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PATENT SPECIFICATION
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1.049.929

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Date of Application and filing Complete Specification: Feb. 4, 1964.
No. 4654/64.

Application made in France (No. 924254) on Feb. 8, 1963.

Complete Specification Published: Nov. 30, 1966.

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The Inventor of this invention in the sense of being the deviser thereof within the meaning of Section 16 of the Patents Act, 1949 is Alfred Champagnat, a French citizen of Societe Francaise des Petroles BP, 10 Quai Paul Doumer, Boite Postale 104, Courbevoie, Seine, France.

Index at acceptance:—C6 F(1, 2); A2 BJ3G1; C5 E12C

Int. Cl.:—C 12 d 13/06//A 23 k, C 10 g

COMPLETE SPECIFICATION

Improvements in or relating to the Treatment of Micro-Organisms and/or to the Treatment of Products Derived from said Micro-organisms

We, THE BRITISH PETROLEUM COMPANY LIMITED, of Britannic House, Finsbury Circus, London, E.C.2., a British joint-stock Corporation, 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 production of nitrogen-containing material. 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 micro-organisms will grow on certain hydrocarbon feedstocks and there have been described methods for the cultivation and harvesting of certain micro-organisms using hydrocarbon feedstocks. Thus British Patent Specification 914567 describes 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, at least 50 parts per million, and preferably 0.02—0.5%, by weight of a surface active agent and thereafter centrifuging, whereby there is obtained a paste comprising yeast and aqueous mineral medium; according to a particular method described in the specification paste recovered by centrifuging is repeatedly washed with water and centrifuged or, said paste is repeatedly washed with water containing a surface active agent and centrifuged and thereafter washed with water to eliminate surface active agent.

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The process now described provides for the cultivation of a micro-organism and its conversion to a material which is more digestible than is the micro-organism itself.

According to one aspect of this invention there is provided a process which comprises cultivating a micro-organism capable of growing on a feedstock consisting of or containing straight chain hydrocarbons, said micro-organism being cultivated in the presence of said feedstock, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen and thereafter subjecting a fraction containing said micro-organism to conditions such that cell rupture takes place and thereafter recovering a fraction consisting of or containing one or more nitrogen-containing compounds selected from proteins, polypeptides and amino-acids, (a) which have been produced by said micro-organism in the course of its metabolism and which have been released from the micro-organism or (b) which have been derived from nitrogen-containing compounds of said micro-organism in the course of cell rupture.

Preferably the fraction containing the micro-organism is subjected to autolysis, plasmolysis or hydrolysis.

Autolysis may be brought about by means of the enzymes contained in the micro-organism or by the addition of other enzymes.

Autolysis, which is the favoured method for breaking down the cells, leads to a product in the form of a liquid the viscosity of which depends on the concentration of dry matter. The autolysate which contains proteins of a much

5 lower molecular weight than the dry yeast is much more digestible. Since it has been obtained without sever heating, the unstable amino acids and the vitamins may be completely or substantially preserved.

Furthermore, the liquid form of the autolysate permits of an easy extraction of the lipids which it contains, using an appropriate solvent.

10 Thus the autolysate may be treated with a suitable solvent to extract from it the lipids and the traces of hydrocarbons which it may still contain. It is then preferably concentrated in vacuo at a temperature lower than 70°C. 15 so as to preserve from destruction the desired constituents, for example, amino-acids and vitamins.

According to another aspect of this invention there is provided a process which comprises cultivating a micro-organism in the presence of a feedstock consisting of or containing straight chain hydrocarbons, separating the product into a fraction containing the micro-organism, a fraction containing aqueous nutrient medium and, if hydrocarbons are present in the product in substantial amount, a fraction containing said hydrocarbons; thereafter subjecting a fraction containing said micro-organism to a series of alternate washing stages and separation stages, in the first washing stage there being used an aqueous solution of a surface active agent and in the last washing stage there being used an aqueous medium which is free of surface active agents, the separation stages being operated to separate the micro-organism from an aqueous phase, the separated micro-organism then passing on for further treatment, the washing and separation stages being carried out in such manner that the micro-organism recovered from said series of stages has a hydrocarbon content less than 7% by wt. based on the weight of the dry micro-organism; thereafter subjecting the washed micro-organism to conditions such that cell rupture takes place.

