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PROCESS FOR THE PRODUCTION OF YEASTS

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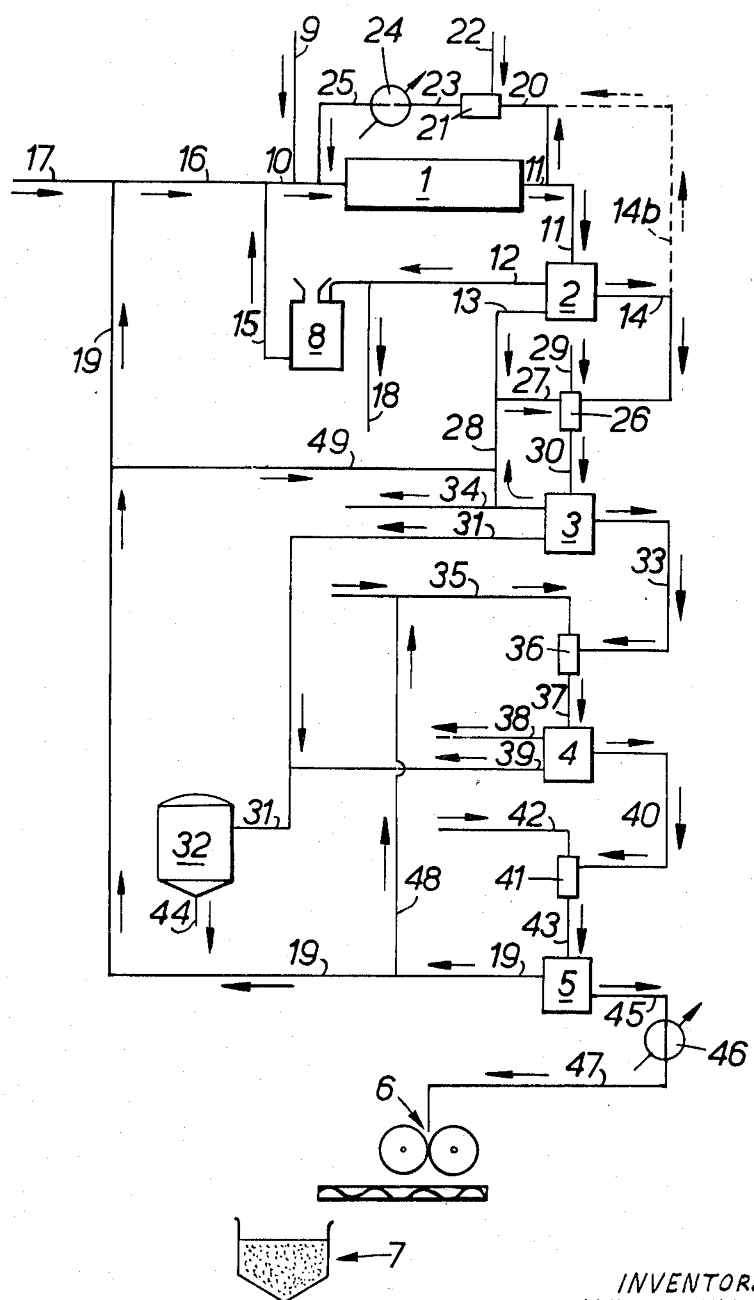

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PROCESS FOR THE PRODUCTION OF YEASTS

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This invention relates to a process for the removal of straight chain hydrocarbons, wholly or in part, from petroleum fractions in which they are contained and, more particularly, to a combined process for effecting said removal of hydrocarbons from petroleum fractions with the production of edible yeasts.

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.

It is also well-known that food yeasts, suitable for consumption by cattle, or in some cases by human beings, are prepared using as starting materials carbohydrates, for example molasses, wood hydrolysis sugars and lyes obtained in the course of paper manufacture.

On the basis of published disclosures it would appear that the replacement of carbohydrates by hydrocarbons for the production of yeasts has hitherto been studied only on the theoretical plane, starting from pure hydrocarbons or mixtures of synthetic paraffin hydrocarbons. No consideration has been given to the development of an industrial process; this is not surprising since it would appear that the use of a second liquid phase (which is immiscible with the aqueous phase in which the yeast grow) would impose a serious restriction on the rate at which the yeast could assimilate oxygen necessary for its normal growth; further, it would appear to be difficult for yeast in an aqueous phase to receive an adequate supply of food from an immiscible phase to support rapid growth.

It is an object of the present invention to provide a process for the production of yeasts from hydrocarbons. It is a further object to provide a process for the removal wholly or in part of straight chain hydrocarbons from petroleum fraction with the recovery of a valuable product based on the original straight chain hydrocarbon content of the petroleum fractions. It is a further object to provide a process for the dewaxing of a petroleum gas oil fraction with the production of edible yeast. Other objects will appear hereinafter.

