

PATENT SPECIFICATION

NO DRAWINGS

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Date of filing Complete Specification: Sept. 18, 1964.

Application Date: Oct. 3, 1963.

No. 38942/63

Complete Specification Published: Nov. 1, 1967.

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1089.093

1089.093



Index at acceptance:—C6 F(1, 2); C5 E12C

Int. Cl.:—C 12 d 13/00

COMPLETE SPECIFICATION

Improvements in or relating to the production of Derivatives from Micro-Organisms

We, THE BRITISH PETROLEUM COMPANY LIMITED, of Britannic House, Finsbury Circus, London, E.C.2, a company incorporated in accordance with the Laws of England, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the production of nitrogenous material from micro-organisms. This invention also relates to a process for the removal of straight chain hydrocarbons, wholly or in part, from mixtures of said hydrocarbons with other hydrocarbons.

According to one aspect of this invention there is provided a process which comprises cultivating a hydrocarbon-consuming micro-organism in the presence of a feedstock comprising or consisting of a hydrocarbon on which the micro-organism is capable of growth, separating from the product a cream or paste comprising the micro-organism, water and optionally small amounts of residual feedstock, thereafter subjecting the cream or paste, either directly or after reducing the proportion of residual feedstock if present, to treatment to rupture at least some of the cell walls and thereby liberate nitrogenous material, thereafter reducing the proportion of water in the product from the rupture of the cell walls or a fraction derived therefrom and comprising nitrogenous material, and thereafter subjecting the fraction containing a reduced proportion of water to solvent extraction wherein an extract containing at least part of the lipids of the micro-organism together with hydrocarbon contaminants is separated from a raffinate fraction containing nitrogenous material.

Preferably the micro-organism is cultivated in the presence of a feedstock consisting

wholly or in part of straight chain hydrocarbons. Preferably the hydrocarbons have a mean molecular weight corresponding to at least 10 carbon atoms per molecule. When the feedstock consists only in part of straight chain hydrocarbons there will usually be present, in the product of the growth stage, the hydrocarbons which, under the conditions of growth are not metabolisable. These hydrocarbons will be recovered from the product of the growth stage, although as described hereinbefore there will at this stage be some contamination of the separated micro-organism by hydrocarbons.

For the growth of the micro-organism it will be necessary to provide, in addition to the feedstock, an aqueous nutrient medium and a supply of oxygen, preferably in the form of air.

The yeasts in this specification are classified according to the classification system outlined in "The Yeasts, a Taxonomic Study" by J. Lodder and N. J. W. Kreger-Van Rij, published by North Holland Publishing Co. (Amsterdam) (1952).

Micro-organisms which are cultivated as herein described may be yeasts, moulds or bacteria.

Preferably when a yeast is employed this is of the family Cryptococcaceae and particularly of the sub-family Cryptococcoideae; however, if desired there may be used, for example, ascosporeogeneous yeasts of the sub-family Saccharomycoideae. Preferred genera of the Cryptococcoideae sub-family are *Torulopsis* (also known as *Torula*) and *Candida*. Preferred strains of yeast are as follows. In particular it is preferred to use the specific stock of indicated Baarn reference number; these reference numbers refer to CBS stock held by the Centraal Bureau voor Schimmelcultuur, Baarn, Holland and to INRA stock held by the Institut National de la Recherche Agronomique, Paris, France,

		Preferred strain
Candida	lipolytica	
Candida	pulcherrima	CBS 610
Candida	utilis	
Candida	utilis, Variati major	CBS 841
Candida	tropicalis	CBS 2317
Torulopsis	colliculosa	CBS 133
Hansenula	anomala	CBS 110
Oidium	lactis	
Neurospora	sitophila	
Mycoderma	cancoillote	INRA; STV 11

Of the above *Candida lipolytica* is particularly preferred.

5 If desired the micro-organism may be a mould, for example *Penicillium expansum*, or a bacterium.

10 The bacteria mentioned in this specification are classified according to the classification system outlined in "Bergey's Manual of Determinative Bacteriology" by R. S. Breed, E. G. D. Murray and N. R. Smith, published by Bailliere, Tindall and Cox (London) 7th Edition (1957).

15 Suitably the bacteria are of one of the orders:—

Pseudomonadales, *Eubacteriales* and *Actinomycetales*.