45 Preferably proteins or polypeptides or polypeptides or amino-acids contained in said micro-organism or derived therefrom are separated from cell wall material consisting of cell walls and/or material which constituted the cell walls.

According to another aspect of this invention there is provided a process which comprises cultivating a micro-organism in the presence of a feedstock consisting of or containing straight chain hydrocarbons; preferably thereafter, if hydrocarbons are still present, separating a fraction containing the micro-organism from said hydrocarbons; and thereafter subjecting a fraction containing said micro-organism to conditions such that proteins or polypeptides or polypeptides or amino-acids contained in said micro-organism or derived therefrom are separated from cell wall material consisting of cell walls and/or

material which constituted the cell walls, and thereafter treating said cell wall material which is contaminated with hydrocarbons, with or without a preliminary modifying treatment, by solvent extraction to recover at least part of the hydrocarbons from the cell wall material.

Usually the cell wall material, obtained after separation of proteins, polypeptides and/or amino-acids, contains glucides, lipids and some further proteins as well as the hydrocarbons which are to be removed. In the solvent extraction stage the lipids will usually be removed from the cell wall material. Thus with the cell wall material there will usually be left the glucosides and some proteins.

Preferably solvent extraction of the cell wall material is carried out by the use of a solvent consisting of or containing a hydrocarbon. Preferably the hydrocarbon has 4—7 carbon atoms per molecule. Preferably the hydrocarbon is a paraffin and preferably, the paraffin is a straight chain paraffin. Normal pentane and normal hexane are suitable solvents.

If desired the extraction of the cell wall material by means of a hydrocarbon solvent is preceded by extraction by means of an alcohol, preferably ethanol.

If desired they may be used as extraction stage using, as solvent, a mixture of a hydrocarbon, for example, as hereinbefore described together with an alcohol, for example ethanol. Thus there may be used a solvent consisting of 80% by wt. of hexane and 20% by wt. of ethanol.

The hydrocarbons recovered in the extract phase by solvent extraction, if metabolisable, may be recycled to the micro-organism cultivation stage.

The solid phase obtained by solvent extraction consisting of the purified cell wall material may contain of the order of 4% by wt. of fixed nitrogen and is a valuable cattle foodstuff.

The preferred manner of cultivation and subsequent treatment of the micro-organism will now be described in further detail.

Preferably the feedstock is a straight chain hydrocarbon or a mixture of hydrocarbons containing a straight chain hydrocarbon. Preferably the hydrocarbon is 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.

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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.

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 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 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, ascosporeogeneous yeasts of the sub-family Saccharomyceloidae. Preferred genera of the Cryptococcoidae 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 voor Schimmelcultuur, Baarn, Holland:—

100	<i>Candida lipolytica</i>	
	<i>Candida pulcherrima</i>	CBS 610
	<i>Candida utilis</i>	
	<i>Candida utilis</i> , Variati major	CBS 841
	<i>Candida tropicalis</i>	CBS 2317
105	<i>Torulopsis collisculosa</i>	CBS 133
	<i>Hansenula anomala</i>	CBS 110
	<i>Oidium lactis</i>	
	<i>Neurospora sitophila</i>	

Of the above *Candida lipolytica* is particularly preferred.

110 If desired, the micro-organism may be a bacterium. Suitably the bacteria are of one of the orders:—

Pseudomonales, *Eubacteriales* and *Actinomycetales*.

Preferably the bacteria which are employed are of the family *Bacillaceae* and *Pseudomonadaceae*. Preferred species are *Bacillus*

megatherium, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Other strains which may be employed include:—

- 5 *Bacillus amylolactes*
Pseudomonas natriegens
Arthrobacter sp.
Micrococcus sp.
Corynebacterium michiganense
10 *Pseudomonas syringae*
Xanthomonas begoniae
Flavobacterium sp. *devorans*
Acetobacter sp.
Actinomyces sp.
Agrobacterium sp.
15 *Aplanobacter* sp.

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

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

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

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

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

- 30 *Penicillium roqueforti*, *Penicillium notatum*, *Aspergillus fustigatus*, *Aspergillus niger* and *Aspergillus versicolor* may be used for cultivation on a solid agent containing hydrocarbons as feedstock.

- 35 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.

