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PATENT SPECIFICATION

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The inventors of this invention in the sense of being the devisers thereof within the meaning of Section 16 of the Patents Act, 1949 are:—ALFRED CHAMPAGNAT & BERNARD MAURICE LAINE, both French Citizens of 21, rue de la Bienfaisance, Paris VIII, France.

COMPLETE SPECIFICATION

Improvements in the production of Micro-Organisms from Hydrocarbons

We, THE BRITISH PETROLEUM COMPANY LIMITED, of Britannic House, Finsbury Circus, London, E.C.2, a British joint-stock Corporation 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 micro-organisms, for example, yeasts by cultivation on hydrocarbons. 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.

The cultivation of yeasts on hydrocarbons, and in particular on petroleum hydrocarbons, as the carbonaceous substratum, generally gives yeasts which after washing and drying possess a peculiar taste which is sharp and rancid and which may interfere with their use in foods for human consumption. The traditional yeasts, cultivated on molasses, residuary liquors or bisulphite liquors, also have a peculiar taste, different from that of yeasts cultivated on hydrocarbons, and certain processes have been proposed for attenuating this taste — notably to attenuate their bitterness, but none of these has succeeded completely.

It has now been found that yeasts cultivated with hydrocarbons as the sole source of carbon have a distinctly higher lipid content than yeasts cultivated on the traditional substrata: molasses, residuary liquors, bisulphite liquors. Lipids consist essentially of fatty acids, esters (with greater or lesser degrees of oxidation) including fats and sterols. It has now been found that extraction of these lipids, wholly or in part, leads to a substantial reduction in this characteristic taste of yeasts cultivated

on hydrocarbons or to the elimination of this taste.

It is an object of this invention to provide an improved process for the production of micro-organisms. It is a further object to provide a process for the production of a yeast. It is a further object to provide a process for the removal of straight chain hydrocarbons, wholly or in part, from mixtures of said hydrocarbons with other hydrocarbons. Other objects will appear hereinafter.

According to one aspect of this invention there is provided a process which comprises cultivating a micro-organism capable of growing on a feedstock consisting wholly or in part of straight chain hydrocarbons with formation of lipids, said micro-organisms being cultivated in the presence of said feedstock, separating from the product a fraction comprising the micro-organism and extracting said fraction by means of a solvent to remove lipids in the extract phase.

Suitably, in accordance with the invention straight chain hydrocarbons are removed from a petroleum fraction with production of a micro-organism, wherein the feedstock employed for the growth of the micro-organism is a petroleum fraction consisting in part of straight chain hydrocarbons wherein there is recovered from the product of the growth of the micro-organism 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 particularly suitable for the removal, wholly or in part, of waxes from a wax-containing petroleum gas oil, wherein the feedstock is a wax-containing petroleum gas oil and wherein there is recovered, from the product of the growth of the micro-organism, a

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gas oil of reduced content of wax.

- 5 Solvents which may be employed in the solvent extraction stage in accordance with the invention include ethyl alcohol, isopropanol, light hydrocarbons, including benzene and light platformate fractions, ethyl ether, acetone, chlorinated solvents and liquefied petroleum gases, such as butane and propane.

- 10 Within the term "micro-organism" used herein we include mixtures of micro-organisms.

- 15 Micro-organisms which are cultivated as herein described may be yeasts, moulds or bacteria capable of growing on a straight chain hydrocarbon.

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 family are *Torulopsis* (also known as *Torula*), *Candida* and *Mycoderma*. Preferred strains of yeast are as follows. In particular it is preferred to use the specific stock of indicated reference number; these reference numbers refer to CBS stock held by the Centraal Bureau voor Schimmelfcultuur, Baarn, Holland and to INRA stock held by the Institut National de la Recherche Agronomique, Paris, France.

<i>Candida</i>	<i>lipolytica</i>	CBS 599
<i>Candida</i>	<i>pulcherrima</i>	CBS 610
<i>Candida</i>	<i>utilis</i>	CBS 890
<i>Candida</i>	<i>utilis</i> , Variati major	CBS 841
<i>Candida</i>	<i>tropicalis</i>	CBS 2317
<i>Candida</i>	<i>arborea</i>	
<i>Torulopsis</i>	<i>colliculosa</i>	CBS 133
<i>Hansenula</i>	<i>anomala</i>	CBS 110
<i>Oidium</i>	<i>lactis</i>	
<i>Neurospora</i>	<i>sitophila</i>	
<i>Mycoderma</i>	<i>cancoillote</i>	INRA; STV 11

Of the above *Candida lipolytica* is particularly preferred.