Preferably the bacteria which are employed are of the family *Bacillaceae* and *Pseudomonadaceae*. Preferred species are *Bacillus megatherium*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Other strains which may be employed include:—

Bacillus amylobacter
Pseudomonas natriegens
Arthrobacter sp.
Micrococcus sp.
Corynebacterium sp.
Pseudomonas syringae
Xanthomonas begoniae
Flavobacterium devorans
Acetobacter sp.
Actinomyces sp.
Nocardia opaca

These bacteria grow in the presence of the following aqueous nutrient medium:—

NH_4Cl	0.5 grams
NaCl	4 grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 grams
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	0.5 grams
KH_2PO_4	0.5 grams
water to make up to:	1000 cc.

Preferably the pH of this medium is maintained at 7. Another aqueous nutrient medium is:

K_2HPO_4	1 gram
KH_2PO_4	0.5 grams
$MgSO_4 \cdot 7H_2O$	0.5 grams
$CaCl_2$	0.1 grams
NaCl	0.1 grams
water to make up to:	1000 cc.

A suitable nutrient medium for yeasts (and moulds) has the composition:

$(NH_4)_2HPO_4$	2 grams
KCl	1 gram
$MgSO_4 \cdot 7H_2O$	0.65 gram
$ZnSO_4$	0.17 gram
$MnSO_4 \cdot 10H_2O$	0.045 gram
$FeSO_4 \cdot 7H_2O$	0.068 gram
Yeast extract	0.030 gram
Tap water	1000 mls.

Micro-organisms, and in particular yeasts, when first cultivated with the use of hydrocarbon fractions as feedstock sometimes grow with difficulty and it is sometimes necessary to use an inoculum of a micro-organism which has previously been adapted for growth on the hydrocarbon fraction which it is intended to use. Furthermore the micro-organism although cultivated in the presence of an aqueous mineral medium containing the appropriate nutrient elements may grow with difficulty, because the hydrocarbon fraction does not contain the growth factors which exist in carbohydrate feedstocks, unless these growth factors are added.

When batch operation is employed, the micro-organism will usually grow initially at a low rate of increase in cellular density. (This period of growth is referred to as the 'lag phase'). Subsequently the rate of growth will increase to a higher rate of growth; the period at the higher rate of growth is referred to as the 'exponential phase' and subsequently again the cellular density will become constant (the 'stationary phase').

A supply of the micro-organism for starting the next batch will preferably be removed

before the termination of the exponential phase.

The growth operation will usually be discontinued before the stationary phase.

At this stage, the micro-organism will usually be separated from the bulk of the aqueous nutrient medium and from the bulk of the unused feedstock fraction.

The growth of the micro-organism used is favoured by the addition to the culture medium of a very small proportion of extract of yeast (an industrial product rich in essential nutrilites or growth factors obtained by the hydrolysis of a yeast) or more generally of the essential nutrilites. The essential nutrilites include biotin; pantothenic acid, nicotinic acid, thiamine, inositol and pyridoxine. The quantity of yeast extract added is preferably of the order of 25 parts per million. The quantity of each nutrilitite required varies between about 0.1 parts per million for biotin and about 10 parts per million for inositol.

Preferably the aqueous nutrient medium is maintained at a desired pH by the step-wise or continuous addition of an aqueous medium of high pH value. Usually, when using

moulds or yeasts and in particular when using *Candida lipolytica*, the pH of the nutrient medium will be maintained in the range 3—6 and preferably in the range 4—5. (Bacteria require a higher pH usually 6.5—3). Suitable alkaline materials for addition to the growth mixture include sodium hydroxide, potassium hydroxide, disodium hydrogen phosphate and ammonia, either free or in aqueous solution.

The optimum temperature of the growth mixture will vary according to the type of micro-organism employed and will usually lie in the range 25—35°C. When using *Candida lipolytica* the preferred temperature range is 28—32°C.

The take-up of oxygen is essential for the growth of the micro-organism. The oxygen will usually be provided as air. In order to maintain a rapid rate of growth the air, used to provide oxygen, should be present in the form of fine bubbles under the action of stirring. The air may be introduced through a sintered surface. However there may be used the system of intimate aeration known as "vortex aeration".

It has been found that by the use of yeast of the strain *Candida lipolytica* in a process according to the invention in which aeration is effected by "vortex aeration", a high growth rate is achieved whereby the generation time lies in the range 2—5 hours and the cell concentration is increased by a factor of up to 12 in two days.

