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NO DRAWINGS

1095.183

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Date of filing Complete Specification: Oct. 15, 1964.

Application Date: Nov. 14, 1963.

No. 45009/63.

Complete Specification Published: Dec. 13, 1967.

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1095.183

Index at acceptance:—C5 E12C; C6 F (1, 2)

Int. Cl.:—C 10 g 31/12

COMPLETE SPECIFICATION

Process for the removal, wholly or in part, of Straight Chain Hydrocarbons from Hydrocarbon Mixtures

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:—

This invention relates to a process for the removal, to a controlled extent, of straight chain hydrocarbons 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 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 naphthas 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.

According to the present invention there is provided a process which comprises cultivating a straight chain hydrocarbon-consuming micro-organism in the presence of a hydrocarbon feedstock containing straight chain hydrocarbons and other hydrocarbons, in the presence of an aqueous nutrient medium, and in the presence of a gas containing free oxygen,

[Price 4s. 6d.]

wherein the amount of an essential component of the nutrient medium which is provided and/or the amount of oxygen which is provided is such that only a desired amount of straight chain hydrocarbon is consumed.

Preferably the hydrocarbon feedstock contains C_{10} or higher hydrocarbons.

In batchwise operation the overall amount of the essential component and/or the overall amount of oxygen will be controlled to give the desired consumption of straight chain hydrocarbons.

In continuous operation the rate of feed of the essential component and/or oxygen will be subject to this control.

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.

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, ~~ascoporeogeneous~~ 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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		Preferred Strain
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 Reference 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.

5 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:—

15 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:—

25 *Bacillus amylobacter*
Pseudomonas natriegens
Arthrobacter sp.
Micrococcus sp.
Corynebacterium sp.
Pseudomonas syringae
Xanthomonas begoniae
Flavobacterium devorans
30 *Acetobacter* sp.
Actinomyces sp.
Nocardia opaca

35 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 of the order of 25 parts per million. The quantity of each nutritive 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



Bacillaceae



- 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 times 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
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	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.)	

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 gram
tap water. (trace elements)	100 mls
Yeast extract	0.025 gram
made up to 1000 mls with distilled water	

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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
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	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)	

However, as hereinbefore described, either oxygen or at least one essential component is controlled, in quantity or rate of feed, below the optimum level in order to achieve a limited growth of the micro-organism and hence to obtain only limited removal of straight-chain hydrocarbons.

Essential components which are suitable for control in accordance with the invention are phosphate ion, magnesium ion, potassium ion and sulphate ion.

Preferably the amount of phosphate ion present in the medium is in the range 0.53 to 0.19 gram/litre. Preferably the amount of potassium ion present in the medium is in the range 0.07 and 0.15 gram/litre.

If desired, the oxygen may be supplied to the fermenter in the form of air. Preferably the rate of aeration is in the range 5 to 60 vols/vol/hour.

Employing the ranges mentioned above, gas-oils may be dewaxed to give products with pour points varying over a wide range.

Preferably control is exercised by limitation of an ion other than ammonium ion since, in general, it is preferred to employ the introduction of ammonia as a means of controlling pH.

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 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



the By this 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.

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 must 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.

By the process of the invention the pour point of product gas oils may be maintained at a predetermined value (within the limits of operability of the process).

Suitably the recovered hydrocarbons, for example gas oils, are further treated by hydrogenation, as described in British Patent Specification No. 1059884, or by caustic wash,

as described in British Patent Specification No. 1059885, to improve the cloud point 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.

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 Application No. 45004/63 (Serial No. 1095182).

The invention is illustrated but not limited by the following examples.

In Examples 1—3 a yeast, *Candida lipolytica*, was cultivated on an Iraq gas-oil feedstock (cloud point +19° C; pour point +17° C; containing 14% n-paraffins) in the presence of an aqueous nutrient medium having 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
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	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.

and in the presence of air. The yeast was cultivated in a continuous 5 litre fermenter.

In Example 1 the phosphorus in the medium was varied, in Experiment 2 the potassium concentration and in Example 3 the amount of oxygen supplied to the fermenter was varied. The purpose of varying the amounts of these materials was so that the extent of the removal of n-paraffins from the gas-oil could be controlled and in this way products with pour points varying over a wide range could be obtained.