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

- Ammonium sulphate 1 gram
Magnesium sulphate 0.20 grams
Ferrous sulphate, 7H₂O 0.005 grams
Manganese sulphate, 1H₂O 0.002 grams
45 Monopotassium phosphate 2 grams
Disodium phosphate 3 grams
Calcium chloride 0.1 grams
Sodium carbonate 0.1 grams
Yeast extract 0.008 grams
50 Distilled water (to make up to 1000 mls).

For other bacteria a suitable nutrient medium has the composition:

- 55 Monopotassium phosphate 7 grams
Magnesium sulphate, 7H₂O 0.2 grams
Sodium chloride 0.1 grams
Ammonium chloride 2.5 grams
Tap water (trace elements) 100 mls
Yeast extract 0.025 grams
60 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 65
Magnesium sulphate, 7H₂O 0.65 grams
Zinc sulphate 0.17 grams
Manganese sulphate, 1H₂O 0.045 grams
Ferrous sulphate, 7H₂O 0.068 grams
Tap water 200 grams 70
Yeast extract 0.025 grams
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*

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lipolytica the preferred temperature range is 28—32°C.

5 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".

10 It has been found that by the use of yeast of the strain *Candida lipolytica* in a process according to the invention 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.

15 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').

20 A supply of the micro-organism for starting the next batch will preferably be removed before the termination of the exponential phase.

25 The growth operation will usually be discontinued before the stationary phase.

30 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.

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

40 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.

45 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) an aqueous 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.

80 Preferably the micro-organism fraction is vigorously mixed with the aqueous surface active agent and, without a further period of growth of the micro-organism, subjected to further separation, preferably by centrifuging, to recover a micro-organism fraction and a spent aqueous phase containing hydrocarbon impurities removed from the micro-organism. If necessary, the washing and separation steps may be repeated, once or more, using an aqueous surface active agent in the washing stage. After the washing with surface active agent it is necessary to wash with an aqueous medium which is free of surface active agent; preferably this medium will be water. Again if desired, a series of washing and separation stages may be employed. Overall the washing stages are carried out until the hydrocarbon content of the micro-organism is less than 7% based on the weight of the micro-organism (as calculated for the dry state). Preferably said content of hydrocarbons will be less than 5%; usually the hydrocarbons will be substantially eliminated; however, for some applications this would not be necessary and in some cases therefore it would not be necessary to reduce said content to a value below 0.1%.

As the surface active agent employed for washing there may be used cationic surface active agents such as stearyl-trimethyl ammonium chloride, non-ionic surface active agents, for example the condensates of oleic acid and ethylene oxide, or anionic surface active agents, for example sodium alkyl sulphates.

The micro-organism, after washing as hereinbefore described, is preferably recovered as a cream; preferably the cream contains 10—20% by wt., e.g. 15% by wt. of dry matter.

The micro-organism, preferably in the form of a cream, is subjected to treatment for the separation of proteins or polypeptides or amino acids contained in the micro-organism from cell wall material.

The initial stage of the separation of the protein or polypeptide or polypeptides or amino-acids may be carried out by autolysis, plasmolysis or hydrolysis.

Autolysis may be effected by means of enzymes contained in the micro-organism or by

the use of added enzymes. Usually pH will be maintained at a value determined by the nature of the enzymes used.

5 The autolysate is usually obtained in the form of a liquid the viscosity of which depends upon the concentration of dry substances which it contains. As it is obtained without violent heating the amino-acids which are not very stable and the vitamins are preserved, usually completely.

10 Preferably when an autolysate is prepared for cattle feeding stuff a yeast is autolysed for three hours. Preferably this autolysate will contain 40—50% by wt. of dry matter.

15 Preferably when an autolysate is prepared for human consumption a yeast is autolysed for 10 to 20 hours. Preferably this autolysate will contain 55—65% by wt. preferably 60% by wt. of dry matter.

20 Preferably autolysis is carried out at a temperature in the range 45—55°C., preferably at 50°C. Preferably before autolysis the cream is treated with a water soluble salt, for example sodium chloride. Preferably the salt is added in an amount less than 2% by weight of the extract (Dry material).

According to another aspect of this invention there are manufactured instead of autolysates, hydrolysates obtained by the action of acids or alkalis on the cells of micro-organisms or plasmolysates by the diffusion of the proteins out of the cells as a result of the action of a change in osmotic pressure. This change is achieved, for example, by the addition of sodium chloride to the aqueous medium. The operations of autolysis, hydrolysis and plasmolysis may be carried out in the same type of apparatus. Preferably this apparatus is stirred.

