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SOME SPECIFIC FEATURES OF THE METHODS OF PRODUCTION OF BACTERIAL FOOD PROTEIN FROM DIESEL FUEL AND PURIFIED NORMAL PARAFFINS 1/

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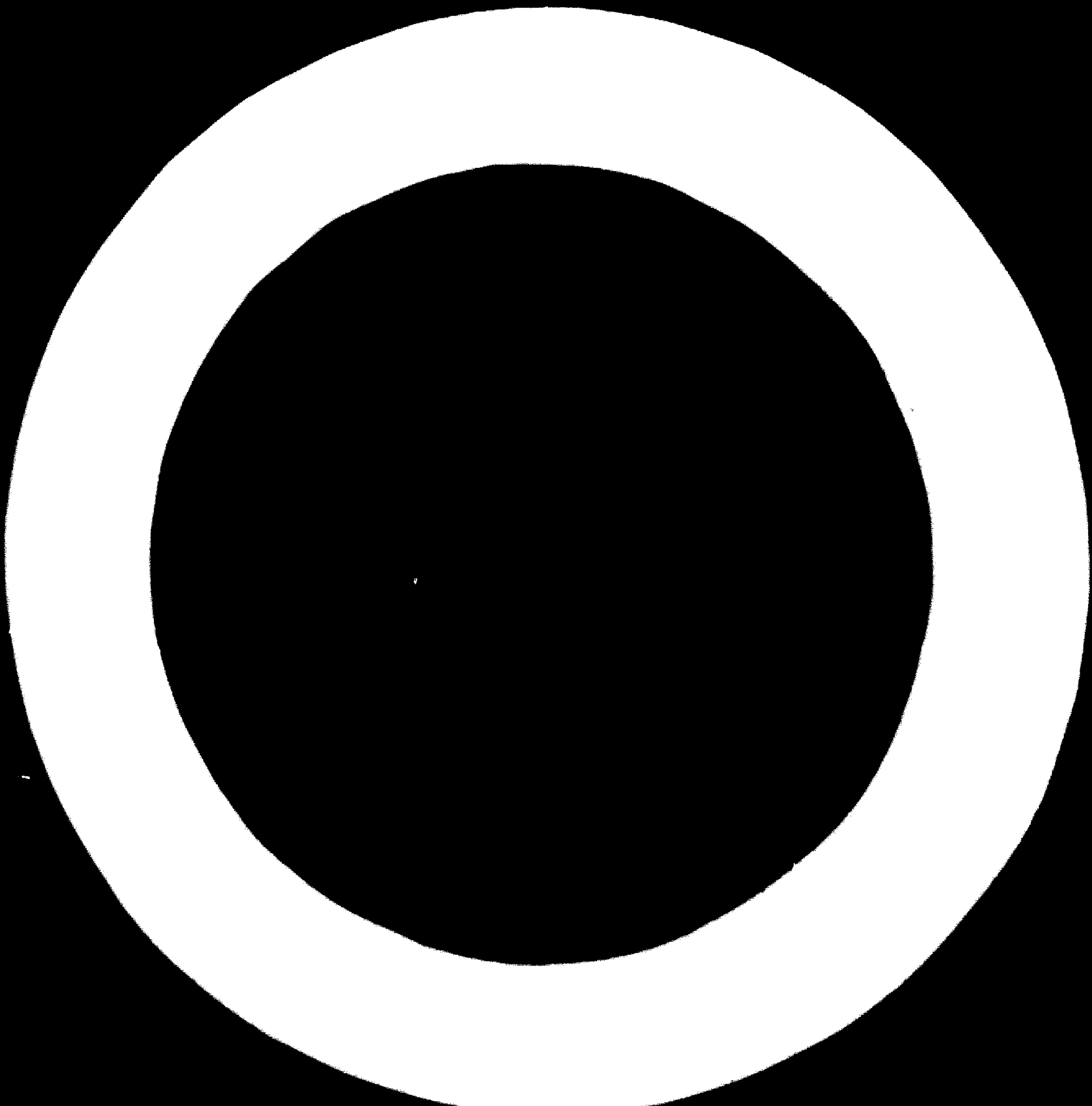