The micro-organism is preferably separated from the bulk of the liquid phase when possible by centrifuging and is recovered as a cream or paste. However, in some cases separation will be accomplished by filtration or to some extent by decantation.

The micro-organism fraction, in the form of a paste or cream, and containing hydrocarbons as impurities may be subjected to a treatment with an aqueous solution of a surface active agent. Preferably the micro-organism fraction is vigorously mixed with the aqueous surface active agent and, without a further period of growth of the micro-organism, subjected to further separation, preferably by centrifuging, to recover a micro-organism fraction and a spent aqueous phase, containing hydrocarbon impurities removed from the micro-organism. If necessary, the treatment and separation steps may be repeated, once or more, using an aqueous surface active agent in the treatment stage. After the treatment with surface active agent it is necessary to wash with an aqueous medium which is free of surface active agent; preferably this medium will be water. Again if desired, a series of treatment and separation stages may be employed. Overall, the treatment stages are carried out until the hydrocarbon content of the micro-organism is less than 7% based on the weight of micro-

organism (as calculated for the dry state). Preferably said content of hydrocarbons will be less than 5%; usually the hydrocarbons will be substantially eliminated; however, for some applications this would not be necessary and in some cases therefore it would not be necessary to reduce said content to a value below 0.1%.

As the surface active agent employed for treatment there may be used cationic surface active agents such as stearyl-trimethyl ammonium chloride, non-ionic surface active agents, for example the condensates of oleic acid and ethylene oxide, or anionic surface active agents, for example sodium alkyl sulphates.

The micro-organism with or without treatment as hereinbefore described, is subjected to treatment for the separation of proteins, polypeptides or amino-acids contained in the micro-organism from cell wall material.

The initial stage of the separation of the protein, polypeptides or amino-acids may be carried out by autolysis, plasmolysis or hydrolysis.

Autolysis may be effected by means of enzymes contained in the micro-organism or by the use of added enzymes. Usually pH will be maintained at a value determined by the nature of the enzymes used.

The autolysate is usually obtained in the form of a liquid the viscosity of which depends upon the concentration of dry substances which it contains. As it is obtained without violent heating the amino-acids which are not very stable and the vitamins are preserved, usually completely.

Preferably the autolysis is carried out for at least 30 minutes.

Preferably when a yeast autolysate is prepared for cattle feeding stuff the yeast is autolysed for up to three hours.

Preferably when a yeast autolysate is prepared for human consumption, the yeast is autolysed for up to 20 hours.

According to another aspect of this invention there are manufactured, instead of autolysates, hydrolysates obtained by the action of acids or alkalis on the cells of micro-organisms or plasmolysates by the diffusion of the proteins out of the cells as a result of the action of a change in osmotic pressure. This change is achieved, for example, by the addition of sodium chloride to the aqueous medium. The operations of autolysis, hydrolysis and plasmolysis may be carried out in the same type of apparatus. Preferably this apparatus is a stirred vat.

It has been found that by reducing the hydrocarbon content of the micro-organism by treatment as hereinbefore described, the rate of operation of the treatment for the rupture of the cell walls, for example, by autolysis, is increased; in consequence the residence time of the micro-organism in this

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stage can be much reduced and the economies in plant for this stage will out-weigh the costs of the treatment stages. The reason for this increase in the rate of operation is that the enzymes responsible for the rupture of the cell walls operate more efficiently when the proportion of hydrocarbons present is low.

The use of a step involving rupturing the cell walls in the process of the present invention has the advantage that the product is more easily freed from hydrocarbons and lipids than is the intact micro-organism.

The severity of the treatment for the rupture of cell walls will preferably be controlled to avoid conditions which are more severe than is required to rupture the walls of a substantial proportion of the cells of the micro-organism. An unnecessarily severe treatment will lead to a highly degraded product which is of reduced protein content, high flavour and which is deliquescent.

The proportion of water in the product of this stage (that is, from the rupture of the cell walls) is now reduced, either with or without prior removal of all or part of the cell wall material.

The reduction of the proportion of water may be carried out by drum driers. Preferably steam is admitted sufficiently slowly to that the temperature of the exterior of the drum is maintained at a low value for example 60–100°C and the quantity of water removed is limited to the amount required to permit miscibility of the solvent with the partially dried material. It is desirable to maintain the temperature of the exterior of the drum at a low value to avoid degradation of amino-acids and polypeptides.