40 It has been found that by reducing the hydrocarbon content of the micro-organism by washing as hereinbefore described, the rate of operation of the treatment for the rupture or extraction of the cell walls, for example, by autolysis, is increased; in consequence the residence time of the micro-organism in this stage will out-weight the costs of the washing stages.

50 The product of this stage for the rupture of the cell walls is now treated for the separation of the nitrogenous materials, for example proteins and/or polypeptides and/or amino-acids from cell wall material.

55 This separation may be carried out by centrifuging and/or filtration. The nitrogenous fraction so obtained will contain as impurities hydrocarbons and usually, also lipids. This fraction will preferably be subjected to solvent extraction. However if desired solvent extraction may be carried out prior to or without separation of the cell wall material.

60 Preferably solvent extraction is carried out by the use of a solvent consisting of or containing a hydrocarbon. Preferably the hydrocarbon has 4—7 carbon atoms per molecule.

Preferably the hydrocarbon is a paraffin and preferably, the paraffin is a straight chain paraffin. Normal pentane and normal hexane are suitable solvents.

If desired the extraction of the hydrocarbons by means of a hydrocarbon solvent is preceded by extraction by means of an alcohol, preferably ethanol.

If desired there may be used an extraction stage using, as solvent, a mixture of a hydrocarbon, for example, as hereinbefore described together with an alcohol, for example ethanol. Thus there may be used a solvent consisting of 80% by wt. of hexane and 20% by wt. of ethanol.

The solution of hydrocarbons and lipids in solvent may be distilled to recover, separately, hydrocarbons and lipids.

The hydrocarbons recovered, if metabolisable, may be recycled to the micro-organism cultivation stage.

The lipids can further be treated for the recovery of sterols.

The autolysate recovered as raffinate may be dried as hereinbefore described, preferably at a temperature below 70°C. In the drying stage, traces of solvent will be eliminated from the autolysate.

The hydrocarbons recovered from the extract phase, if metabolisable, may be recycled to the micro-organism cultivation stage.

The fraction comprising the cell wall material, which will contain impurities, is then treated for the removal of these impurities as hereinbefore described.

EXAMPLE

40 litres of aqueous mineral nutrient medium, having the composition given hereinafter, was introduced into a stainless steel fermenter having an effective capacity of 60 litres.

The composition of the aqueous nutrient medium was as follows:—

Diammonium phosphate	2	grams	
Potassium chloride	1.15	grams	110
Magnesium sulphate, 7H ₂ O	0.65	grams	
Zinc sulphate	0.17	grams	
Manganese sulphate, 1H ₂ O	0.045	grams	
Ferrous sulphate, 7H ₂ O	0.068	grams	
Yeast extract	0.025	grams	115
Tap water	200	grams	
Distilled water to	1000	ml.	

20 litres of a 24 hour inoculum of *Candida lipolytica* on mixed C₉—C₁₆ normal hydrocarbons were then added, such that the cellular density was about 1gm of dry matter per litre.

15 grams/litre of heavy petroleum gas oil was introduced into the fermenter, that is 1.03 litres, which is enough to carry the cellular density to 2 grams/litre.

The temperature of the culture was held at 30°C±1°C, pH 4, the aeration and agitation were such that the rate of aeration was 3

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millimoles of O₂ per litre of medium per minute.

10N aqueous ammonia was introduced through an automatic pH controlling system. When the flow of ammonia reached 20 ml the addition of the gas oil was started, at a rate determined by the theoretical needs of the culture and assuming a yield of

$$\left\{ 100 \times \frac{\text{yeast produced}}{\text{gas oil feedstock}} \right\} \text{ of } 10\% \text{ and a}$$

cellular division time of 3 hours. This addition was made every hour until the total amount of gas oil added reached 250 grams/litre, i.e. 17 litres.

This addition was continued up to and including the fifteenth hour.

Starting with a cellular density of 1 gram/litre there was obtained a cellular density of 8 grams/litre at 15 hours.

A culture product so obtained was centrifuged and a yeast paste was recovered.