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SUMMARY

SOME SPECIFIC FEATURES OF THE METHODS OF PRODUCTION OF BACTERIAL FOOD PROTEIN FROM DIESEL FUEL AND PURIFIED NORMAL PARAFFINS 1/

K. Kostadinov*

Efforts to develop microbiological methods for the production of food protein from non-nutritive raw materials are recently made in many countries. Most wide attention is paid to microbial oxidation of n-paraffins and to microbial paraffin removal. The reasons for this are the practically non -limited raw material reserves and the high level of development of technological decisions reached by different companies. In spite of the uniform biological and biochemical basis of these methods, differences in technological decisions are justified by unequal behaviour of raw materials. The most important differences are in the amount of investments, operation costs, composition of the end product and the way of inclusion of the above mentioned methods in the existing petrochemical plants.

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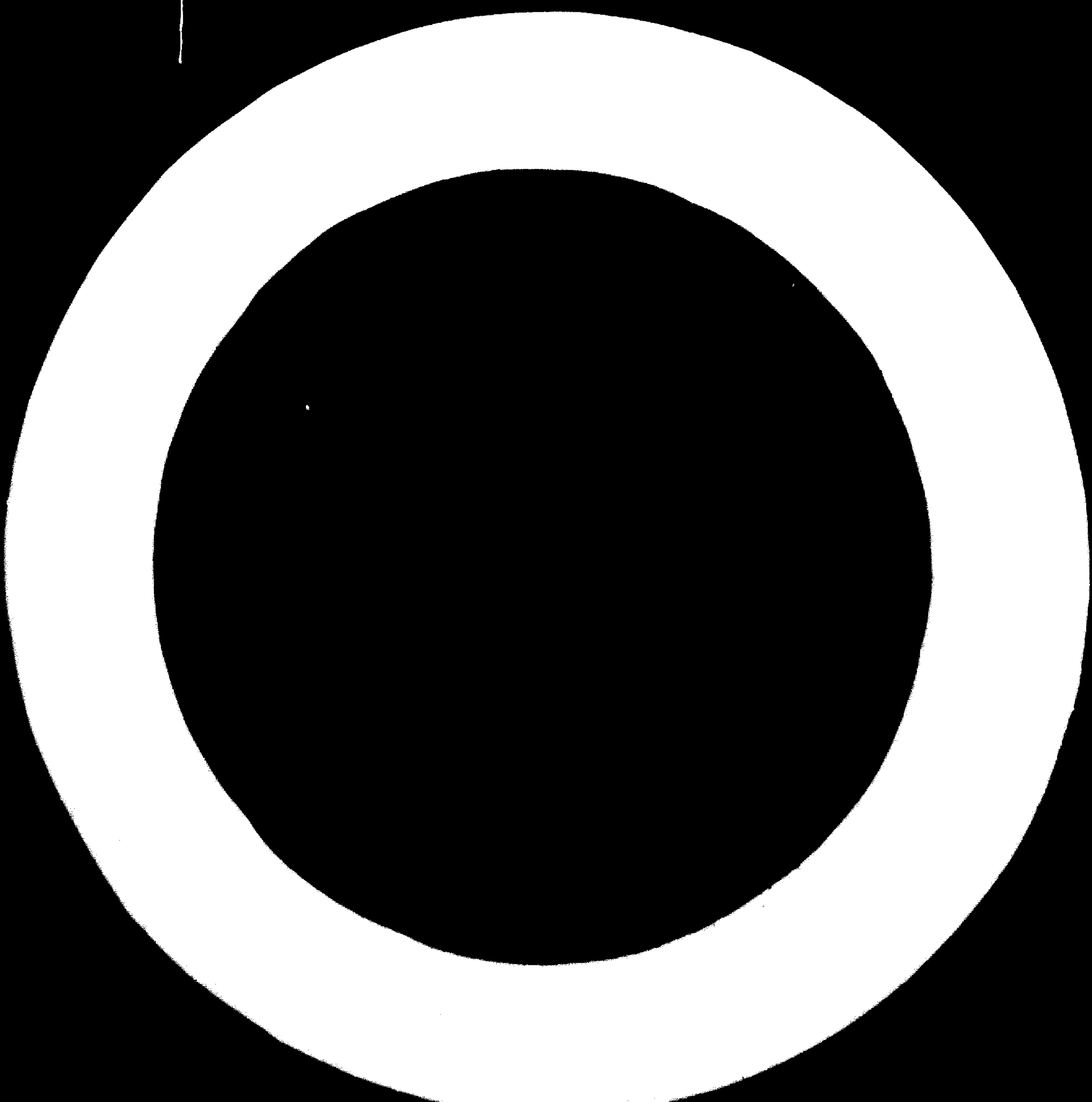
Most accurate mathematical assessment of these differences may be announced by investigators who have already studied all problems of these methods. But semi-quantitative assessments may be made by every investigator depending on the extent of his knowledge on these processes.

