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PROCESS FOR IMPROVING CLOUD POINT OF PETROLEUM GAS OIL BY HYDROGENATION THEREOF FROM HYDROCARBON MIXTURES

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13 Claims. (Cl. 195—3)

This invention relates to a process for the production of micro-organisms, for example, yeasts. 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.

It is well-known that certain petroleum fractions, particularly gas oils, contain straight chain hydrocarbons, mainly paraffins which are waxes and which have an adreverse 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 naphthenes 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 contained, before they can be converted to other products.

In a process in which a micro-organism is used for the removal, wholly or in part, of straight chain hydrocarbons from hydrocarbon mixtures it has been found that, in general, the process gives rise to the formation of carbon, hydrogen and oxygen containing by-products, for example esters. These by-products may remain in the hydrocarbon fraction which is separated from the micro-organism and constitute a contaminant. In particular, when the feed stock is a wax-containing gas oil, the recovered gas oil may have an unacceptably high cloud point by reason of the presence of these by-products.

In accordance with this invention it has been found that these by-products may be converted at least in part to less detrimental products by a process of hydrogenation.

According to the present invention there is provided a process which comprises, in a micro-organism growth stage, cultivating a micro-organism in the presence of a hydrocarbon feedstock consisting of a mixture of straight chain hydrocarbons with other hydrocarbons; in the presence of an aqueous nutrient medium; and in the presence of a gas containing free oxygen, thereafter separating the micro-organism from the hydrocarbon residue and subjecting said hydrocarbon residue to hydrogenation.

Preferably the feedstock is a petroleum fraction.

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.

It is an important feature of this invention that when cultivating yeasts in the presence of the feedstocks here-

inbefore 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 the continuous process, this high percentage conversion can be achived 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 will usually be required to 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.

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 Cryptoccoccoideae; 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 numbers; these reference numbers refer to stock held by the Centraal Bureau vor Schimmelculture, Baarn, Holland:

Candida lipolytica
Candida pulcherrima CBS 610
Candida utilis
Candida utilis, Variati major CBS 841
Candida tropicalis CBS 2317
Torulopsis collisculosa CBS 133
Hansenula anomala
Oidium lactis
Neurospora sitophila

Of the above *Candida lipolytica* is particularly preferred.

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

If desired, the micro-organism may be a bacterium. 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 megaterium, Bacillus subtilis* and *Pseudomonas aeruginosa.* Other strains which may be employed include:

Bacillus amylebacter
Pseudomonas natriegens
Arthrobacter sp.
Micrococcus sp.
Corynebacterium sp.
Pseudomonas syringae
Xanthomonas begoniae

Flavobacterium devorans
Acetobacter sp.
Actinomyces sp.

Suitable moulds are of the family Aspergillaceae. A suitable genus is Penicillium .

Preferably there is used *Penicillium expansum*. Another suitable genus is Aspergillus.

Usually the cultivation is carried out in the presence of an aqueous nutrient medium. If desired, certain solid nutrient media may be employed.

In either case, a gas containing free oxygen must be provided.

Penicillium expansum is suitable for cultivation in an aqueous nutrient medium containing hydrocarbons.

Penicillium roqueforti, penicillium notataum, Aspergillus fussigatus and *Aspergillus niger, Aspergillus versicolor* may be used for cultivation on a solid agent containing hydrocarbons as feedstock.

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, a genus in the Actinomycetales order, has the following composition:

	Grams
Ammonium sulphate	1
Magnesium sulphate	0.20
Ferrous sulphate, $7H_2O$	0.005
Manganese sulphate, $1H_2O$	0.002
Monopotassium phosphate	2
Disodium phosphate	3
Calcium chloride	0.1
Sodium carbonate	0.1
Yeast extract	0.008

Distilled water (to make up to 1000 mls).

For other bacteria a suitable nutrient medium has the composition:

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

Made up to 1000 mls. with distilled water.

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

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

Distilled 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.

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, that is 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.

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 un-used feedstock fraction.

If desired the micro-organism may be subjected to autolysis before further purification of the product.

According to one method of treating the product the major part of the continuous aqueous phase is first separated; preferably this is carried out by centrifuging or decanting. The separated aqueous phase will usually contain a greater concentration of non-nutritive ions than can be tolerated in the recycle stream and when this is so, only a proportion of the recovered aqueous phase can be recycled. Thus it will usually be possible to separate ca. 96% by wt. of the aqueous phase which is present in the product, of which on the same percentage basis, ca. 20% by wt. will be discarded. The recycle stream is supplied with make-up quantities of the necessary nutrients and is returned to the fermenter;

if desired the make-up materials may be fed to the fermenter as a separate stream.