- 35 If desired, the micro-organism may be a mould. A suitable strain is *Penicillium expansum*.

If desired, the micro-organism may be a bacterium.

- 40 Suitably the bacteria are of one of the orders:— *Pseudomonales*, *Eubacteriales* and *Actinomycetales*.

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

<i>Bacillus amylobacter</i>	50
<i>Pseudomonas natriegens</i>	
<i>Arthrobacter</i> sp.	
<i>Micrococcus</i> sp.	
<i>Corynebacterium michiganense</i>	
<i>Pseudomonas syringae</i>	
<i>Xanthomas begoniae</i>	55
<i>Flavobacterium devorans</i>	
<i>Acetobacter</i> sp.	
<i>Actinomyces</i> sp.	
<i>Agrobacterium</i> sp.	
<i>Aplanobacter</i> sp.	60

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.

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

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Diammonium phosphate	2	grams
Potassium chloride	1.15	grams
Magnesium sulphate, $7H_2O$	0.65	grams
Zinc sulphate	0.17	grams
Manganese sulphate, $1 H_2O$	0.045	grams
Ferrous sulphate, $7 H_2O$	0.068	grams
Tap water	200	grams
Yeast extract	0.025	grams

Distilled water (to make up to 1000 mls.)

A typical nutrient medium for the growth of *Nocardia*, a genus in the Actinomycetales order, has the following composition:—

Ammonium sulphate	1	gram
Magnesium sulphate	0.20	grams
Ferrous sulphate, $7H_2O$	0.005	grams
Manganese sulphate, $1H_2O$	0.002	grams
Monopotassium phosphate	2	grams
Disodium phosphate	3	grams
Calcium Chloride	0.1	grams
Sodium Carbonate	0.1	grams
Yeast Extract	0.008	grams

Distilled water (to make up to 1000 mls.)

For other bacteria a suitable nutrient medium has the composition:

Monopotassium phosphate	7	grams
Magnesium sulphate, $7H_2O$	0.2	grams
Sodium Chloride	0.1	grams
Ammonium Chloride	2.5	grams
Tap water (trace elements)	100	mls
Yeast extract	0.025	grams

Made up to 1000 mls. with distilled water

Another suitable nutrient medium for the growth of bacteria has the composition:—

NH ₄ Cl	0.5 grams
NaCl	4 grams
MgSO ₄	0.5 grams
Na ₂ HPO ₄	0.5 grams
KH ₂ PO ₄	0.5 grams

Water to make up to:

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.

In batch operation, 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 vitamins of group B obtained by the hydrolysis of a yeast) or more generally of vitamins of group B and/or biotin. This quantity is preferably of the order of 25 parts per million with reference to the aqueous fermentation medium. It can be higher or lower according to the conditions chosen for the growth.

The growth of the micro-organism takes place at the expense of the feedstock fraction with the intermediate production of bodies

having an acid function, principally fatty acids, in such manner that the pH of the aqueous mineral medium progressively diminishes. If one does not correct it the growth is fairly rapidly arrested and the concentration of the micro-organism in the medium, or cellular density, no longer increases so that there is reached a so-called stationary phase.

Preferably therefore 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—8). 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 may be recovered

as a cream or paste. However, in some cases separation will be accomplished by filtration or to some extent by decantation.

5 This cream or paste which contains aqueous material may be treated 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 continuously fed back to the extractor. Under these conditions the yeast may be introduced and withdrawn continuously or batchwise.

20 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.

25 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.

30 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.

40 Preferably the number of pulses lies between 1 and 60 per minute.

45 Suitable solvents for use in the process have been described hereinbefore. 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 micro-organism can be further treated in a second extraction stage using a hydrocarbon solvent, for example normal hexane or a light platformate fraction or benzene.

50 Preferably in the second stage there is used as solvent a mixture of a 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.