EXAMPLE 1

The amount of phosphorus, in the form of di-ammonium hydrogen phosphate, in the medium was varied in a series of fermentations performed at pH 6, at a dilution rate of 0.1 and at a temperature of 30° C. (The dilution rate is the ratio of the volume of feedstock fed to the fermenter/hour to the volume of liquid phase). The quantity of gas-oil present in the medium was 100 grams/litre. The following results for the pour points of the products from each fermentation were obtained:

Di-ammonium Hydrogen Phosphate Concentration in medium (grams/litre)	Cellular Density (grams/litre)	Pour Point (originally +17° C.)
1	10	-2
0.7	10	-2
0.5	10	+7
0.25	5	+15

5 It can be seen that good control of the pour point of the product could be obtained by arranging the concentration of the ammonium hydrogen phosphate in the medium to be between 0.7 and 0.25 grams/litre. There was only a small drop in cellular density when the phosphate level was reduced over this range.

EXAMPLE 2

10 The amount of potassium, in the form of potassium chloride, in the medium was varied in a series of fermentations performed at pH 6, at a dilution rate of 0.1 and at a temperature of 30° C. The quantity of gas-oil present in the medium was 100 grams/litre. The following results for the pour points of the resulting gas-oils obtained using the various concentrations of potassium is shown below:

Potassium Chloride Concentration in medium (grams/litre)	Cellular Density (grams/litre)	Pour Point (originally +16° C.)
1.15	9	-20° C.
0.30	9.2	-18° C.
0.15	7	-6° C.

25 It can be seen that varying the potassium chloride concentration between 0.15 and 0.30 gram/litre enabled the pour point of the product to be controlled without seriously affecting the cellular density.

EXAMPLE 3

30 In this Example the degree of aeration was varied in a series of fermentations performed at pH 4 and at a temperature of 30° C with a dilution rate of 0.2. The quantity of gas-oil present in the medium was 120 grams/litre.

Aeration vols/vol/hours	Cellular Density (grams/litre)	Pour Points (originally +17° C.)
5	3.6	+11° C.
10	5.3	+10° C.
20	6.3	+9° C.
30	6.3	+7° C.
65	6.7	+7° C.
110	7.6	+6° C.

It can be seen that varying the aeration rate between 5 and 65 vols/vol/hour enabled gas-oil products with pour points varying between +7 and +11° C to be obtained. Gas-

oils with a wider range of pour points could be obtained if the dilution rate was reduced to 0.1, while keeping the other factors the same.

Aeration vols/vol/hour	Cellular Density (grams/litre)	Pour Points (originally +17° C.)
10	5.2	+ 8
20	6.3	- 6
60	7.6	-10
110	7.8	-10

- 10 Here it can be seen that varying the aeration rate between 10 and 60 vols/vol/hour gives gas-oil products with a pour point range of 18° C without seriously affecting the cellular density.

15 **EXAMPLE 4**

In a modification of the method used in Example 1 (in which the yeast, *Candida lipolytica*, was cultivated on a medium containing

varying amounts of phosphorus in the form of di-ammonium hydrogen phosphate) further experiments were performed using two other yeasts, *Candida pulcherrima* and *Hansenula anomala*, and also a bacterium, *Bacillus megaterium*. In each case two runs were performed: one with a medium containing phosphorus and the other with a medium containing no phosphorus. The media used were:

For the yeasts	
(NH ₄) ₂ HPO ₄	2 grams
KCl	1.15 grams
MgSO ₄ ·7H ₂ O	0.65 gram
MnSO ₄ ·H ₂ O	0.045 gram
ZnSO ₄	0.17 gram
Yeast extract	0.015 gram
Water	1 litre

For the medium containing no phosphorus the ammonium phosphate was replaced by 1.72 grams per litre of ammonium chloride.



For the bacterium:

Na_2HPO_4	0.4 gram
KH_2PO_4	0.5 gram
NH_4Cl	0.5 gram
NaCl	4 grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 gram
Yeast Extract	0.015 gram
Water	1 litre

5 For the medium containing no phosphorus the phosphates were replaced by 1 gram per litre of potassium hydrogen sulphate. Otherwise all the fermentation conditions were the same as were used in Example 1.

The gas-oil used in the experiments with media containing no phosphorus had a pour point of $+11^\circ \text{C}$ and that used in the control experiments had a pour point of $+17^\circ \text{C}$.
The following results were obtained:

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Phosphorus in medium	Micro-organism	Initial Cell population (millions per ml)	Final Cell population (millions per ml)	Final cellular density (grams per litre)	Initial pour point (°C.)	Final pour point (°C.)
With phosphorus	C. pulcherrima	—	—	10	+17	-22
Without phosphorus	C. pulcherrima	0.7	3.7	—	+11	+11
With phosphorus	H. anomala	—	—	16	+17	-19
Without phosphorus	H. anomala	1.3	2	—	+11	+11
With phosphorus	B. megaterium	—	—	20	+17	-34
Without phosphorus	B. megaterium	0.9	8	—	+11	+11

It can be seen from these results that the quantity of phosphorus present can be used in a similar way to that described in Example 1 to control the degree of dewaxing and thus the pour point of the gas-oil produced.