8.4 kilograms of this paste contained 2 kilograms of dry material (excluding retained hydrocarbons), 1 kilogram of non-metabolisable hydrocarbons and 1 kilogram of proteins; to this paste was added 14.5 litres of water containing 180 grams of sodium chloride.

The mixture was maintained at 50°C for 10 hours under continuous slow agitation without controlling the pH. The product so obtained contained about 600 grams of protein and 1200 grams of solubilised dry material.

This product was centrifuged in two stages in a "Sharples" centrifuge at 11000 G to recover an oil phase containing cell wall material; a limpid liquid autolysate (containing 500 grams of protein) and a residue containing cell wall material, slightly impregnated with hydrocarbons. "Sharples" is a Registered Trade Mark.

The cell wall material recovered as residue was subjected to partial drying. After drying to a product containing 20% by wt. of water, this product was de-oiled by extraction at 55°C by means of an azeotropic mixture of normal hexane and isopropanol at a ratio of 5 litres of solvent per kilogram of dry material in the product treated. The de-oiled material can be used as an animal foodstuff.

In British Patent Application 25210/63, to The British Petroleum Company Limited, there is claimed a process which comprises cultivating a micro-organism capable of growing on a feedstock consisting wholly or in part of straight chain hydrocarbons with formation of lipids, said micro-organism being cultivated in the presence of said feedstock, separating from the product a fraction comprising the micro-organism and extracting said fraction by means of a solvent to remove lipids in the extract phase.

In British Patent Application 18179/66, to The British Petroleum Company Limited,

there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism, which during cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium, subjecting said fraction to drying, recovering a dry impure micro-organism and subjecting the dry impure micro-organism to solvent extraction for the removal, at least in part from the micro-organism of lipids together with hydrocarbons associated with said micro-organism.

In British Patent Application 18180/66, to The British Petroleum Company Limited, there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism which during cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium and residual hydrocarbon, mixing said fraction with a surface active agent and subjecting the mixture with or without washing to a phase separation to recover (a) a treated fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said treated fractions being subjected to solvent extraction for the removal, at least in part, from the micro-organism, of lipids together with hydrocarbons associated with said micro-organism.

WHAT WE CLAIM IS:—

1. A process which comprises cultivating a micro-organism capable of growing on a feedstock consisting of or containing straight chain hydrocarbons, said micro-organism being cultivated in the presence of said feedstock, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen and thereafter subjecting a fraction containing said micro-organism to conditions such that cell rupture takes place and thereafter recovering a fraction consisting of or containing one or more nitrogen-containing compounds selected from proteins, polypeptides and amino-acids, (a) which have been produced by said micro-organism in the course of its metabolism and which have been released from the micro-organism or (b) which have been derived from nitrogen-containing compounds of said micro-organism in the course of cell rupture.

2. A process which comprises cultivating a micro-organism capable of growing on a feedstock consisting of or containing straight chain hydrocarbons, said micro-organism being cul-