Another method of reducing the proportion of water which may be employed is azeotropic distillation in the presence of a suitable solvent, such as ethanol or isopropanol. Another method which may be employed in washing with a suitable solvent such as ethanol or isopropanol.

Preferably the product from this stage contains 5 to 25% by wt. of water.

This product, with or without an intervening refining treatment is extracted with a solvent. This may be achieved by continuous solvent extraction or by successive washings with solvent followed by phase separation. Suitably the extraction is carried out in a stationary vessel equipped with paddle stirrers, preferably rotating at less than 10 revs per minute or in a vessel which rotates on a horizontal axis. When operating a continuous solvent extraction, the extract is withdrawn continuously and distilled, continuously or batchwise, at atmospheric or reduced pressure, and solvent is continuously fed back to the extractor. Under these con-

ditions the yeast may be introduced and withdrawn continuously or batchwise.

Preferably the solvent extraction is effected while feeding solvent to the extractor at a periodically varying rate to create pulsations in the flow of said liquid stream.

The pulses of the liquid passing through the solid material bring about oscillations and limited displacements of each grain of solid material in relation to its neighbours, and this is equivalent to a mechanical agitation of the whole. For this reason the whole of the products to be extracted is much more rapid and complete.

Suitably there is arranged in the feed of the liquid stream a device which imparts to it pulses whose amplitude and frequency are regulated experimentally at the most favourable value for each particular case. These pulses are produced by any suitable processes already known, and preferably an alternating pump is used whose valves have been removed.

Preferably the number of pulses lies between 1 and 60 per minute. The operation of the process under the action of pulses is further described in British Patent Specification No. 1,059,890.

Suitable solvents for use in the process include ethyl alcohol, iso-propanol, light hydrocarbons, including benzene and light platformate fractions, ethyl ether, acetone, chlorinated solvents and liquefied petroleum gases, such as butane and propane.

If desired a first extraction stage can be operated using a polar solvent, for example an alcoholic solvent, for example ethanol or isopropanol and then the partially purified raffinate can be further treated in a second extraction stage using a hydrocarbon solvent, for example normal hexane or a light platformate fraction or benzene.

Preferably in the second stage there is used as solvent, a mixture of hydrocarbon in major amount with a polar solvent in minor amount. Preferably there is used the azeotropic mixture of hexane with isopropanol or ethanol. If desired both extraction stages can be operated in continuous manner.

When using a solvent consisting of a mixture of hydrocarbon and a polar solvent, it is believed that the function or one function of the polar solvent is to weaken the bonding of the material to be extracted (even the bonding of hydrocarbons which are not themselves soluble in the polar solvent).

By the use of any alcohol in the first stage of a two stage extraction as hereinbefore described, the water content of the material treated is considerably reduced. As a result the material which is fed to the second stage has a sufficiently low content of water to ensure that the non-aqueous contaminants

which are still present are miscible with the solvent used in the second extraction stage. Each extraction stage may consist of either one or more substages consisting of washing with the solvent used in the stage followed by separation.

If a single washing is employed in the first stage the amount of ethanol or isopropanol which is used should be 1.5—3 times the volume of water which is present in the material subjected to treatment. However if desired two washings with ethanol or isopropanol may be employed using in the first washing a volume of solvent equal to the volume of water in the cream or paste and in the second washing a smaller amount of ethanol or isopropanol for example, one half of the amount used in the first washing.

Between washings of each stage or substage the raffinate is allowed to drain, for example by filtering and part of the residual solvent is then preferably removed by vacuum filtration.

In the second stage the amount of solvent used in the (or each) washing will usually be 2—20 times the volume of the resulting dry material.

Preferably the final stage employed for the removal of solvent is evaporation, suitably under reduced pressure and suitably in a stream of inert gas, for example, nitrogen or super-heated steam.