The choice of a successful method and technology depends to a high degree on the real conditions in each country, particularly on the level of industrial development, the number and qualification of the available specialists and the material and power resources.

From the point of view of petrochemical industry microbial removal of paraffins may facilitate transportation of crude oils of high paraffin content or the production of high quality gasoline from middle petroleum fractions or may allow the production of heavy fuels of special properties.

Combination of protein production from purified normal paraffins and alkyl benzene sulphonate production may lead to a reasonable scheme for using the available raw materials. The possibility of increasing the capacity of units for the production of n-paraffins may contribute to the improvement of both kinds of production.

Microbial paraffin removal may be carried out according to requirements in such a way that non-edible fats properly purified may be obtained and thus the balance of raw materials for detergents may be improved.



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INTRODUCTION

There are many reports on a world scale on protein deficiency and the problem is still under study from various aspects. In the striving for producing more and more protein work is carried out on the intensification of agricultural production, utilisation of underwater resources, direct processing of plant products for obtaining protein and production of microbial proteins by employing non-nutritive raw materials. Each one of these trends has its advantages and defects on account of which no one of them can be accepted as unique and universal. Although we are accustomed to speaking about protein problems, it does not refer, in fact, to satisfying the needs of protein in general, but to balanced nutrition of people and animals by using the full value of foods and fodders. At the same time, these are needs which step out beyond the limits of what is physiologically needed and which penetrate into the sphere of human psychology. The word is about food as an element of the complex of sensory and emotional perceptions. Solution of the problems of nutrition as a psychological necessity, in the economically uneven developing human society, is performed at different levels in a scientific, production and consumer's relation, it being necessary to satisfy certain requirements for overcoming the psychological barrier. The food must have a certain agreeable taste, aesthetic appearance, attracting smell, giving also to some extent a physical satisfaction. The purpose is to satisfy the arising needs and not to influence them by the force of prices because of the deficiency of surpluses. It can easily be assumed that the low price of non-conventional protein and the high price of animal protein may lead to deformation of the needs and prices and to an alteration of the structure and forms of human nutrition therefrom. But, is this our purpose?

Considering the problem of satisfying the natural nutritive needs of man, we would like to draw our attention to the two basic microbiological methods for production of protein fodder from petroleum products: microbial deparaffinization of petroleum fractions and microbial transformation of purified normal paraffins.

I. MICROBIAL PROTEIN FROM PETROLEUM FRACTIONS

Microbial deparaffinization is distinguished for its greater possibilities in adapting itself to the concrete conditions of each country and its economics. Depending on the structure of the prices and character of the needs we may accept different variants of the method by means of which to satisfy different needs and to achieve in a different way a maximum economic effect. Which are the main technological specific features of the method?

The basic requirement to the raw material is that it contains normal paraffins with chain length practically greater than C_{IO}. The volatility of the raw material is, of course, not unimportant since the high degree of aeration causes the carrying away in the atmosphere of considerable amounts of hydrocarbons which may have noxious effect, above all, on the environment and exert, to a lesser degree, an unfavourable effect for increasing the value of the final products. This may be avoided by selecting petroleum fractions with an initial boiling point of over 250°C at which the quantity of hydrocarbons eliminated in the air becomes practically insignificant.

Selection of the most adequate raw material is realized through search of an optimum among the price of the initial fraction, content of favourable n-paraffins, price of the deparaffinized fraction and working expenses for the microbial deparaffinization. As regards the raw material, particular attention deserves the dilemma of the criteria: optimization of the degree of deparaffinization, opposed to the bioproductivity of an unit volume fermenter. The compromise solution between the two alternatives depends on the specific conditions in each country.