1 and chain exane		millimoles of O ₂ per litre of medium per minute.	there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism, which during cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium, subjecting said fraction to drying, recovering a dry impure micro-organism and subjecting the dry impure micro-organism to solvent extraction for the removal, at least in part from the micro-organism of lipids together with hydrocarbons associated with said micro-organism.	65
hydro- ent is alco-	5	10N aqueous ammonia was introduced through an automatic pH controlling system. When the flow of ammonia reached 20 ml the addition of the gas oil was started, at a rate determined by the theoretical needs of the culture and assuming a yield of		70
action hydro- tribed anol. isting wt. of	75	$\left\{ 100 \times \frac{\text{yeast produced}}{\text{gas oil feedstock}} \right\}$ of 10% and a cellular division time of 3 hours. This addition was made every hour until the total amount of gas oil added reached 250 grams/litre, i.e. 17 litres.		75
ds in ately,	80	This addition was continued up to and including the fifteenth hour.		
bolis- inism	85	Starting with a cellular density of 1 gram/litre there was obtained a cellular density of 8 grams/litre at 15 hours.	In British Patent Application 18180/66, to The British Petroleum Company Limited, there is claimed a process which comprises cultivating a straight chain paraffinic hydrocarbon-consuming micro-organism which during cultivation produces lipids, in the presence of a feedstock consisting at least in part of a straight chain hydrocarbon and an aqueous nutrient medium, recovering a fraction comprising the micro-organism and at least some aqueous medium and residual hydrocarbon, mixing said fraction with a surface active agent and subjecting the mixture with or without washing to a phase separation to recover (a) a treated fraction comprising the micro-organism together with an aqueous phase and some residual hydrocarbon fraction and (b) a hydrocarbon fraction of reduced content of straight chain paraffins or which is free of normal paraffins; the said treated fractions being subjected to solvent extraction for the removal, at least in part, from the micro-organism, of lipids together with hydrocarbons associated with said micro-organism.	80
r the	20	A culture product so obtained was centrifuged and a yeast paste was recovered.		85
may rably rying from	90	8.4 kilograms of this paste contained 2 kilograms of dry material (excluding retained hydrocarbons), 1 kilogram of non-metabolisable hydrocarbons and 1 kilogram of proteins; to this paste was added 14.5 litres of water containing 180 grams of sodium chloride.		90
ex- cycled	95	The mixture was maintained at 50°C for 10 hours under continuous slow agitation without controlling the pH. The product so obtained contained about 600 grams of protein and 1200 grams of solubilised dry material.		95
wall then es as	100	This product was centrifuged in two stages in a "Sharples" centrifuge at 11000 G to recover an oil phase containing cell wall material; a limpid liquid autolysate (containing 500 grams of protein) and a residue containing cell wall material, slightly impregnated with hydrocarbons. "Sharples" is a Registered Trade Mark.		100
rient ere- steel	105	The cell wall material recovered as residue was subjected to partial drying. After drying to a product containing 20% by wt. of water, this product was de-oiled by extraction at 55°C by means of an azeotropic mixture of normal hexane and isopropanol at a ratio of 5 litres of solvent per kilogram of dry material in the product treated. The de-oiled material can be used as an animal foodstuff.		105
110	45	In British Patent Application 25210/63, to The British Petroleum Company Limited, there is claimed a process which comprises cultivating a micro-organism capable of growing on a feedstock consisting wholly or in part of straight chain hydrocarbons with formation of lipids, said micro-organism being cultivated in the presence of said feedstock, separating from the product a fraction comprising the micro-organism and extracting said fraction by means of a solvent to remove lipids in the extract phase.		110
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120	55			120
oil 1.03 mial	125			125
d at tion s 3	60			

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- tivated in the presence of said feedstock, in the presence of an aqueous nutrient medium and in the presence of a gas containing free oxygen, separating the product into a fraction containing the micro-organism, a fraction containing aqueous nutrient medium and, if hydrocarbons are present in the product in substantial amount, a fraction containing said hydrocarbons; thereafter subjecting a fraction containing said micro-organism to a series of alternate washing stages and separation stages, in the first washing stage there being used an aqueous solution of a surface active agent and in the last washing stage there being used an aqueous medium which is free of surface active agents, the separation stages being operated to separate the micro-organism from an aqueous phase, the separated micro-organism then passing on for further treatment, the separated micro-organism then passing on for further treatment, the washing and separation stages being carried out in such manner that the micro-organism recovered from said series of stages has a hydrocarbon content less than 7% by wt. based on the weight of the dry micro-organism; thereafter subjecting the washed micro-organism to conditions such that cell rupture takes place and thereafter recovering a fraction consisting of or containing one or more nitrogen-containing compounds selected from proteins, polypeptides and amino-acids, (a) which have been produced by said micro-organism in the course of its metabolism and which have been released from the micro-organism or (b) which have been derived from nitrogen-containing compounds of said micro-organism in the course of cell rupture.
3. A process according to claim 1 or 2 in which the micro-organism is subjected to cell rupture by a method selected from autolysis, plasmolysis and hydrolysis.
4. A process according to any one of the preceding claims which comprises cultivating a micro-organism in the presence of a feedstock consisting of or containing straight chain hydrocarbons; preferably thereafter, if hydrocarbons are still present, separating a fraction containing the micro-organism from said hydrocarbons; and thereafter subjecting a fraction containing said micro-organism to conditions such that proteins or polypeptides or amino-acids contained in said micro-organism or derived therefrom are separated from cell wall material consisting of cell walls and/or material which constituted the cell walls, and thereafter treating said cell wall material which is contaminated with hydrocarbons, with or without a preliminary modifying treatment, by solvent extraction to recover at least part of the hydrocarbons from the cell wall material.
5. A process according to claim 4 in which solvent extraction of the cell wall material is

carried out by the use of a solvent consisting of or containing a hydrocarbon.