By the use in the second extraction stage of a solvent which is a mixture of hydrocarbon and a polar solvent the composition of the second stage solvent, which will in any case acquire polar solvent from the first stage extraction, can be stabilised. Build-up of polar solvent can be avoided in the course of a distillation stage, in which the second stage solvent is recovered by the removal of separate streams consisting of (a) polar solvent for recycle to the first extraction stage and (b) a mixture of hydrocarbon and polar solvent for recycle to the second stage. Suitably in a distillation stage the extract obtained by the second extraction stage is distilled to recover (a) overhead a mixture of hydrocarbon, polar solvent and water for recycle to the second extraction stage and (b) a bottoms fraction containing polar solvent, water and the extracted materials; this fraction is preferably blended with the extract obtained in the first extraction stage before this is fed to distillation whereby all contaminants recovered by solvent extraction are removed as a bottoms fraction in this distillation stage. Suitably the polar solvent is ethanol or isopropanol. Suitably the second stage solvent is an azeotropic mixture.

Optimum contact time will usually vary inversely with the temperature of extraction. It will usually be undesirable to use a temperature above 70°C since higher tempera-

tures will lead to some degradation of the product.

If the proportion of water in the micro-organism derivative has been sufficiently reduced before solvent extraction it will then usually be possible to operate the first extraction stage with only a single washing with an alcoholic solvent (and with the use of a smaller amount of solvent than would be required if no drying had taken place). If the proportion of water in the micro-organism derivative has been reduced nearly to zero, a first extraction stage using a polar solvent, for example an alcoholic solvent, is not necessary; in this case the single stage extraction process can be operated by the use of a solvent which is entirely hydrocarbon or which is a mixture of hydrocarbon and polar solvent, for example an alcohol, ketone or chlorinated hydrocarbon.

In general it is desirable to avoid drastic methods for reducing the proportion of water since this will lead to partial decomposition of the micro-organism, for example by destruction of vitamins and oxidation of unsaturated compounds; furthermore the products of decomposition will be soluble in the solvent used in extractive distillation thus being lost from the product or requiring further stages for their recovery.

A yeast which has been freed from the whole or part of its lipids and the contaminating hydrocarbons by one of the methods described hereinbefore and whose taste has been improved is a new industrial product of value for human nutrition.

The lipid extract which has been recovered by the evaporation of the solvent is also a new industrial product which can be used either as such or as a raw material for the separation of its sterols, fatty acids (either before saponification or after) or of its other constituents.

It is well-known that certain petroleum fractions, particularly gas oils, contain straight chain hydrocarbons, mainly paraffins which are waxes and which have an adverse effect upon the pour point of the fraction; that is to say, when these hydrocarbons are removed wholly or in part, the pour point of the fraction is lowered. Usually the wax is removed by precipitation by means of solvents, the wax originally present in the fraction being recovered as such, that is, without conversion to more valuable products.

The petroleum fractions boiling below the gas oils, for example, heavy naphthas and kerosines also contain straight chain hydrocarbons which are potentially valuable for conversion to other products but hitherto, in general, utilisation of these hydrocarbons has been rendered difficult by the necessity of recovering these hydrocarbons from the petroleum fractions, in which they are con-

tained, before they can be converted to other products.

According to a preferred feature of this invention there is provided a process which comprises cultivating and treating a micro-organism in the manner as hereinbefore described in the presence of a petroleum fraction consisting in part of straight chain hydrocarbons and having a mean molecular weight corresponding to at least 10 carbon atoms per molecule, and in the presence of an aqueous nutrient medium; and in the presence of a gas containing free oxygen and separating from the mixture, on the one hand, the micro-organism and, on the other hand, a petroleum fraction having a reduced proportion of straight chain hydrocarbons or which is free of said straight chain hydrocarbons.

The process of the invention is of particular value for the treatment of petroleum gas oil fractions which contain straight hydrocarbons in the form of waxes, since by the process of the invention a gas oil of improved pour point is obtained while the waxes are converted to a valuable product.

Usually the straight-chain hydrocarbons will be present in the feedstocks according to the invention as paraffins; however, the straight-chain hydrocarbons may be present as olefins; also there may be used a mixture containing straight chain paraffins and olefins.

It is an important feature of this invention that when cultivating yeasts in the presence of the feedstocks hereinbefore described under conditions favouring the growth of the yeasts at the expense of the straight-chain hydrocarbons, the other hydrocarbons, for example isoparaffins, naphthenes and aromatics are not metabolised or, at most, the proportion which is metabolised is very small. Furthermore, unlike conventional chemical processes governed by the law of mass action, the rate of removal of straight chain hydrocarbons is

not substantially reduced as the proportion of these hydrocarbons in the overall mixture of hydrocarbons decreases (except, of course, in the very final stages of removal). Thus, when desired, the percentage conversion of straight chain hydrocarbons which is achieved can be maintained at a value approaching 100% without necessitating a very disproportionate expenditure of contact time to achieve small improvements. Furthermore, in a continuous process, this high percentage conversion can be achieved without resorting to the use of a long reaction path.