60 When using a solvent consisting of a mixture of a 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 micro-organism cream or paste is considerably reduced. As a result the micro-organism containing 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 sub-stages consisting of washing with the solvent used in the stage followed by separation.

80 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 cream or paste of the micro-organism. 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 sub-stage the cream or paste 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 micro-organism.

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 a 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 a 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 a 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

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fraction in this distillation stage. Suitably the polar solvent is ethanol or isopropanol. Suitably the second stage solvent is an azeotropic mixture.

- 5 In accordance with preferred procedure the micro-organism containing fraction is treated in a first stage consisting of one or more sub-stages, in each sub-stage the fraction being washed with a polar solvent and the extract separated, one or more extract fractions being
- 10 blended with a second stage recycle fraction and the blend distilled to recover first stage solvent which is recycled and a residue fraction, the raffinate product of the first stage being passed to a second stage treatment, consisting of one or more sub-stages, in each
- 15 sub-stage the fraction being washed with a mixture of a hydrocarbon solvent and a polar solvent and the extract separated, one or more second stage fractions being distilled with recovery of
 - (a) a first stage solvent which is recycled to the first stage,
 - (b) a fraction containing hydrocarbon solvent
 - 25 and polar solvent which is recycled to the second solvent extraction stage and
 - (c) a bottoms fraction containing extracted materials which constitutes the said second stage recycle fraction.
- 30 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 temperatures will lead to some degradation of the product.
- 35 If the cream or paste of micro-organism is subjected to partial drying 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
- 40 with the use of a smaller amount of solvent than would be required if no drying had taken place. If the extent of drying is considerable a first stage 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 a hydrocarbon and polar solvent, for example an alcohol, ketone or chlorinated hydrocarbon.

In general it is desirable to avoid drying under drastic conditions 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.

The invention is illustrated but not limited by the following Examples 1—3. Experiments 1 and 2 which do not constitute operation according to the invention are provided for purposes of comparison.

EXAMPLE 1.

10 litres of the following aqueous mineral medium was introduced into a 15 litre stirred fermenter; parts are by weight:—

Sodium phosphate, tribasic	3.4
Potassium chloride	0.6
Magnesium sulphate	0.3
Ammonium sulphate	2.5

Made up to 1000 parts with soft water containing trace elements.

A suitable alternative medium has the composition:—

Diammonium phosphate	2	grams
Potassium chloride	1.15	grams
Magnesium sulphate, 7H ₂ O	0.65	grams
Zinc sulphate	0.17	grams
Manganese sulphate, 1 H ₂ O	0.045	grams
Ferrous sulphate, 7 H ₂ O	0.068	grams
Tap water	200	grams
Yeast extract	0.025	grams
Distilled water (to make up to 1000 mls.)		

To the fermenter was added a few parts per million of yeast extract and then 50 grams of *Candida lipolytica* in the form of an aqueous cream containing 20% by wt. of dry material and then 150 grams of a heavy gas oil of petroleum origin containing 20% by wt. of normal paraffins.

When the culture reached the desired concentration of yeast cells for continuous operation, the continuous feed, to the fermenter, of aqueous mineral medium and petroleum oil was started up. The temperature was maintained at 30° C and the pH of the medium was maintained regulated at pH—4 by the addition of aqueous ammonia.

This emulsion was fed to a centrifugal separator from which were recovered three phases, being, in increasing order of density; (a) an oil phase containing the yeast cells, (b) an aqueous mineral medium phase (which may contain traces of oil and yeast) and (c) a yeast cream containing approximately 1 part of yeast, 4 parts of aqueous medium and a certain quantity of oil adhering to the yeast cells.

The yeast cream together with an aqueous solution of a surface active agent, for example a non-ionic detergent having, in the molecule, a condensed ethylene oxide chain, was fed continuously to a mixer and the mixture so obtained was centrifuged to obtain three fractions:— in increasing order of density: (a) an oil phase, (b) an aqueous phase containing the surfactant product which was recycled to the mixer and (c) a second yeast cream containing one part by wt. of yeast (which was still slightly contaminated by the oil) with 4 parts by wt. of aqueous liquid containing the surfactant.