6. A process according to claim 5 in which the hydrocarbon has 4—7 carbon atoms per molecule.

7. A process according to claim 6 in which the hydrocarbon is a paraffin.

8. A process according to claim 7 in which the paraffin is a straight chain paraffin.

9. A process according to claim 8 in which the paraffin is normal hexane.

10. A process according to any one of claims 5—9 in which the extraction of the cell wall materials by means of a hydrocarbon solvent is preceded by extraction by means of an alcohol, preferably ethanol.

11. A process according to claim 4 in which solvent extraction is carried out by means of a mixture of a hydrocarbon and alcohol.

12. A process according to claim 11 in which the solvent is a mixture of 80% by wt. of normal hexane and 20% by wt. of ethanol.

13. A process according to any one of claims 4—12 in which the hydrocarbons recovered by solvent extraction are recycled to the micro-organism cultivation stage.

14. A process according to any one of the preceding claims in which autolysis is carried out with added enzymes.

15. A process according to any one of claims 1—13 in which autolysis is carried out without added enzymes.

16. A process according to any one of the preceding claims in which a fraction obtained from the micro-organism and containing one or more nitrogen-containing compounds selected from proteins, poly-peptides and amino-acids, either with or without prior separation of cell wall material is subjected to solvent extraction.

17. A process according to claim 16 in which solvent extraction is carried out by means of a solvent as defined in any one of claims 5—12.

18. A process according to any one of the preceding claims in which an autolysate is concentrated at reduced pressure at a temperature below 70°C.

19. A process according to any one of the preceding claims in which the micro-organism which is cultivated is a yeast.

20. A process according to claim 19 in which the yeast is of the family Cryptococcaceae.

21. A process according to claim 20 in which the yeast is of the sub-family Cryptococcoideae.

22. A process according to claim 21 in which the yeast is of the genus *Torulopsis*.

23. A process according to claim 21 in which the yeast is of the genus *Candida*.

24. A process according to claim 21 in which the yeast is *Candida lipolytica*.

25. A process according to any one of

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claims 1—18 in which the micro-organism is a bacterium.

26. A process according to any one of the preceding claims in which the feedstock consists wholly or partially of straight chain hydrocarbons.

27. A process according to claim 26 in which the feedstock is a petroleum fraction.

28. A process according to any one of the preceding claims, in which straight chain hydrocarbons are removed from a petroleum fraction with production of a micro-organism, wherein the feedstock employed for the growth of the micro-organism is a petroleum fraction consisting in part of straight chain hydrocarbons, wherein there is recovered from the product of the growth of the micro-organism a petroleum fraction having a reduced proportion of straight chain hydrocarbons or which is free of said straight chain hydrocarbons.

29. A process according to claim 28 for the removal, wholly or in part, of waxes from a wax-containing petroleum gas oil, wherein the feedstock is a wax-containing petroleum gas oil wherein there is recovered, from the product of the growth of the micro-organism, a gas oil of reduced content of wax.

30. A process substantially as hereinbefore described with reference to the foregoing Example.

31. A fraction containing one or more nitrogen-containing compounds selected from proteins, polypeptides and amino acids whenever produced by a process as claimed in any one of claim 1—30.

32. Cell wall material whenever recovered by a process as claimed in any one of claims 1—30.

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Chartered Patent Agents.

Leamington Spa: Printed for Her Majesty's Stationery Office by the Courier Press.—1966.

Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.

ERRATA

SPECIFICATION No. 1,049,929

Page 2, line 3, for "sever" read "severe".

Page 2, line 94, for "theye" read "there".

Page 2, line 94, for "as" read "an".

Page 3, line 97, for "Schimmelculture" read "Schimmelculture".

Page 4, line 116, for "candida" read "Candida".

Page 6, line 47, for "weight" read "weigh".

Page 6, line 51, for "nirtogenous" read "nitrogenous".

Page 6, line 52, for "polpeptides" read "polypeptides".

Page 8, line 78, for "materials" read "material".

Page 9, line 36, for "claim" read "claims".

THE PATENT OFFICE

27th January 1967



* C L N S *