According to the present invention there is provided a process which comprises the steps of continuously cultivating, in a fermenter, a strain of yeast which is adapted to grow on straight chain paraffinic hydrocarbons, 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 continuously separating from the mixture (a) a fraction comprising yeast, (b) a fraction comprising the major part of the aqueous phase and (c) a petroleum fraction having a reduced proportion of straight chain hydrocarbons or which is free of said straight chain hydrocarbons, the fraction (*b*) being recycled, continuously or batchwise, to constitute part of the aqueous nutrient medium.

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, olefins may also be present.

It is an important feature of this invention that when cultivating yeasts in the presence of the feedstocks hereinbefore described under conditions favoring the growth of the yeasts at the expense of the straight chain hydrocarbons, the other hydrocarbons, for example isoparaffins, naphthenes and aromatics are not metabolised or, at most, the proportion which is metabolised is very small. Furthermore, unlike conventional chemical processes governed by the law of mass action, the rate of removal of straight chain hydrocarbons is not substantially reduced as the proportion of these hydrocarbons in the overall mixture of hydrocarbons decreases (except, of course, in the very final stages of removal). Thus, when desired, the percentage conversion of straight chain hydrocarbons which is achieved can be maintained at a value approaching 100% without necessitating a very disproportionate expenditure of contact time to achieve small improvements. Furthermore, in a continuous process, this high percentage conversion can be achieved without resorting to the use of a long reaction path.

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

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.

Preferably the yeast which is grown on the feedstock is of the family Cryptococcaceae and particularly of the subfamily Cryptococcoïdeae; however if desired there may be used, for example ascosporogeneous yeasts of the subfamily Saccharomycoideae. Preferred genera of the Cryptococcoideae subfamily are Torulopsis (also known as Torula) and Candida. Preferred strains of Candida are *Candida tropicalis, Candida utilis* and *Candida pulcherina* and, in particular, *Candida lipolytica* (also known as *Mycotorula lipolytica*). Other suitable strains include

Torulopsis colliculosa, *Hansenula anomala* and *Oidium lactis*.

When starting with a fresh stock of the yeast it will usually be necessary to adapt the yeast to assimilate carbon from hydrocarbons and to use an inoculum of the adapted yeast for the process of growth according to the invention. However these yeasts, when cultivated on an aqueous mineral medium containing the appropriate nutrient elements, grow with difficulty because the petroleum fractions do not contain the growth factors which exist in molasses and wood hydrolysis sugars, for example.

The growth of the yeast used is favoured by the addition to the culture medium of a very small proportion of "bio" factors, for example 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 yeast takes place at the expense of the petroleum 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 yeast 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 stepwise or continuous addition of an aqueous medium of high pH value. Usually, 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. 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 yeast employed and will usually lie in the range 25–40° 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 yeast. 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.

At the end of the growth one obtains an emulsion which contains yeast and nonmetabolised hydrocarbons in a continuous aqueous phase.

The major part of the continuous aqueous phase is first separated; preferably this is carried out by centrifuging. The separated aqueous phase will usually contain a greater concentration of nonnutritive ions that 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 makeup quantities of the necessary nutrients and is returned to the fermenter; if desired the makeup materials may be fed to the fermenter as a separate stream.

By centrifuging the product from the fermenter three fractions may be 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), the remaining fractions (i) and (iii) may be reblended and mixed with an aqueous solution of a surfactant.

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

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

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

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

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

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

As stated hereinbefore, the culture medium will preferably contain added bio factors.

The treated petroleum fractions will be very suited to a number of different uses, depending on boiling range and other characteristics. Thus the kerosenes may be employed as aviation jet engine or gas turbine engine fuels; the heavy gas oils are suitable as heavy diesel fuels. Also there may be obtained oils suitable for use as refrigerator oils or as transmission fluids.

The invention is illustrated but not limited with reference to the accompanying drawing which is a schematic flow sheet of a process as herein described. The process as now particularly described makes provision for certain adverse conditions such as high cost or scarcity of soft water, but with the availability of seawater or brackish water and, on the other hand, difficulties in the centrifugal separation of the pure yeast cells from the unmetabolised oil and the aqueous nutritive medium. It goes without saying that the devices described may be simplified or used only in part if more favourable conditions exist, for example cheap and abundant soft water.

The installation shown in diagram form in the sole illustration comprises essentially the following parts: a fermenter **1**, four washing and centrifugal separation groups **2**, **3**, **4** and **5** and the traditional drying equipment **6** and conditioning equipment **7** for the yeast. The illustration does not include the pumps necessary for transferring the liquids and the apparatus itself is not described because it is all of a known type.

In the interests of clarity in the description, sometimes figures will be given for the rates of flow in the essential circuits, these rates being expressed in parts by volume for the final production of one part by weight of dry yeast. However, these figures are not restrictive and may vary within considerable limits.

The fermenter 1 may be of any type suitable for the continuous cultivation of yeasts on aqueous substratum containing sugars.

The devices for the injection and distribution of air are not shown in the diagram, nor are any cooling appliances or appliances for regulating the pH.

The fermenter 1 contains a synthetic aqueous nutritive medium.

Suitably the aqueous medium (apart from bio factors) has the following composition:

	Parts by weight
Calcium phosphate, tribasic	3.4
Potassium chloride	0.6
Magnesium sulphate	0.3
Ammonium sulphate	2.5

made up to 1000 parts with soft water containing trace elements.