This second yeast cream was passed with water to a mixer and the mixture so obtained was centrifuged to obtain:— (a) an oil phase, (b) an aqueous phase and (c) a thick yeast cream containing 20% by wt. of yeast (estimated as dry yeast) and 80% by wt. of water and which contained only a very small quantity of oil.

This thick yeast contained 44% by wt. of proteins and 18.5% by wt. of lipids. It possessed a sharp and rancid taste. The lipids with residual oil were extracted from the yeast by solvent extraction using a mixture of 80% of hexane and 20% of ethyl alcohol.

After evaporating the solvent, a yeast containing 52% of proteins and 3% of lipids was obtained.

(From 100 gms. of yeast there was obtained by solvent extraction 16 gms. of extract consisting principally of oxidised and unoxidised fatty acids, esters and sterols.)

The yeast which had thus been freed from the major part of its lipids possessed an absolutely neutral taste.

EXAMPLE 2.

A thick cream was prepared as described in Example 1, and treated in a solvent extraction unit in the form of a filtration drum rotated with its axis horizontal.

Yeast cream containing 2 parts by weight of dry yeast and 8 parts by weight of water was charged to the extractor with 16 parts of ethanol. The mixture was maintained at 60° C for 30 minutes while rotating the drum.

Solvent was drawn off, finally under vacuum. There was thus obtained a mixture of 2 parts of yeast and 2 parts of solvent which consisted of $\frac{1}{3}$ water and $\frac{2}{3}$ of ethanol, together with some remaining contaminants.

This mixture was treated with 10 parts by weight of a solvent mixture consisting of:

Normal Hexane	80% by wt.
Ethanol	20% by wt.

The mixture was maintained at 60° C for 15 minutes and solvent drawn off, finally under vacuum. The washing with this solvent mixture was repeated several times. (If a yeast product of high purity is required the washing stage may be repeated up to, for example, 10 times.). Finally the yeast product is dried in superheated steam.

The analysis of the yeast cream before solvent extraction and of the yeast product obtained is shown in the following Table.

TABLE

	Yeast cream	Yeast product
Nitrogen, by wt. of dry yeast	7%	7.8%
Total wt. of Lipids based on wt. of dry yeast	10%	0.5%

EXAMPLE 3.

The yeast cream described in Example 2 was continuously extracted in a drum having its axis vertical and having a paddle stirrer which rotated at 10 revs/minute. In separate runs the solvent was respectively ethanol; ethanol followed by normal hexane; and isopropanol.

The extract was continuously distilled for the recovery of lipids and hydrocarbons and the solvent recycled to the extractor.

Conditions and results obtained were as shown in the following Table:

TABLE

Run No.	4		7		8	
Solvent and Period of treatment	Ethanol (11 hours)		Ethanol (20 hours) Hexane (9 hours)		Isopropanol (40 hours)	
Rate of Feed of Solvent	600 mls/hr.		600 mls/hr.		600 mls/hr.	
Temperature	60°C.		60°C.		60°C.	
Weight of Yeast Paste (grams)	1000		1000		1000	
Analysis	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Dry Yeast						
Wt. (grams)	210	140	210	200	210	200
% Nitrogen	6.05	9.0	5.65	7.45	5.65	8.50
Total % wt. of Lipids in Yeast	41.2	5.0	42.9	4.2	42.9	6.9
Extract						
Wt. (grams)		72		200		100

By way of comparison then is provided the following description of Experiments 1 and 2 which do not constitute operation according to the invention.

EXPERIMENT 1.

The process described in Example 1 was repeated except that the yeast employed was a yeast *Saccharomyces cerevisiae* cultivated in the known manner on a substratum of beet molasses. It is found that no perceptible improvement in the characteristic taste of this yeast is obtained.

EXPERIMENT 2.

A similar experiment is carried out starting off from a yeast *Torula* cultivated in a known manner on cellulose bisulphite liquor. It is found in like manner that no perceptible improvement in the characteristic taste of this yeast is obtained.

In British Patent Application 18179/66 to the British Petroleum Company Ltd., there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism, which during

cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium, subjecting said fraction to drying, recovering a dry impure micro-organism and subjecting the dry impure micro-organism to solvent extraction for the removal, at least in part from the micro-organism of lipids together with hydrocarbons associated with said micro-organism.