The high degree of extraction of n-paraffins is partly associated with the problem of complete utilization of the resources, but first of all as a method for production of a new type of petroleum fraction- such with a low cooling point. The processing of the oil fractions or of the fuel for the northern regions still seems to be in the perspective. It seems reasonable to distribute the material expenses on microbial deparaffinization between the produced protein and low-cooling fraction since the latter has a considerably higher price than the one of the initial fraction.

The processes of microbial deparaffinisation, optimised according to the cooling temperature indicator, however, require higher energy expenditures and greater investments since the productivity of the fermenter, expressed in kg of biomass/m^3. hour/ is considerably lower in comparison with the processes optimised according to the indicator of biomass produced. We are not able to present a numerical expression of these differences since this depends on a composite complex of mutually-conditioned factors: temperature, boiling interval of the fraction, composition and amount of the n-paraffins in the fraction, wanted lowering of the cooling point and not in the last place - on the properties of the microorganisms. The contradiction in the essence of the bioprocess is related to the circumstance that the specific rate of reproduction of the microorganisms decreases along with the increased length of the hydrocarbon chain. A repeated study has been made on the change of the generation time of the strain, depending on the length of the hydrocarbon chain. It has been demonstrated (I) that owing to the differing generation time of the strain with regard to the different n-paraffins, the latter are depleted to a different degree from the culture medium. Whereas, for example, C_{I2} is metabolized 95%, C_{I9} is metabolized only 30% on condition that they have been present in the nutrient medium in equal quantities. Most recent investigations (2) have revealed that certain strains are characterized by reverse dependence. Upon cultivation in a shaking machine at 37°C on a model mixture of equal quantities of n-paraffins within the limit of C_{I2} - C_{20}, the following results have been obtained:

Table I

Strain	Degree of metabolization(%)			Productivity (g/l 48 h)
	C_{I3}	C_{I7}	C_{I9}	
Candide melini 42	I7	40	75	4
Candide melini 44	25	52	83	2
Candide zeylanoides	90	87	82	7

The multivariant approach to the raw material for microbial deparaffinization suggests that this method makes possible a more complete linking of microbial protein production with petroleum chemical technologies. Of particular practical interest is the microbial deparaffinization of medium petroleum fractions intended for the cracking process. The relative increase of the content of aromatics and isoparaffins in the deparaffinized fraction sharply improves the octane benzine number obtained after the cracking. Besides, it is not necessary to optimize the process for achieving a maximum decrease of the n-paraffins content (or of the cooling point) which would not lead to an increase of the investments and direct expenditures per unit of production. The countries producing and transporting petroleum may find acceptable the preliminary microbial deparaffinization of petroleum so as to realize a decrease of its viscosity and improve the conditions for its transportation.

Since we have no reason to point out only the advantages of this method, let us also mention the difficulties which arise in stirring up the culture medium containing a large percentage of petroleum fraction. The relatively stable emulsion of water into oil, non-soluble in water, in which microbial deparaffinization is realized, requires particular care for reducing the concentration gradients in accordance with the level of the fermentor. The increased viscosity of the culture medium presumes higher energy expenditure for stirring, while the stability of the air bubbles worsens the separation of the deparaffinized fraction from the water medium and biomass. While discussing the problems connected with the raw material we mentioned that microbial deparaffinization could be optimized according to the cooling point of the deparaffinized petroleum fraction or in accordance with the productivity of the fermenter. In the first case we are forced to select such technological parameters of the process that would help in achieving full decomposition of the n-paraffins and partial cooxidation of certain alkylated aromatics and isoparaffins. From the viewpoint of the physiology of the microorganisms it is only the metabolizable hydrocarbons out of the entire petroleum fraction which play the role of a substrate. Their diminution down to a minimum value is associated with substrate limitation which in its part affects the productivity of the fermenter since a smaller substrate quantity is processed for a time unit in an unit of volume. In cases when we aim at maximum productivity of the fermenter (depending on the type and composition of the raw material) an incomplete metabolisation of the contained n-paraffins is expected, the prevailing part of n-paraffins with chain length over C_{19} remaining in the partly deparaffinized fraction. The lower n-paraffins also incompletely metabolize and in this way it is possible to secure reproduction of the microorganisms only by oxygen limitation, without any practical hydrocarbon limitation. Under these conditions, the relative growth rate is high and the productivity, therefrom.