The process, as applied to the cultivation of a yeast, may incorporate product separation stages as follows. In some cases micro-organisms other than yeasts may be separated in this manner.

By centrifuging the product from the fermenter three fractions are recovered. These are in order of increasing density:

(i) An oil phase containing yeast cells
(ii) A naqueous phase containing traces of oil and yeast, and
(iii) A yeast "cream" consisting of yeast, having a quantity of oil fixed on to the cells, together with aqueous phase.

After recovery of fraction (ii), fraction (iii) or a blend of fractions (i) and (iii) is mixed with an aqueous solution of a surfactant.

The purpose of this treatment is to separate the oil from the yeast cells; the oil being apparently held to the cells by adsorption.

It may be advantageous to employ an edible surfactant, for example a saccharose ester, which makes it possible to reduce the subsequent washing required to remove from the yeast a surfactant which is not edible.

The emulsion so formed is broken down by centrifuging to obtain three fractions.

(iv) An oil phase
(v) An aqueous phase containing surfactant ,which phase is recycled for the treatment of fractions (i) and (iii), and
(vi) A yeast "cream," consisting of yeast still contaminated by oil together with an aqueous surfactant phase.

In order to reduce as far as possible the consumption of surfactant product, the aqueous washing solution containing it is recycled.

Fraction (vi) may be further treated by alternate washing with surfactant and centrifuging until the oil content of the yeast has reached a desired low value. The yeast "cream" now consisting of yeast and aqueous surfactant may now be washed with water and again centrifuged. If desired two or more washings may be given to this yeast "cream." If desired, one or more of these water washings (but preferably not the last) may make use of salt water (for example sea water); preferably the final wash is with soft water. With a view to economising the soft water necessary for the process, the whole of this water coming from the last washing is employed for making up the nutritive medium for the fermentation, where necessary at the stage of washing with the solution of surfactant, and the rest is sent to the salt water used for washing with a view to reducing its salt concentration. Finally the yeast may be dried under conditions suitable for its subsequent use as a foodstuff.

Other steps which may be taken to obtain a purified micro-organism or a product derived therefrom or to improve the process in respect of the production of the unmetabolised hydrocarbon fraction are described in the applications set out hereinafter.

The recovered unmetabolised hydrocarbon with or without an intervening refining stage is subjected to hydrogenation.

Suitable catalysts for use in the hydrogenation stage are compounds of cobalt and molybdenum with or without iron, nickel metal, nickel/tungsten sulphide, or any other conventional hydrogenation or desulphurising catalyst.

Preferably hydrogenation will be carried out under the following conditions.

The temperature may lie in the range 100–500° C. according to the catalyst; pressure from 10–70 kgs./sq. cm.; space velocity from 1–10 vol./vol./hour, hydrogen/hydrocarbon ratio in the range 0.1/1 to 5/1. The hydrogenation stage may be carried out in liquid, gas or mixed phase.

The invention is illustrated but not limited with reference to the following examples.

Throughout these examples cellular density is expressed as dry weight of yeast per litre of culture.

Example 1

40 litres of an aqueous mineral nutrient medium having the composition given below were introduced in a stainless steel fermenter having an effective capacity of 60 litres.

In order to keep the temperature in the fermenter constant at 30° C., water was circulated in an annulus constituted by the space between two concentrical cylinders, the smaller one being the fermenter itself.

The aqueous nutrient medium had the composition:

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

Distilled water add. 1000 ml.

20 litres of inoculum from a 24 hr. culture of *Candida lipolytica* on mixed C_9–C_{16} normal paraffinic hydrocarbons were then added such that the cellular density was about 1 gram/litre.

1.03 litres of heavy gas-oil, (15 grams/litre of fermenter) were then introduced into the fermenter, that is enough to carry the cellular density to 2 grams/litre.

The temperature of the culture was controlled at 30±1° C., pH 4, agitation and aeration were such that the rate of aeration was 3 millimoles O_2 per litre of medium per minute. An automatic pH controller added 10 N ammonia.

When the flow of ammonia reached 20 ml. the batchwise addition of gas-oil was started at a rate determined by the theoretical needs of the culture, assuming a yield

$$\left(100 \times \frac{\text{dry wt. of yeast produced}}{\text{gas-oil required}}\right)$$

of 10% and a cell division time of 3 hours. This addition took place every hour until a total of 250 grams/litre of gas-oil had been added, i.e. 17 litres.

