

1095.182

PATENT SPECIFICATION

1095.182



NO DRAWINGS

Inventor: BERNARD MAURICE LAINE

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COMPLETE SPECIFICATION

Improvements in or relating to the Cultivation of a Micro-Organism and to the Removal, wholly or in part, of a Straight Chain Hydrocarbon from a Mixture in which it is contained

5 We, THE BRITISH PETROLEUM COMPANY LIMITED, of Britannic House, Finsbury Circus, London, E.C.2., England, 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:—

10 This invention relates to a process for the cultivation of a micro-organism and to the removal of straight chain hydrocarbons from a petroleum fraction.

15 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.

25 It is an object of the present invention to provide a commercially viable process for the removal of straight chain hydrocarbons from petroleum fractions with the resulting upgrading of the petroleum fraction accompanied by the conversion of the straight chain hydrocarbons into a readily saleable food supplement.

30 According to one aspect of this invention there is provided a process which comprises distilling a petroleum fraction to obtain at least two distillate fractions comprising a heavy distillate fraction and a light distillate fraction, thereafter treating said heavy distillate fraction with a straight chain hydrocarbon-consuming micro-organism whereby said micro-organism grows using, as feedstock, straight chain hydrocarbons contained in said heavy distillate frac-

tion, and thereafter separating the treated heavy distillate fraction. Preferably the treated heavy distillate fraction is blended with the light distillate fraction. 45

The terms 'heavy fraction' and 'light fraction' are used in a relative sense without limitation upon the absolute boiling points of the fractions. 50

Preferably the petroleum fraction is distilled to obtain a heavy gas-oil fraction and a light gas-oil fraction.

Preferably the treated heavy gas-oil fraction is blended with the light gas-oil fraction. 55

If it is intended to blend back the treated heavy gas-oil fraction, the cut point between the heavy gas-oil fraction and the light gas-oil fraction will preferably be in the range 300—350°C. 60

If it is not intended to blend back the treated heavy gas-oil fraction, the 90% distillation point of the heavy gas-oil fraction should be below 380°C.

65 It is a particular advantage of the process as hereinbefore described that, since the straight chain hydrocarbons which are responsible for a high pour point, are found in the heavy distillate fraction after the distillation a smaller quantity of petroleum fraction has to be subjected to the treatment with micro-organisms with the resulting reduction in fermenter volume. At the same time the drop in pour point and cloud points of the blend of the treated and untreated petroleum fractions are at least as low as those obtained in the case where the unrefined petroleum fraction is subjected to the treatment with micro-organisms. 75

80 Micro-organisms which are cultivated as herein described may be yeasts, moulds or bacteria. Within the term "micro-organism" used herein we include mixtures of micro-organisms.

[Price 4s. 6d.]



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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).

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.

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CBS

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Preferred Strain

<i>Candida</i>	<i>lipolytica</i>	
<i>Candida</i>	<i>pulcherrima</i>	CBS 610
<i>Candida</i>	<i>utilis</i>	
<i>Candida</i>	<i>utilis</i> , Variati major	CBS 841
<i>Candida</i>	<i>tropicalis</i>	CBS 2317
<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 Reference No. STV 11

Of the above *Candida Lipolytica* is particularly preferred.

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

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).

Suitably the bacteria are of one of the orders:—
Pseudomonadales, Eubacteriales and Actinomycetales.

Preferably the bacteria which are employed are of the families Bacillaceae and Pseudomonadaceae. Preferred species are *Bacillus megaterium*, *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.

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.

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 nutritives, that is, growth factors, obtained by the hydrolysis of a yeast) or more generally of the essential nutritives. The essential nutritives include biotin; pantothenic acid, nicotinic

acid, thiamine, inositol and pyridoxine. The quantity of yeast extract added is preferably about 25 parts per million. The quantity of each nutrilit required varies between about 0.1 parts per million for biotin and about 10 parts per million for inositol.

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 1000 in two days.