The productivity of the fermenter is influenced not only by the dilution rate but also by the concentration of the biomass in the culture liquid. Besides this, both the rate of dilution and concentration of the biomass depend on the concentration of the n-paraffins in the nutrient medium. The specific features of microbial deparaffinization secure a maximum density of the population by means of which two new advantages are achived: Increased resistance of the culture and reduced losses in isolating the biomass from the culture medium.

The productivity of the fermenter in microbial deparaffinisation practically depends on the systems of aeration and on the specific features of the strain. These two moments make the process adequate for programmed management.

The existence of a system for extraction purification of the biomass removes the danger of arising of an incorrigible waste at the stage of reproduction. The qualities of the final product are not also influenced in medicobiological relation by possible deviations of the technological regime.

The stability of the emulsion water in oil is determined to a great degree from the biomass included in it. The first stage of biomass isolation is the separation of the foam from the culture medium, by which practically full extraction of the biomass from the culture medium is achieved, but the separation of the deparaffinized fraction and water from the biomass proves to be quite a complex problem. The method most frequently employed is the centrifugal one by use of chemical deemulsifiers. The use of this method involves certain difficulties caused by the change in the properties of the emulsion, depending on the admitted violations of the technological regime. Besides this, there exists a direct connection between the quantity of chemical deemulsifier and rate of separation. A number of possibilities for separation of the 3-component system without utilization of centrifugal machinery, has been studied. One of the possible ways for separating the system (3) is thermal dissociation of the emulsion by which a biomass, containing I0 - I2% hydrocarbons and I5 - 20% water, is obtained. This biomass is efficiently purified through combined or successive extraxtion with non-polar solvents (4). Besides, the application of centrifugal machinery and chemical deemulsifiers is avoided. The expenditure of energy for drying up is low and the quality of the raw biomass is not influenced either by violations of the technological regime, nor from the type of microorganisms. The various research teams are ,of course, having their preferences toward different methods of isolating the biomass. The basic requirements as regards the methods are: they must not be influenced by the conditions of obtaining the biomass and the type of strain; it would be also necessary that the isolated biomass possesses the properties facilitating its extraction purification.

The extract obtained from extraction of the biomass contains hydrocarbons and lipides. Depending on the specific economic conditions, the utilisation of this extract may essentially influence the extraction expenditures of the process. The whole problem is reduced to the question what is more important: the biomass as a complex or the protein contained in it? In case the price of the microbial fodder is determined from the content of digestible protein, including its aminoacid composition, then the fats separated after extraction appear to be a product without any working expenses up to the moment of extraction. Besides this, the protein quantity does not diminish as a result of the extraction.

The purified microbial fats may serve as an important reserve for the development of chemistry of the surface-active substances, as well as in other fields of chemical synthesis (5). It would be important to point out that, under certain economic conditions, the price of the fats may exceed the one of the biomass.

Since in the extraction process we obtain a mixture of lipides and hydrocarbons and only well purified lipides may have a high market value, the entire problem is reduced to the working expenses for their purification.

There exist methods (6) for extraction of 70% of the cellular lipides with hydrocarbon content under I%.

The lipide extract, containing a considerable amount of hydrocarbons, is separated in two fractions: a hydrocarbon fraction containing unutilised lipides and a purified lipide fraction. The result of lipide purification may be seen in table 2.

Table 2

Content of the fatty acids in the utilised pure lipides and in the unutilised lipides

Fraction	Fatty acids (% of the sum)									
	C_{10}-C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}
Hydrocarbons containing unutilized lipides	-	-	2,34	28,4	37,2	2,5	3,0	3,34	II,8	I4
Purified lipides	5,45	2,84	I3,22	23,8	39,75	4,5	0,97	I,07	2,I4	5,20

Where the balance of the raw materials is favourable, the selling price of the lipides may influence in a positive way the prime cost of the protein obtained.

As mentioned in the beginning, microbial deparaffinization as a process allows a greater variety in utilizing the raw materials and is more completely bound to the existing petroleum-chemical complex. In fact, this is the basic difference from the other microbiological methods of production of microbial fodder protein.