By the application of this process under conditions which limit the metabolisation of the straight-chain hydrocarbons it is possible to operate with the removal of only a desired proportion of these hydrocarbons.

Suitable feedstocks to the process of the invention include kerosine, gas oils and lubricating oils; these feedstocks may be unrefined or may have undergone some refinery treatment, but must contain a proportion of straight-chain hydrocarbons in order to fulfil the purpose of this invention. Suitably the petroleum fraction will contain 3—45% by weight of straight-chain hydrocarbons.

Preferred methods for use in the cultivation of the micro-organism and for the recovery of the product are described in British Patent Specification Nos. 914,567, 914,568, 1,017,584, 1,017,585, 1,021,697, 1,021,968, 1,049,065, 1,049,066, 1,049,067 and in 1,059,881 to 1,059,890 inclusive.

EXAMPLE

Candida lipolytica was cultivated in a continuous fermenter (volume 120 litres) on an Iraq gas-oil. The gas oil had an initial boiling point of 280°C, a final boiling point of 380°C and contained 13% by weight of n-paraffins. The following aqueous nutrient medium was employed:—

(NH ₄) ₂ HPO ₄	2 grams/litre
KCl	1 gram/litre
Mg SO ₄ 7H ₂ O	0.65 gram/litre
ZnSO ₄	0.17 gram/litre
MnSO ₄ 10H ₂ O	0.045 gram/litre
FeSO ₄ 7H ₂ O	0.068 gram/litre
Yeast extract	0.030 gram/litre

Dissolved in tap water



The medium, containing 120 grams/litre of gas-oil, was fed to the fermenter at the rate of 1.2 litres/hour. The pH was maintained at 4 and the temperature at 30°C. The mixture in the fermenter was initially inoculated with 0.5 grams of *Candida lipolytica* culture in 50 mls of aqueous nutrient medium. The mean residence time was 5 hrs. at a dilution level of 0.2. The cellular density rose to 8 grams/litre.

The following purification stages were then carried out on the yeast so obtained.

1. Treatment of the yeast paste with a surface-active agent

The product from the fermentation (con-

taining 300 grams/litre of gas-oil and 25 grams/litre of dry yeast) was subjected to a decantation in which an upper phase consisting of residual hydrocarbon, yeast and a little water was separated from an aqueous phase containing the unconsumed mineral salts. The proportion of residual gas-oil in the yeast cream was reduced by agitating the cream with an aqueous solution of a non-ionic surface-active agent consisting of the nonyl-phenyl ether of a polyoxy-ethylene polymer containing, on average, 8.75 ethylene oxide units, and then centrifuging. The effect of various concentrations of surface-active agent on the level of gas-oil in the yeast cream is shown below.

Concentration of surface-active agent present	0	0.1 gram/litre	1.0 gram/litre
% Residual gas-oil (based on dried yeast)	39.3	18.1	3.0
Paste Number	—	I	II

The yeast pastes so obtained were washed once with water (enough water was added to reduce the yeast concentration to 1% by weight based on the dry material) and the mixture centrifuged again. The pastes so obtained contained about 85% by weight of water.

2. Autolysing the Yeast Cream

The yeast pastes I and II obtained in Stage 1 were each subjected to autolysis at 55°C for 3 hours with the pH held at 7.5. The autolysis stage was more efficient, as far as the amount of protein solubilised was concerned, if the amount of gas-oil adsorbed on the yeast had been reduced to less than 5—8% by weight (based on the dry material) before autolysis, as is shown in these figures:

	I	II
% gas-oil	18.1	3
Nitrogen content of autolysate (% weight)	11.5	12
Solubilised material (% weight of dry material)	7.5	15

3. Drying the Pastes

The autolysate obtained in Stage 2 contains 75—90% water; the amount of water was reduced to less than 15% weight before extraction with a water-immiscible solvent. Two methods of drying were used.

(1) Rotary dryer under the following conditions:

Drum rotation rate 5 r.p.m.

