United States Patent Office 3,522,147 Patented July 28, 1970

3,522,147

GROWTH AND SEPARATION OF HYDROCARBON CONSUMING MICROORGANISMS

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No Drawing. Filed Mar. 29, 1967, Ser. No. 626,700
Claims priority, application Great Britain, May 13, 1966, 21,340/66
The portion of the term of the patent subsequent to Aug. 2, 1983, has been disclaimed
Int. Cl. C12c *11/00;* C12k *3/00*
U.S. Cl. 195—28 22 Claims

ABSTRACT OF THE DISCLOSURE

Cultivation of a hydrocarbon-consuming micro-organism in the presence of a hydrocarbon feedstock consisting wholly or in part of the straight chain hydrocarbons, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen, thereafter, in a purification stage, maintaining said micro-organism with an aqueous nutrient medium and a gas containing free oxygen whereby the hydrocarbon contaminating the micro-organism is reduced in quantity, thereafter subjecting the fraction containing the micro-organism to a separation stage, thereafter reducing the proportion of water in the fraction containing the micro-organism and thereafter subjecting the fraction containing the micro-organism to solvent extraction.

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.

In a process in which a micro-organism is cultivated in the presence of a hydrocarbon it has now been found that the micro-organism may be contaminated by sorbed hydrocarbons. Sometimes other materials formed in the fermentation process may be present, thus yeasts may be contaminated by lipids.

In accordance with the present invention, there is provided a process which comprises the following stages:

(1) Cultivating, in a growth stage, a micro-organism in the presence of a hydrocarbon feedstock consisting wholly or in part of straight chain hydrocarbons, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen,

(2) Thereafter, maintaining, in a purification stage, said micro-organism with an aqueous nutrient medium and a gas containing free oxygen whereby the hydrocarbon contaminating the micro-organism is reduced in quantity,

(3) Thereafter subjecting the fraction containing the micro-organism to a separation stage and,

(4) Thereafter reducing the proportion of water in the fraction containing the micro-organism and,

(5) Thereafter subjecting the fraction containing the micro-organism to solvent extraction.

The separation process in stage (3) may be decantation; additionally or alternatively it may be a centrifuging.

Solvents which may be employed 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. Preferably the hydrocarbon feedstock contains hydrocarbons of C_{10} or higher. Suitably there may be used a hydrocarbon fraction derived from petroleum.

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

The petroleum fractions boiling below the gas oils, for example, heavy naphthenes and kerosines also contain straight chain hydrocarbons which are potentially valuable for conversion to other products but hitherto, in general, utilization 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.

According to a preferred feature of this invention there is provided a process which comprises cultivating 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.

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 iso-paraffins, 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 if 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 achieved without resorting to the use of a long reaction path.

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

Suitable feedstocks to the process of the invention include kerosine, gas oils and lubricating oils; these feedstocks may be unrefined or may have undergone some refinery treatment, but 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 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 species 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 vor Schimmelculture, Baarn, Holland, and to INRA stock held by the Institut National de la Recherch Agronomique, Paris, France.

	Preferred strain
Candida lipolytica.	
Candida pulcherrime	CBS 610
Candida utilis.	
Candida utilis, Bariati 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.

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

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

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 species 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.

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 order Actinomycetales, has the following composition:

	Grams
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—7 grams
Magnesium sulphate, $7H_2O$—0.2 gram
Sodium chloride—0.1 gram
Ammonium chloride—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:

Diammonium phosphate—2 grams
Potassium chloride—1.15 grams
Magnesium sulphate, $7H_2O$—0.65 gram
Zinc sulphate—0.17 gram
Manganese sulphate, $1H_2O$—0.045 gram
Ferrous sulphate, $7H_2O$—0.068 gram
Tap water—200 mls.
Yeast extract—0.025 gram
Distilled water (to make up to 1000 mls.).

Micro-organism, 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 1000 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 unused feedstock fraction.

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.

By centrifuging the product from the fermenter (preferably after decanting as described) three fractions are recovered. These are in order of increasing density:

(1) an oil phase containing micro-organism cells,
(2) an aqueous phase containing traces of oil and micro-organism, and
(3) a micro-organism "cream" consisting of micro-organism, having a quantity of oil fixed on to the cells, together with aqueous phase.

Fraction (3) will then be mixed with an aqueous nutrient medium, which may be the same as or different from the aqueous nutrient medium employed in the growth stage, and is maintained in admixture with a gas containing free oxygen whereby the hydrocarbon contaminating the micro-organism is reduced in quantity or is eliminated.

Again, preferably, the major part of the continuous aqueous phase is separated with recycle of a proportion of the recovered aqueous phase, as hereinbefore described; recycle may be to either the growth stage or purificatiton stage as desired.

By centrifuging the product from the fermenter, preferably after separating the major part of the aqueous phase by decanting, there are obtained:

(i) an aqueous phase containing traces of micro-organism
(ii) a micro-organism cream.

At this stage the proportion of water in the micro-organism cream is reduced. If desired the proportion of water may be reduced to a very low level although it is normally preferable to reduce the proportion of water only to such levels that the next stage (that is, the solvent purification stage as desired.

The cream or paste produced in the last stage, still containing some water, is then subjected to solvent extraction. This solvent extraction may be performed continuously 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.

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

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

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

Preferably the number of pulses lies between 1 and 60 per minute. The operation of the process under the action of pulses is further described in British patent application 2,234/63 (SFP 1404).

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.

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

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

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.

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

all contaminants recovered by solvent extraction are removed as a bottoms fraction in this distillation stage. Suitably the polar solvent is ethanol or isopropanol. Suitably the second stage solvent is an azeotropic mixture.