In British Patent Application 18180/66 (Serial No. 1,049,067), to the British Petroleum Company Ltd., there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism which during cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium

and residual hydrocarbon, mixing said fraction with a surface active agent and subjecting the mixture with or without washing to a phase separation to recover (a) a treated fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said treated fraction being subjected to solvent extraction for the removal, at least in part, from the micro-organism, of lipids together with hydrocarbons associated with said micro-organism.

WHAT WE CLAIM IS: —

1. A process which comprises cultivating a micro-organism capable of growing on a feedstock consisting wholly or in part of a straight chain hydrocarbons with formation of lipids, said micro-organism being cultivated in the presence of said feedstock, separating from the product a fraction comprising the micro-organism and extracting said fraction by means of a solvent to remove lipids in the extract phase.

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 4 in which the yeast is *Candida lipolytica*.

8. A process according to any one of the preceding claims in which the micro-organism is grown in the presence of a nutrient medium containing vitamins of group B.

9. A process according to any of the preceding claims in which the micro-organism is grown in the presence of a nutrient medium containing extract of yeast.

10. A process according to claim 1 in which the micro-organism is a bacterium.

11. A process according to any one of the preceding claims in which the product obtained by cultivating the micro-organism is treated for the separation of a micro-organism containing fraction from the bulk of the liquid phase, and in which the micro-organism containing fraction so separated or a modified fraction obtained by further treating said fraction, for example by drying or partial drying, is subjected to solvent extraction.

12. A process according to claim 11 in which the modified micro-organism containing fraction is a micro-organism containing fraction which has been at least partially dried at a temperature not greater than 70° C.

13. A process according to claim 11 in which the micro-organism containing fraction is extracted with a hydrocarbon solvent, or a mixture of a hydrocarbon solvent and a polar solvent.

14. A process according to claim 11 in which the micro-organism containing fraction is in the form of a water-containing cream or paste and wherein solvent extraction is carried out in two stages, the first stage being carried out in the presence of a polar solvent and the second stage being carried out in the presence of a mixed solvent consisting of a hydrocarbon solvent and a polar solvent.

15. A process according to claim 14 in which the micro-organism containing fraction is treated in a first stage consisting of one or more sub-stages, in each sub-stage the fraction being washed with a polar solvent and the extract separated, one or more extract fractions being blended with a second stage recycle fraction and the blend distilled to recover first stage solvent which is recycled and a residue fraction, the raffinate product of the first stage being passed to a second stage treatment, consisting of one or more sub-stages, in each sub-stage the fraction being washed with a mixture of a hydrocarbon solvent and a polar solvent and the extract separated, one or more second stage fractions being distilled with recovery of

(a) a first stage solvent which is recycled to the first stage,

(b) a fraction containing hydrocarbon solvent and polar solvent which is recycled to the second solvent extraction stage and

(c) a bottoms fraction containing extracted materials which constitutes the said second stage recycle fraction.

16. A process according to any one of claims 13—15 in which the polar solvent is ethanol or isopropanol.

17. A process according to any one of claims 13—15 in which the hydrocarbon solvent is normal hexane, benzene or a light platformate fraction.

18. A process according to any one of claims 13—17 in which the mixed hydrocarbon solvent and polar solvent is an azeotropic mixture.

19. A process according to claim 11 in which a fraction obtained from the product of the cultivation stage, said fraction comprising the micro-organism, and at least some aqueous medium and residual hydrocarbon is mixed with an aqueous solution of a surface active agent and the mixture treated to recover (a) a washed fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said washed fraction, with or without an intervening treatment being treated by solvent extraction for



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and residual hydrocarbon, mixing said fraction with a surface active agent and subjecting the mixture with or without washing to a phase separation to recover (a) a treated fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said treated fraction being subjected to solvent extraction for the removal, at least in part, from the micro-organism, of lipids together with hydrocarbons associated with said micro-organism.

15 WHAT WE CLAIM IS: —

1. A process which comprises cultivating a micro-organism capable of growing on a feedstock consisting wholly or in part of a straight chain hydrocarbons with formation of lipids, said micro-organism being cultivated in the presence of said feedstock, separating from the product a fraction comprising the micro-organism and extracting said fraction by means of a solvent to remove lipids in the extract phase.