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 typical nutrient medium for the growth of *Nocardia* has the following composition:—

$(\text{NH}_4)_2\text{SO}_4$	1 gram
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.20 gram
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.005 gram
$\text{MnSO}_4 \cdot 10\text{H}_2\text{O}$	0.002 gram
KH_2PO_4	2 grams
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	3 grams
CaCl_2	0.1 gram
Na_2CO_3	0.1 gram
Yeast Extract	0.008 gram
Distilled water (to make up to 1000 mls.)	

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For other bacteria a suitable nutrient medium has the composition:

KH_2PO_4	7 grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 gram
NaCl	0.1 gram
NH_4Cl	2.5 grams
Tap water (trace elements)	100 mls.
Yeast extract	0.025 gram
Made up to 1000 mls. with distilled water	

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

$(\text{NH}_4)_2\text{HPO}_4$	2 grams
KCl	1.15 grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.65 gram
$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$	0.17 gram
$\text{MnSO}_4 \cdot 10\text{H}_2\text{O}$	0.045 gram
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.068 gram
Tap water	200 grams
Yeast extract	0.025 gram
Distilled water (to make up to 1000 mls.)	

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.

If desired the micro-organism may be sub-

jected to autolysis before further purification of the product.

The fraction containing the micro-organism is, with or without further treatment, a potential source of food for both animals and humans.

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 chain 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. Suitably the petroleum fraction will contain 3—45% by weight of straight-chain hydrocarbons.

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 metabolism of the straight-chain hydrocarbons it is possible to operate with the removal of only a desired proportion of these 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. 914567, 914568, 1017584, 1017585, 1021697, 1021698, 1049065, 1049066, 1049067, 1049929 and in 1059881 through to 1059890; also in British Patent Application No. 45009/63 (Serial No. 1095183).

The invention is illustrated but not limited by the following examples. Experiment 1 is included in Example 1 for comparative purposes only and does not constitute operation in accordance with the invention.

EXAMPLE 1

In Experiment 1, the gas-oil is dewaxed without a preliminary distillation and in Experiment 2 the gas-oil is distilled and the heavy fraction so obtained is subjected to the dewaxing process before being blended back with the light fraction from the distillation.

The gas-oil used was obtained from an Iraq crude oil and boiled between 220 and 380°C. It contained 13% by weight of n-paraffins. In Experiment 2, the gas-oil was distilled at atmospheric pressure in a column of 14 theoretical plates to obtain two fractions: one (75% by volume) boiling between 220 and 320°C and the other (25% by volume) boiling between 300 and 380°C. The yeast used for the dewaxing stage was *Candida lipolytica*. The fermentations were performed in a 60 litre continuous fermenter. The following aqueous nutrient medium was used:

$(\text{NH}_4)_2\text{HPO}_4$	2 grams
KCl	1.15 grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.65 gram
$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$	0.17 gram
$\text{MnSO}_4 \cdot 10\text{H}_2\text{O}$	0.045 gram
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.068 gram
Yeast extract	0.025 gram
Tap water	1000 mls.

The amount of gas-oil present in the medium in Experiment 1 was 180 grams/litre while the amount of gas-oil heavy distillate fraction

present in Experiment 2 was 90 grams/litre — the difference in the amount of petroleum fractions present was arranged so that the pour

point of the product in each Experiment would be the same after the cultivation. The pH was maintained at 4 and the temperature at 30°C. The mean residence time was 10 hr. at a dilution rate of 0.1. (The dilution rate is ratio of the volume of feedstock fed to the fermenter/hour to the volume of the liquid

phase in the fermenter). The cellular density rose to 10 grams/litre in Experiment 1 and 8 grams/litre in Experiment 2.

The cloud and pour points of the various fractions obtained in the two experiments were determined and are shown below.