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.

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 separated either as such or as a raw material for its separation of its sterols, fatty acids (either before saponification or after) or of its other constituents.

Finally the yeast may be dried under conditions suitable for its subsequent use as a foodstuff.

The process may be operated continuously or batchwise.

When operating continuously it will usually be necessary to provide separate fermenters or separate zones in the same fermenter for the operation of the growth and purification stages.

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 following applications; the use of any process herein described lies within the scope of the present invention.

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.

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

EXAMPLE

Candida lipolytica was grown in a 12 cu. m. fermenter under continuous conditions in the presence of a culture medium which, as fed to the fermenter, had the composition shown below, the feed rate being 2400 litres/hour:

	Grams/litre
$(NH_4)_2HPO_4$	2.0
KCl	1.15
$MgSO_4.7H_2O$	0.65
$MnSO_4.4H_2O$	0.06
$FeSO_4.7H_2O$	0.124
$ZnSO_4.7H_2O$	0.306
Yeast extract	0.030
Tap water to 1000 ml.	

The hydrocarbon feed to the fermenter was a heavy Iraq gas oil containing 12% normal paraffins and having the following characteristics:

Initial boiling point (° C.)	217
5% B.P.	284
50% B.P.	343
95% B.P.	377
Final boiling point	385

The gas oil was fed to the fermenter at the rate of 120 grams per litre of total gas oil and aqueous medium fed.

The pH in the fermenter was maintained at 4 by the continuous addition of aqueous ammonia and the fermenter temperature was maintained at 130° C. Aeration was at the rate of 60 vol./vol./hr. of air.

The product of the fermentation was continuously removed and decanted to obtain 800 litres/hour of yeast cream (containing 25 grams/litre of yeast). The cream was diluted with fresh water at the rate of 1600 litres/hour and mixed vigorously with 0.5 gram/litre of a non-ionic surface active agent (obtained by condensing ethylene oxide with a mixture of lauric alcohol and myristic alcohol to give an ethylene oxide chain of an average of 8.5 units per molecule); the surface active agent is marketed under the trade designation NI 29. The mixture was centrifuged in a Sharples DG 2 autojector type centrifuge to recover the following three phases: Yeast cream, Residual gas oil, Aqueous phase.

The yeast cream so obtained was mixed with fresh water to which had been added 10% of its volume of the culture medium as used in the fermenter and the mixture was continuously fed to a vessel in which the residence time was 3 hours while maintaining gentle aeration at the rate of 10 vol./vol./hour of air. The product of this stage was centrifuged in a simple nozzle-separator Alfa-Laval QX centrifuge. The yeast paste so obtained was drum dried at a temperature of 140° C. and the yeast product was solvent extracted with normal hexane at a rate of 2 litres of normal hexane per kilogram of yeast (measured as dry yeast) present in the yeast products; the solvent extraction was repeated 5 times to give a substantially hydrocarbon-free yeast product.

What we claim is:

1. A process which comprises cultivating a hydrocarbon-consuming micro-organism in the presence of a hydrocarbon feedstock comprising of straight chain hydrocarbons, an aqueous nutrient medium and a gas containing free oxygen thereafter, in a purification stage, maintaining said micro-organism with an aqueous nutrient medium and a gas containing free oxygen whereby the hydrocarbon contaminating the micro-organism is reduced in quantity, thereafter subjecting a product of said stage, said product containing the micro-organism, to a separation stage, thereafter recovering a fraction containing the micro-organism thereafter subjecting the fraction to drying and thereafter subjecting the dried fraction to solvent extraction.

2. A process according to claim **1** in which the micro-organism is a paraffinic hydrocarbon consuming micro-organism.

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

4. A process according ot claim **3** in which the yeast is of the family Cryptococcaceae.

5. A process according to claim **4** in which the yeast is of the sub-family Crytococcoideae.

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

7. A process according to claim **5** in which the yeast is of the genus Candida.

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

9. A process according to claim **7** in which the yeast is *Candida tropicalis.*

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

11. A process according to claim **1** in which the separation stage consists wholly or in part of decantation.

12. A process according to claim **1** in which a paste of a micro-organism is subjected to solvent extraction.

13. A process according to claim **1** in which a dry micro-organism is subjected to solvent extraction.

14. A process according to claim **1** in which the solvent extraction is effected by means of a solvent comprising a hydrocarbon.

15. A process according to claim **14** in which the solvent comprising a hydrocarbon is a mixture of a hydrocarbon and a polar solvent.

16. A process according to claim **15** in which the polar solvent is an alcohol.

17. A process according to claim **16** in which the polar solvent is ethanol.

18. A process according to claim **16** in which the polar solvent is isopropanol.

19. A process according to claim **14** in which the hydrocarbon is normal hexane.

20. A process according to claim **14** in which the hydrocarbon is benzene.

21. A process according to claim **14** in which the hydrocarbon is a light platformate fraction.

22. A process according to claim **15** in which the mixed hydrocarbon and polar solvent is an azeotropic mixture.

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LIONEL M. SHAPIRO, Primary Examiner

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U.S. Cl. X.R.

195—3, 82, 96

'96 PO-1050
1945 (5/69)

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,522,147 Dated JULY 28, 1970

Inventor(s) Jean Antoine Filosa

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 3, between lines 55 and 56, an entry should be made in each of the columns, as follows:
-- Ammonium sulphate 1 --;

Column 5, line 61, cancel "purification stage as desired" and insert -- extraction) may be satisfactorily performed --; and

Column 7, line 17, cancel "its" (first occurrence) and insert -- the --.

SIGNED AND
SEALED
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FEB. 9,1971

(SEAL)
Attest:

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