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 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.

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,

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:—

1. A process which comprises cultivating a straight chain hydrocarbon-consuming micro-organism in the presence of a hydrocarbon feedstock containing straight-chain hydrocarbons and other hydrocarbons, in the presence of an aqueous nutrient medium, and in the presence of a gas containing free oxygen wherein the amount of an essential component of the nutrient medium which is provided and/or the amount of oxygen which is provided is such that only a desired amount of straight-chain hydrocarbon is consumed.

2. A process according to claim 1 in which oxygen and/or at least one essential component, is controlled in quantity or rate of feed below the optimum level in order to achieve

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Phosphorus in medium	Micro-organism	Initial Cell population (millions per ml)	Final Cell population (millions per ml)	Final cellular density (grams per litre)	Initial pour point (°C.)	Final pour point (°C.)
With phosphorus	C. pulcherrima	—	—	10	+17	-22
Without phosphorus	C. pulcherrima	0.7	3.7	—	+11	+11
With phosphorus	H. anomala	—	—	16	+17	-19
Without phosphorus	H. anomala	1.3	2	—	+11	+11
With phosphorus	B. megaterium	—	—	20	+17	-34
Without phosphorus	B. megaterium	0.9	8	—	+11	+11

It can be seen from these results that the quantity of phosphorus present can be used in a similar way to that described in Example 1 to control the degree of dewaxing and thus the pour point of the gas-oil produced.

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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,

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:—

1. A process which comprises cultivating a straight chain hydrocarbon-consuming micro-organism in the presence of a hydrocarbon feedstock containing straight-chain hydrocarbons and other hydrocarbons, in the presence of an aqueous nutrient medium, and in the presence of a gas containing free oxygen wherein the amount of an essential component of the nutrient medium which is provided and/or the amount of oxygen which is provided is such that only a desired amount of straight-chain hydrocarbon is consumed.

2. A process according to claim 1 in which oxygen and/or at least one essential component, is controlled in quantity or rate of feed below the optimum level in order to achieve





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Cryptococcaceae

- limited growth of the micro-organism and hence to obtain only limited removal of the straight-chain hydrocarbons.
3. A process according to either of the preceding claims wherein the essential component is phosphate ion, potassium ion, magnesium ion, or sulphate ion.
4. A process according to any of the preceding claims wherein the quantity of phosphate ion present is between 0.53 and 0.19 gram/litre.
5. A process according to claims 1—3 wherein the quantity of potassium ion present is between 0.07 and 0.15 gram/litre.
6. A process according to claims 1 and 2 wherein the oxygen fed to the fermenter is in the form of air.
7. A process according to claim 6 in which the aeration rate is between 5 and 65 vols/vol/hour.
8. A process according to any one of the preceding claims in which the micro-organism which is cultivated is a yeast.
9. A process according to claim 8 in which the yeast is of the family *Cryptococcaceae*.
10. A process according to claim 9 in which the yeast is of the sub-family *Cryptococcoidae*.
11. A process according to claim 10 in which the yeast is of the genus *Torulopsis*.
12. A process according to claim 10 in which the yeast is of the genus *Candida*.
13. A process according to claim 12 in which the yeast is *Candida lipolytica*.
14. A process according to any one of claims 1—7 in which the micro-organism is a bacterium.
15. A process according to any of the preceding claims which comprises cultivating a micro-organism 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, 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.
16. A process according to claim 15 wherein the feedstock is a wax containing petroleum gas-oil and wherein there is recovered from the product of the growth of the micro-organism a gas-oil of reduced content of wax.
17. A process substantially as described with reference to any of the foregoing Examples.
18. A micro-organism whenever produced by a process as claimed in any of the preceding claims.
19. A gas-oil whenever produced by a process as claimed in any of the claims 1—17.

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

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.
—1967. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2.
from which copies may be obtained.

ERRATUM

SPECIFICATION No. 1,095,183

Page 1, Heading, *Inventors:* for "BERNARD MAURICE LAING" read "BERNARD MAURICE LAINE"

THE PATENT OFFICE
29th January 1968