Temperature of the steam = 80—100°C

(2) Washing with a hydrophilic solvent such as isopropanol.

4. Solvent Extraction of the Paste Containing a Reduced Amount of Water

The pastes in which the proportion of water had been reduced by Method I were washed a number of times with an azeotropic mixture of hexane (80 parts) and isopropanol (20 parts) (3 litres of mixed solvent per kg. of dry material). The temperature was maintained at 55°C and the mixture was stirred for about 20 mins during each washing.

The residual lipid and hydrocarbon contents of the nitrogenous material so obtained

amount

were compared with their levels in materials prepared by the same method except that, in one case, the yeast was not autolysed and,

in the other, the yeast was neither autolysed nor treated with surface active agent. The following results were obtained. 5

		Not treated with Surface-active Agent or Autolysed	Treated with Surface-active Agent but not Autolysed	Treated with Surface-active Agent and Autolysed
Nitrogenous Material before extraction	% water (by weight)	20	17	18
	% hydrocarbons (by weight)	20	1.4	3
	% lipids (by weight)	40	12	10
Extracted with solvent	No. of washings	7	7	5
	% of hydrocarbons (by weight)	1.0	0.3	0.3
	% of lipids (by weight)	6.4	5.3	4

10 It can be seen that the case where the yeast was both treated with surface-active agent and autolysed gave a nitrogenous product which contained after two fewer washings, the lowest levels of hydrocarbon and lipids.

If a nitrogenous product containing an even lower proportion of lipids is required then

the proportion of water in the product of the autolysis stage is reduced by washing with the hydrophilic solvent (see Stage 3 Method 2) rather than by Method 1 prior to solvent extraction. 15

The results of this are shown below: 20

Material before extraction	% water by weight	70
	% hydrocarbon by wt.	3.7
	% lipids by wt.	12
After extraction with isopropanol and 7 washings with hexane/alcohol	% of hydrocarbon by wt.	0.7
	% of lipids by wt.	0.7

WHAT WE CLAIM IS:—

1. A process which comprises cultivating a hydrocarbon-consuming micro-organism in the presence of a feedstock comprising or consisting of a hydrocarbon on which the micro-organism is capable of growth, separating from the product a cream or paste comprising the micro-organism, water and optionally small amounts of residual feedstock, thereafter subjecting the cream or paste, either directly or after reducing the proportion of residual feedstock if present, to treatment to rupture at least some of the cell walls and thereby liberate nitrogenous material, thereafter reducing the proportion of water in the product from the rupture of the cell walls or a fraction derived therefrom and comprising nitrogenous material, and thereafter subjecting the fraction containing a re-

duced proportion of water to solvent extraction wherein an extract containing at least part of the lipids of the micro-organism together with hydrocarbon contaminants is separated from a raffinate fraction containing nitrogenous material. 45

2. A process according to claim 1 in which the micro-organism is a yeast.

3. A process according to claim 2 in which the yeast is of the family Cryptococcaceae. 50

4. A process according to claim 3 in which the yeast is of the sub-family Cryptococcaceae.

5. A process according to claim 4 in which the yeast is of the genus Torulopsis. 55

6. A process according to claim 4 in which the yeast is of the genus Candida.

7. A process according to claim 6 in which the yeast is Candida lipolytica.



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were compared with their levels in materials prepared by the same method except that, in one case, the yeast was not autolysed and,

in the other, the yeast was neither autolysed nor treated with surface active agent. The following results were obtained.

		Not treated with Surface-active Agent or Autolysed	Treated with Surface-active Agent but not Autolysed	Treated with Surface-active Agent and Autolysed
Nitrogenous Material before extraction	% water (by weight)	20	17	18
	% hydrocarbons (by weight)	20	1.4	3
	% lipids (by weight)	40	12	10
Extracted with solvent	No. of washings	7	7	5
	% of hydrocarbons (by weight)	1.0	0.3	0.3
	% of lipids (by weight)	6.4	5.3	4

It can be seen that the case where the yeast was both treated with surface-active agent and autolysed gave a nitrogenous product which contained after two fewer washings, the lowest levels of hydrocarbon and lipids.

If a nitrogenous product containing an even lower proportion of lipids is required then

the proportion of water in the product of the autolysis stage is reduced by washing with the hydrophilic solvent (see Stage 3 Method 2) rather than by Method 1 prior to solvent extraction.