Experiment	Fraction	Boiling Range	Volume (based on initial gas-oil)	Cloud Point	Pour Point
	Initial Gas-oil	220 — 380°C.		+1°C.	-1°C.
II	Light Fraction	220 — 320°C.	75%	-9°C.	-15°C.
	Heavy Fraction	300 — 380°C.	25%	—	+17°C.
	Dewaxed Heavy Fraction	—	—	-6°C.	-13°C.
	Blend of Light and Dewaxed Heavy Fractions	—	—	-7°C.	-13°C.
I	Dewaxed Initial Gas-oil	—	—	1°C.	-13°C.

15 It can be seen that a lower cloud point and the same pour point is obtained in Experiment 2 as in Experiment 1. Also, since in Experiment 1 there is 4 times as much feedstock to be treated in the fermenter as there is in Experiment 2 and since in addition it is necessary in Experiment 1 to supply the feedstock to the fermenter at twice the rate as in Experiment 2 in order to obtain the same pour point as in Experiment 1 it can be seen that there is a very considerable saving in the fermenter volume and/or time of fermentation.

EXAMPLE 2

There were performed three further sets of experiments, similar to those described in Example 1 except that, instead of using *C. lipolytica*, a different micro-organism was employed in each case. The micro-organisms used were the yeasts, *Candida pulcherrima* and *Hansenula anomala*, and the bacterium, *Bacillus megaterium*. The distillation and fermentation conditions were identical to those used in Example 1.

The following results were obtained:

Experiment	Fraction	H. anomala		C. pulcherrima		B. megaterium	
		Pour Pt.	Cloud Pt.	Pour Pt.	Cloud Pt.	Pour Pt.	Cloud Pt.
	Initial Gas-Oil	-1°C.	—	-1°C.	—	-1°C.	—
II	Light Fraction	-14°C.	-8°C.	-14°C.	-8°C.	-14°C.	-8°C.
	Dewaxed Heavy Fraction	-10°C.	-6°C.	-16°C.	-8°C.	-22°C.	-13°C.
	Blend of Light and Dewaxed Heavy Fractions		-8°C.		-8°C.		-10°C.
I	Dewaxed Initial Gas-Oil	-10°C.	0	-16°C.	-3°C.	-22°C.	-6°C.

It can be seen that very similar results were obtained using these other micro-organisms as was obtained with *C. lipolytica*.

5 In British Patent No. 914567 there is described and claimed a process for the recovery of yeasts which have been grown in the presence of hydrocarbons, which comprises adding to a mixture of yeast, aqueous mineral medium and hydrocarbon, so obtained, at
10 least 50 parts per million by weight of a surface active agent and thereafter centrifuging, whereby there is obtained a paste comprising yeast and aqueous mineral medium.

15 In British Patent No. 914568 there is described and claimed a process which comprises maintaining a yeast in a nutrient medium in the presence of a paraffinic feedstock of petroleum origin and a free oxygen-containing gas under conditions favouring growth of the yeast,
20 said feedstock having an average molecular weight corresponding to at least 10 carbon atoms per molecule, and separating the yeast from the aqueous medium.

WHAT WE CLAIM IS:—

25 1. A process which comprises distilling a petroleum fraction to obtain at least two distillate fractions comprising a heavy distillate fraction and a light distillate fraction, thereafter treating the said heavy distillate fraction
30 with a straight chain hydrocarbon-consuming micro-organism whereby said micro-organism grows using as feedstock, straight-chain hydrocarbons contained in said heavy distillate fraction, and thereafter separating the treated
35 heavy distillate fraction.

2. A process according to claim 1 in which the treated heavy distillate fraction is blended with the untreated light distillate fraction.

40 3. A process according to claim 1 in which the petroleum fraction is distilled to obtain a heavy gas-oil fraction and a light gas-oil fraction.

45 4. A process according to claim 3 in which the treated heavy gas-oil fraction is blended with the light gas-oil fraction.

50 5. A process according to any of the preceding claims which comprises cultivating a micro-organism in the presence of a petroleum fraction having a mean molecular weight corresponding to at least 10 carbon atoms per molecule.

