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PROCESS FOR CULTIVATING MICRO-ORGANISMS ON HEAVY DISTILLATE FRACTION CONTAINING STRAIGHT-CHAIN HYDROCARBONS

Bernard Maurice Laine, Lavera, France, assignor to The British Petroleum Company Limited, London, England, a company of England

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12 Claims. (Cl. 195—3)

This invention relates to cultivating micro-organisms on a heavy distillate fraction containing straight chain hydrocarbons.

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.

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.

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 micro-organism whereby said micro-organism grows using, as feedstock, straight chain hydrocarbons contained in said heavy distillate fraction, and thereafter separating the treated heavy distillate fraction. Preferably the treated heavy distillate fraction is blended with the light distillate fraction.

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

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.

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 of 300–350° C.

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.

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.

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

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, ascosporogeneous 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 vor Schimmelculture, Baarn, Holland, and to INRA stock held by the Institut National de la Recherche Agronomique, Paris, France.

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.

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 Determining 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 exists 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 nutrilites, that is, 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 nutrilite required varies between about 0.1 part 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, of 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 1,000 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:

	Grams
$(NH_4)_2SO_4$	1
$MgSO_4 \cdot 7H_2O$	0.20
$FeSO_4 \cdot 7H_2O$	0.005
$MnSO_4 \cdot 10H_2O$	0.002
KH_2PO_4	2
$Na_2HPO_4 \cdot 7H_2O$	3
$CaCl_2$	0.1
Na_2CO_3	0.1
Yeast extract	0.008

Distilled water (to make up to 1000 mls.).

For other bacteria a suitable nutrient medium has the composition:

KH_2PO_4, grams	7
$MgSO_4 \cdot 7H_2O$, gram	0.2
NaCl, gram	0.1
NH_4Cl, grams	2.5
Tap water (trace elements), mls.	100
Yeast extract, gram	0.025

Made up to 1000 mls. with distilled water.

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

	Grams
$(NH_4)_2HPO_4$	2
KCl	1.15
$MgSO_4 \cdot 7H_2O$	0.65
$ZnSO_4 \cdot H_2O$	0.17
$MnSO_4 \cdot 10H_2O$	0.045
$FeSO_4 \cdot 7H_2O$	0.068
Tap water	200
Yeast extract	0.025

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 subjected 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 metabolisation 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. 914,567 and 914,568—also in British application Nos.:

36,873/62 (SFP 1125)
44,606/63 (SFP 1300)
46,906/62 (SFP 1300–A)
19,918/63 (SFP 1400)
25,210/63 (SFP 1401)
2,234/63 (SFP 1404)
49,049/62 (SFP 1407)
49,050/62 (SFP 1408)
49,052/62 (SFP 1410)
45,011/63 (SFP 1611)
49,055/62 (SFP 1413)
49,056/62 (SFP 1414)
49,057/62 (SFP 1415)
45,009/62 (SFP 1616)
49,060/62 (SFP 1418)
49,061/62 (SFP 1419)
49,062/62 (SFP 1420)
49,063/62 (SFP 1421)
7,623/63 (SFP 1629)
19,271/63 (SFP 1440)
45,001/63 (SFP 1641)
25,229/64 (SFP 1644)
38,942/63 (SFP 1508)
183/64 (SFP 1512)
184/64 (TD 1513)
11,860/64 (SFP 1516)
182/64 (SFP 1522)
46,410/63 (SFP 1528)
46,411/63 (SFP 1532)
21,209/64 (SFP 1574)
26,498/64 (SFP 1575)
22,743/64 (RSO 1618)
37,985/64 (RSO 1643)
37,988/64 (SFP 1683)

Also in the specification of French application No. 924,254 (SFP 1402).

The invention is illustrated but not limited by the following example. Experiment I is included in the example for comparative purposes only and does not illustrate the invention.

Example

We show below the results of two experiments illustrating the advantages of refining a gas-oil before dewaxing it by means of yeast cultivation. In Experiment I, the gas-oil is dewaxed without a preliminary distillation and in Experiment II 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 II, 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:

$(NH_4)_2HPO_4$, grams	2
KCl, grams	1.15
$MgSO_4 \cdot 7H_2O$, gram	0.65
$ZnSO_4 \cdot H_2O$, gram	0.17
$MnSO_4 \cdot 10H_2O$, gram	0.045
$FeSO_4 \cdot 7H_2O$, gram	0.068
Yeast extract, gram	0.025
Tap water, mls.	1000

The amount of gas-oil present in the medium in Experiment I was 180 grams/litre while the amount of gas-oil heavy distillate fraction present in Experiment II 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 I and 8 grams/litre in Experiment II.

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

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

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

I claim:

1. A process which comprises distilling a petroleum fraction to obtain at least two distillate fractions comprising a heavy distillate fraction containing straight-chain hydrocarbons and a light distillate fraction containing straight-chain hydrocarbons of a lighter nature than those contained in the heavy distillate fraction, thereafter treating the said heavy distillate fraction with a micro-organism in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen whereby said micro-organism grows using as feedstock, straight-chain hydrocarbons contained in said heavy distillate fraction, thereafter separating the treated heavy distillate fraction, and blending the treated heavy distillate fraction with the untreated light distillate fraction.

2. 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.

3. A process according to claim 1 wherein the petroleum fraction is a fraction having a mean molecular weight corresponding to at least 10 carbon atoms per molecule.

4. A process according to claim 1 in which the petroleum fraction is a gas-oil fraction which contains straight-chain hydrocarbons in the form of waxes.

5. A process according to claim 2 in which the cut point between said light gas-oil fraction and said heavy gas-oil fraction is in the range 300–350° C.

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

7. A process according to claim 6 in which the yeast is of the family Cryptococcaceae.

8. A process according to claim 7 in which the yeast is of the sub-family Cryptococcoideae.

9. A process according to claim 8 in which the yeast is of the genus Torulopsis.

10. A process according to claim 8 in which the yeast is of the genus Candida.

11. A process according to claim 10 in which the yeast is *Candida lipolytica*.

12. A process according to claim 1 in which the yeast is grown in the presence of a nutrient medium containing extracts of yeast.

References Cited by the Examiner

UNITED STATES PATENTS

2,697,061	12/1954	Harris et al.	195—1
3,186,922	6/1965	Champagnat	195—82

A. LOUIS MONACELL, *Primary Examiner.*

D. M. STEPHENS, *Assistant Examiner.*