Starting with a cellular density of 2 grams/litre, after 24 hours (at the end at the exponential growth phase) a cellular density of 18 grams/litre was obtained.

Characteristics of the heavy gas-oil used:

Density 15° C./4° C.	0.876
Flash point, Afnor ° C.	Above 120
Cloud point, ° C.	+20
Pour point, ° C.	+18
Percent wt. N-paraffins	10
Saybolt distillation	
Initial boiling point, ° C.	221
5%, ° C.	228
50%, ° C.	363
95%, ° C.	402
Final, BP,	Above 405
Percent, volume	1
Percent loss in volume	0

At 25 hours a quantity of culture was withdrawn from the fermenter, from which 300 ml. of non-metabolised gas-oil was obtained by centrifuging at +5° C. The characteristics of this residual gas-oil are given below:

Density, 15° C./4° C.	0.890
Flash point, Afnor, ° C.	Above 120
Cloud point, ° C.	+5

Pour point	−13
Percent N-paraffins, by wt.	2.1
Saybolt distillation:	
Initial boiling point, ° C.	248
5%, ° C.	300
50%, ° C.	368
70%, ° C.	381
90%, ° C.	395
95%, ° C.	402
Final BP, ° C.	Above 405
Percent residual, volume	1
Percent loss in volume	0

The gas-oil recovered was washed with water and dried by means of phosphorus pentoxide.

It was then passed over 100 ml. of a conventional Co/Mo hydrogenation catalyst at a rate of 400 ml. per hour at 390° C. and a pressure of 70 kg., with hydrogen at 100 ml. per hour.

After treatment the gas-oil showed a cloud point of −10° C.

Example 2

A continuous culture in a 5 litre fermenter containing 3 litres of culture was fed at a rate of 300 ml./hr. with a medium containing 10% by weight of heavy gas-oil emulsified in 90% of a mineral medium as given in Example 1.

The gas-oil feed had the following characteristics:

Density, 15° C./4° C.	0.875
Pour point, ° C.	+17
Cloud point, ° C.	+20
Sulphur, percent wt.	1.6
N-paraffins, percent wt.	12
Afnor flash point	135
Saybolt distillation:	
Initial BP, ° C.	139
5%, ° C.	304
50%, ° C.	362
95%, ° C.	398
Final BP, ° C.	Above 400
Residual volume, percent	1
Loss in volume, percent	0

The pH was held at 4±0.1 by the automatic addition of 10 N ammonia using a suitable apparatus. Temperature was maintained at 30° C., and agitation and aeration were such that the aeration rate was 3 millimoles O_2 per litre of medium per minute.

The cellular density became stabilised at the end of a few days at 10±1 gram dry matter per litre. Sufficient culture was then withdrawn to obtain 300 ml. of non-metabolised gas-oil by centrifuging at +5° C.

The residual gas-oil had the following characteristics:

Density, 15° C./4° C.	0.888
Pour point, ° C.	−5
Cloud point, ° C.	+21
Total sulphur, percent wt.	1.95
N-paraffins percent wt.	2.1
Afnor flash point	150
Saybolt distillation:	
Initial BP, ° C.	265
5%, ° C.	310
50%, ° C.	365
95%, ° C.	398
Final BP, ° C.	Above 400
Residual volume, percent	1
Loss in volume, percent	0

This non-metabolised gas-oil was hydrogenated as described in Example 1.

The cloud point was lowered from +21 to 0° C.

I claim:

1. A process for the removal, at least in part, of waxes from a wax-containing petroleum gas oil which comprises, in a micro-organism growth stage, cultivating a straight chain hydrocarbon consuming-micro-organism in the presence of said wax-containing petroleum gas oil; in the presence of an aqueous nutrient medium; and in the presence of gas containing free oxygen, thereafter separating the micro-organism from the gas oil of reduced content of wax, and subjecting the gas oil to hydrogenation to reduce its cloud point.

2. A process according to claim 1 in which the hydrogenation is carried out in the presence of a catalyst containing cobalt and molybdenum.

3. A process according to claim 1 in which the hydrogenation temperature lies in the range 100–500° C.

4. A process according to claim 1 in which the hydrogenation is carried out at a pressure in the range 10–70 kgs./sq. cm.

5. A process according to claim 1 in which the hydrogenation is carried out at a hydrogen/hydrocarbon ratio in the range 0.1/1 to 5/1.

6. A process according to claim 1 in which the hydrogenation is carried out at a space velocity in the range 1–10 vol./vol./hour.

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

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

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

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

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

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

13. A process according to claim 1 in which the micro-organism is a bacteria.

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