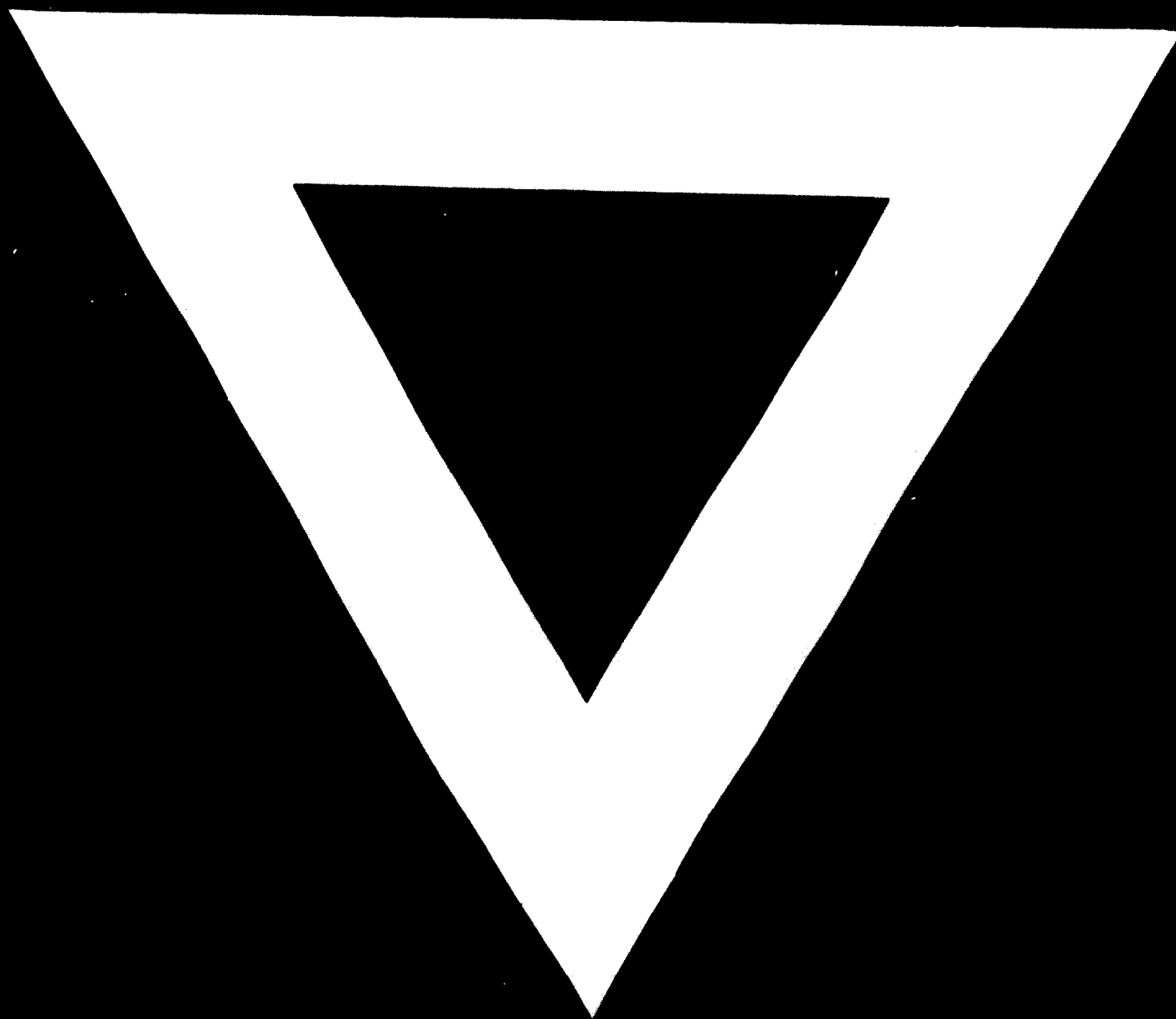
Further possibilities of the SCP production from various raw materials for feeding purposes are systematically followed and economically advantageous combinations of the production of fodder and of edible protein from the yeast are studied.

This experience enables Czechoslovakia to offer assistance to developing countries either independently or in the cooperation with other partners and that in education of experts, in research and in production.

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