The results of this are shown below:

Material before extraction	% water by weight	70
	% hydrocarbon by wt.	3.7
	% lipids by wt.	12
After extraction with isopropanol and 7 washings with hexane/alcohol	% of hydrocarbon by wt.	0.7
	% of lipids by wt.	0.7

WHAT WE CLAIM IS:—

1. A process which comprises cultivating a hydrocarbon-consuming micro-organism in the presence of a feedstock comprising or consisting of a hydrocarbon on which the micro-organism is capable of growth, separating from the product a cream or paste comprising the micro-organism, water and optionally small amounts of residual feedstock, thereafter subjecting the cream or paste, either directly or after reducing the proportion of residual feedstock if present, to treatment to rupture at least some of the cell walls and thereby liberate nitrogenous material, thereafter reducing the proportion of water in the product from the rupture of the cell walls or a fraction derived therefrom and comprising nitrogenous material, and thereafter subjecting the fraction containing a re-

duced proportion of water to solvent extraction wherein an extract containing at least part of the lipids of the micro-organism together with hydrocarbon contaminants is separated from a raffinate fraction containing nitrogenous material.

2. A process according to claim 1 in which the micro-organism is a yeast.

3. A process according to claim 2 in which the yeast is of the family Cryptococcaceae.

4. A process according to claim 3 in which the yeast is of the sub-family Cryptococcoideae.

5. A process according to claim 4 in which the yeast is of the genus *Torulopsis*.

6. A process according to claim 4 in which the yeast is of the genus *Candida*.

7. A process according to claim 6 in which the yeast is *Candida lipolytica*.

8. A process according to any of the preceding claims in which the yeast is grown in the presence of a nutrient medium containing extracts of yeast.
- 5 9. A process according to claim 1 in which the micro-organism is a bacterium.
- 10 10. A process according to any one of the preceding claims in which the feedstock is of petroleum origin.
- 15 11. A process according to any one of the preceding claims in which the micro-organism is cultivated in the presence of a feedstock consisting wholly or in part of straight chain hydrocarbons.
- 20 12. A process according to any of the previous claims in which the method of rupturing the cell walls is by autolysis, plasmolysis or hydrolysis.
- 25 13. A process according to claim 12 in which the autolysis is performed under mild conditions so that only some of the cell walls are ruptured.
- 30 14. A process according to any one of the preceding claims in which the method of reducing the proportion of water is by means of a drum dryer, by means of azeotropic distillation in the presence of a suitable solvent or by means of washing with a suitable solvent.
- 35 15. A process according to claim 14 in which the solvent is ethyl alcohol or isopropanol.
- 40 16. A process according to claim 10 in which the fraction containing a reduced proportion of water is extracted with a solvent comprising a hydrocarbon.
17. A process according to claim 16 in which the solvent comprising a hydrocarbon is a mixture of a hydrocarbon and a polar solvent.
19. A process according to claim 17 in which the hydrocarbon is n-hexane, benzene or light platformate fraction.
20. A process according to claim 17 in which the solvent comprising a hydrocarbon is an azeotropic mixture of a hydrocarbon and a polar solvent.
21. A process according to claim 16 in which the extraction with a solvent comprising a hydrocarbon is preceded by an extraction with a polar solvent.
22. A process according to any of the preceding claims in which the micro-organism is cultivated in the presence of a petroleum fraction consisting in part of straight chain hydrocarbons and having a mean molecular weight corresponding to at least 10 carbon atoms per molecule, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen and separating from the mixture, on the one hand, the micro-organism and, on the other hand, a petroleum fraction having a reduced proportion of straight chain hydrocarbons or which is free of said straight chain hydrocarbons.
23. A process according to claim 22 in which the petroleum feedstock is a wax-containing gas-oil and there is separated from the product of the cultivation a gas-oil having a reduced proportion of wax or which is free from said wax.
24. A process substantially as hereinbefore described with reference to the foregoing Example.
25. A micro-organism or a fraction derived from a micro-organism whenever produced by a process as claimed in any of the preceding claims.
26. An extract whenever produced by a process as claimed in any of claims 1—23.

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Printed for Her Majesty's Stationery Office, by Unifax (Reprographic) Limited.

—1967. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2,
from which copies may be obtained.