II. MICROBIAL PROTEIN FROM NORMAL PARAFFINS

The microbial oxidation of the purified n-paraffins seems to be, at first glance, a more classical microbiological process arousing less discussions. It is, however, linked more unilaterally with the existing petroleum-chemical complex.

The requirements to the raw material are more strict both with regard to the content of hydrocarbons with branched and cyclic chain, and with respect to the composition of the n-paraffins. On account of the different rate at which the separate n-paraffins metabolize, there exists a direct dependence between the fitness of the raw material and its temperature boiling interval. The latter depends, however, on the temperature boiling interval of the petroleum fraction out of which the purified n-paraffins are obtained. In this way, the question about the quality of the raw material is conditioned mainly on the specificities of the petroleum-chemical complex to which the production of n-paraffins and microbial fodder protein is bound up.

The fraction n-paraffins C_{11}-C_{13} finds an extensive application in the production of surface-active substances, as well as of other chemical transformations whereas the higher n-paraffins are having limited application.Hence,the combination of the raw material base for production of surface-active substances and microbial fodder protein allows to improve the economics of both productions and to utilize more completely the existing fractions.

The necessity of complete removal of the aromatic hydrocarbons deserves particular attention.The high grade definitive purification of the raw material is not unimportant for the price both of the n-paraffins and the protein.The selection of the paraffin-oxidizing microorganisms capable of metabolizing mono- and dicyclic aromatic hydrocarbons in the presence of n-paraffins (7,8)would lead to lowering the requirements to the raw material and to favourable economic results therefrom.

The production and utilization of microbial fodder protein on the basis of pure n-paraffins are acceptable from a medicobiological point of view inasmuch as in defined concentrations they do not exert a toxic effect upon the higher animals. On account of this the technology makes no provision for an additional extraction purification of the biomass.The reproduction is carried out at low concentrations of n-paraffins in the culture medium,a part of which are removed within the next processing.

Same as in each technological process, the reproduction of microorganisms could be influenced in certain periods by various violations of the technological parameters which may result in a considerable deviation of the concentration of the normal paraffins from the optimum value.This may lead to the production of an incorrigible waste.The experience from exploitation of the existing plants for production of fodder yeasts on a sugar basis reveals that it is not possible to forsee all reasons worsening the technological process.Therefore we should not yet neglect in an easy way the probability of worsening the quality of the production.The second specific feature of this process is the requirement concerning the reproduction which ought to be carried out under the conditions of a permanent hydrocarbon limitation.At first glance, there seems to appear insoluble contradictions:from the view point of sureness of the process and production quality it is nicessary to maintain a very low concentration of n-paraffins in the fermenter but this entails a retarded rate of reproduction and diminished effectiveness of the basic device.A precedent is caused giving rise to a constant conflict between the qualitative and economic parameters.

The other technological processes in the production of microbial biomass out of purified n-paraffins are elegant and certain enough not to arouse any anxiety.

Let's come again to the point:which method is the better one? There is no simple answer to that question.The reply depends on the specific features of the petroleum-chemical complex in each country,on the character of the needs,on the structure of the prices,etc.As regards its machinery lay-out microbial deparaffinization is a more complex process,but it is more plastic,more easily bound to the problems of petroleum chemistry and more promising with regard to the quality of the microbial biomass.The microbial oxidation of pure n-paraffins could be connected with the production of surface-active substances,thus increasing its economic advantages.

In conclusion, we should like to say a few words about the problem of the qualities to which microbial fodder protein should respond. The stock-breeders need a bioproduct which is balanced as much as possible with regard to aminoacid and vitamin content. Would it be possible to achieve this from a single microorganism of one and only hydrocarbon material?

In spite of the great achievements of the geneticists it would be quite unreal to expect that one strain could combine a high content of all essential aminoacids. More real seems to be the perspective by which several types of microbial protein will be produced on several substrates with different microorganisms, the defects of the separate bioproducts being corrected by means of an adequate mixing. This presumes, of course, presence of several production lines. The utilisation of various raw materials would alleviate both the general perspective planning of the industrial complexes and would also lead to a complex and economically balanced distribution of the reserve resources.

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