6. A process according to any of the preceding claims in which the petroleum fraction is a gas-oil fraction which contains straight-chain hydrocarbons in the form of waxes. 55

7. A process according to any of the preceding claims in which, in the case where the treated gas-oil fraction is to be blended back with the light gas-oil fraction, the cut point between said light gas-oil fraction and said heavy gas-oil fraction is in the range 300—350°C. 60

8. A process according to claims 1—6 in which in the case where the treated gas-oil fraction is not to be blended with the light gas-oil fraction, the 90% distillation point of the heavy gas-oil fraction is below 380°C. 65

9. A process according to any of the preceding claims in which the micro-organism is a yeast. 70

10. A process according to claim 9 in which the yeast is of the family Cryptococcaceae.

11. A process according to claim 10 in which the yeast is of the sub-family Cruptococcoideae. 75

12. A process according to claim 11 in which the yeast is of the genus *Torulopsis*.

13. A process according to claim 11 in which the yeast is of the genus *Candida*.

14. A process according to claim 13 in which the yeast is *Candida lipolytica*. 80

15. 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. 85

16. A process according to any of claims 1—8 in which the micro-organism is a bacterium.

17. A process according to claim 1 and substantially as hereinbefore described with reference to either of the foregoing Examples. 90

18. A micro-organism or a fraction derived from a micro-organism whenever produced by a process as claimed in any of the preceding claims. 95

19. A gas-oil whenever produced by a process as claimed in any of claims 1—17.

J. WOOLARD,
Agent for the Applicants,
Chartered Patent Agent.

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5 In British Patent No. 914567 there is described and claimed a process for the recovery of yeasts which have been grown in the presence of hydrocarbons, which comprises adding to a mixture of yeast, aqueous mineral medium and hydrocarbon, so obtained, at
10 least 50 parts per million by weight of a surface active agent and thereafter centrifuging, whereby there is obtained a paste comprising yeast and aqueous mineral medium.

15 In British Patent No. 914568 there is described and claimed a process which comprises maintaining a yeast in a nutrient medium in the presence of a paraffinic feedstock of petroleum origin and a free oxygen-containing gas under conditions favouring growth of the yeast,
20 said feedstock having an average molecular weight corresponding to at least 10 carbon atoms per molecule, and separating the yeast from the aqueous medium.

WHAT WE CLAIM IS:—

25 1. A process which comprises distilling a petroleum fraction to obtain at least two distillate fractions comprising a heavy distillate fraction and a light distillate fraction, thereafter treating the said heavy distillate fraction
30 with a straight chain hydrocarbon-consuming micro-organism whereby said micro-organism grows using as feedstock, straight-chain hydrocarbons contained in said heavy distillate fraction, and thereafter separating the treated
35 heavy distillate fraction.

2. A process according to claim 1 in which the treated heavy distillate fraction is blended with the untreated light distillate fraction.

40 3. A process according to claim 1 in which the petroleum fraction is distilled to obtain a heavy gas-oil fraction and a light gas-oil fraction.

45 4. A process according to claim 3 in which the treated heavy gas-oil fraction is blended with the light gas-oil fraction.

50 5. A process according to any of the preceding claims which comprises cultivating a micro-organism in the presence of a petroleum fraction having a mean molecular weight corresponding to at least 10 carbon atoms per molecule.

6. A process according to any of the preceding claims in which the petroleum fraction is a gas-oil fraction which contains straight-chain hydrocarbons in the form of waxes. 55

7. A process according to any of the preceding claims in which, in the case where the treated gas-oil fraction is to be blended back with the light gas-oil fraction, the cut point between said light gas-oil fraction and said heavy gas-oil fraction is in the range 300—350°C. 60

8. A process according to claims 1—6 in which in the case where the treated gas-oil fraction is not to be blended with the light gas-oil fraction, the 90% distillation point of the heavy gas-oil fraction is below 380°C. 65

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