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.

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

35 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 4 in which the yeast is candida lipolytica.

40 8. A process according to any one of the preceding claims in which the micro-organism is grown in the presence of a nutrient medium containing vitamins of group B.

45 9. A process according to any of the preceding claims in which the micro-organism is grown in the presence of a nutrient medium containing extract of yeast.

10. A process according to claim 1 in which the micro-organism is a bacterium.

50 11. A process according to any one of the preceding claims in which the product obtained by cultivating the micro-organism is treated for the separation of a micro-organism containing fraction from the bulk of the liquid phase, and in which the micro-organism containing fraction so separated or a modified fraction obtained by further treating said fraction, for example by drying or partial drying, is subjected to solvent extraction.

60 12. A process according to claim 11 in which the modified micro-organism containing fraction is a micro-organism containing fraction which has been at least partially dried at a temperature not greater than 70° C.

13. A process according to claim 11 in which the micro-organism containing fraction is extracted with a hydrocarbon solvent, or a mixture of a hydrocarbon solvent and a polar solvent.

70 14. A process according to claim 11 in which the micro-organism containing fraction is in the form of a water-containing cream or paste and wherein solvent extraction is carried out in two stages, the first stage being carried out in the presence of a polar solvent and the second stage being carried out in the presence of a mixed solvent consisting of a hydrocarbon solvent and a polar solvent.

75 15. A process according to claim 14 in which the micro-organism containing fraction is treated in a first stage consisting of one or more sub-stages, in each sub-stage the fraction being washed with a polar solvent and the extract separated, one or more extract fractions being blended with a second stage recycle fraction and the blend distilled to recover first stage solvent which is recycled and a residue fraction, the raffinate product of the first stage being passed to a second stage treatment, consisting of one or more sub-stages, in each sub-stage the fraction being washed with a mixture of a hydrocarbon solvent and a polar solvent and the extract separated, one or more second stage fractions being distilled with recovery of

80 (a) a first stage solvent which is recycled to the first stage,

85 (b) a fraction containing hydrocarbon solvent and polar solvent which is recycled to the second solvent extraction stage and

90 (c) a bottoms fraction containing extracted materials which constitutes the said second stage recycle fraction.

100 16. A process according to any one of claims 13—15 in which the polar solvent is ethanol or isopropanol.

105 17. A process according to any one of claims 13—15 in which the hydrocarbon solvent is normal hexane, benzene or a light platformate fraction.

110 18. A process according to any one of claims 13—17 in which the mixed hydrocarbon solvent and polar solvent is an azeotropic mixture.

115 19. A process according to claim 11 in which a fraction obtained from the product of the cultivation stage, said fraction comprising the micro-organism, and at least some aqueous medium and residual hydrocarbon is mixed with an aqueous solution of a surface active agent and the mixture treated to recover (a) a washed fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said washed fraction, with or without an intervening treatment being treated by solvent extraction for

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the removal, wholly or in part, from the micro-organism of lipids, including hydrocarbons associated with said micro-organism.

- 5 20. A process according to any one of the preceding claims in which straight chain hydrocarbons are removed from a petroleum fraction with production of a micro-organism, wherein the feedstock employed for the growth of the micro-organism is a petroleum fraction consisting in part of straight chain hydrocarbons, wherein there is recovered from the product of the growth of the micro-organism a petroleum fraction having a reduced proportion of straight chain hydrocarbons or which is free of said straight chain hydrocarbons.
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21. A process according to claim 20 for the removal, wholly or in part of waxes from a wax-containing petroleum gas oil, wherein the

feedstock is a wax-containing petroleum gas oil and wherein there is recovered, from the product of the growth of the micro-organism, a gas oil of reduced content of wax.

22. A process according to claim 1 and substantially as hereinbefore described with reference to any one of the foregoing Examples.

23. A micro-organism whenever cultivated, separated and treated by a process as claimed in any one of the preceding claims.

24. An extract whenever produced by growth and treatment of a micro-organism by a process as claimed in any one of claims 1—22.

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ERRATUM

SPECIFICATION No. 1,049,065

Page 1, Heading, for "VIII," read "VIIIe,"

THE PATENT OFFICE
13th January 1967



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