When starting off the fermentation, there is added to the nutritive medium together with the inoculum a few parts per million of yeast extract for the purpose of adding the bio growth factors. The requisite quantity of petroleum fraction, for example paraffinic gas oil, is introduced by the pipes 9 and 10. When the culture has reached the desired concentration of yeast cells for continuous operations, the feed of the fermenter 1 with nutritive medium and petroleum oil is started. The temperature is regulated to the desired level, namely from 25 to 40° C., using appropriate means. The pH of the medium is also regulated automatically to the desired value by the addition of an ammoniacal solution.

In the following description relative feed rates are expressed as parts by wt. per unit time. 100 parts of aqueous nutritive medium are introduced into the fermenter 1 through the pipe 10. The pipe 9 feeds the petroleum fraction at a rate of flow which depends on the composition of this oil, for example 5 parts of which 1 part is metabolisable. Air is fed at the rate of 1 to 2 volumes per volume of fermenter per minute. From the fermenter 1 by pipe 11 there passes an emulsion consisting of 100 parts of aqueous nutritive medium, 4 parts of unmetabolised oil and 1 part of yeast (because the growth has given 1 part of yeast for 1 part of metabolised hydrocarbons).

The aqueous nutritive medium of the pipe 11 still contains nutritive mineral elements together with vitamin and other growth factors resulting from a slight autolysis of the yeast which is inevitable in the fermenter (bio factors). It is therefore desirable to recycle this to the inlet to the fermenter. Since it also contains non-nutritive ions, for example SO_3 and Cl ions (which would become concentrated in the nutritive medium and would harm the growth if this recycling were carried out to the extent of 100%) it is therefore necessary to discard part as a bleedoff. On the other hand it is necessary to replace the nutritive elements consumed by the yeast and to make up with soft water the quantity of nutritive medium which has been evacuated. All this is effected as follows:

The effluent leaving the fermenter 1 through the pipe 11 feeds a centrifugal separator 2 designed to separate three phases which are, in increasing order of density: (a) an oil phase containing the yeast cells through the pipe 13, (b) an aqueous nutritive medium phase (which may without trouble also include traces of oil and yeast) through the pipe 12, and (c) a yeast cream phase through the pipe 14. This cream is a fluid paste containing approximately 1 part of yeast, 4 parts of aqueous medium and a certain quantity of oil fixed on to the yeast cells.

The essential part played by the separator 2 is to recover the greatest possible amount of aqueous nutritive medium, usually about 96 parts, so that it can be recycled to the fermenter 1. However, before this is done, as has been stated above, a certain proportion, for example 20 parts, of the aqueous nutritive medium, is discarded to waste via the pipe 18. The rest passes into a small mixer 8 where the nutritive salts necessary for growth are added and dissolved. The nutritive medium which has been reconstituted in this way is fed to the inlet of the fermenter 1 through the pipe 15. A makeup of 20 parts of soft water is introduced by the pipe 16, to compensate for the 20 parts of medium evacuated at 18. This soft water may come from outside via the pipe 17 or, better still, it is soft water which has already been used for the final washing of the yeast and in this case it arrives by the pipe 19.

Despite the recycling of the aqueous nutritive medium, the quantity of bio factors which it contains is generally not sufficient to ensure a satisfactory growth of the yeast from the point of view of yield and quality. Consequently a makeup of bio factors is employed. Optionally this may be produced by the means described below:

A small proportion of the effluent leaving the fermenter 1 through the pipe 11 is taken through the pipe 20 into a small closed vat 21 into which there is a direct injection of steam through the pipe 22. The temperature in 21 is thus raised to 100° C. or over, which brings about the complete autolysis of the yeast cells contained in the medium. The effluent from the vat 21, which makes use of the pipe 23, is therefore a concentrate of bio factors. It is cooled by the cooler 24 and sent to the feed of the fermenter 1 through the pipes 25 and 10.

For the same purpose it is also possible to take a small portion of the yeast cream leaving the separator 2 at 14 and to send it through the pipe 14*b*, shown in dotted lines in the drawing, into the vat 21 which is heated by direct steam. This variant permits of a reduction in the steam injected at 22.

This procedure ensures the continuity and the quality of the yeast production, with the maximum economy of substances supplied from outside: bio factors, nutritive salts, soft water. It is now necessary to recover these yeasts with the greatest possible purity. For this purpose, a mixer 26 receives through the pipe 27 the oil containing the yeast cells of the pipe 13. It also receives the yeast cream contaminated by the oil in the nutritive medium arriving through the pipe 14. An aqueous washing phase containing a surfactant also feeds the vat 26 through the pipes 28 and 27. The purpose of the surfactant is to accomplish the separation of the yeast cells from the oil which is firmly fixed on to them, apparently by adsorption.

The pipe 30 carries the mixture from the mixer 26 to the centrifugal separator 3 of the same type as the separator 2. The following leave the separator 3 in increasing order of density: (a) an oil phase which the pipe 31 carries to the storage 32, (b) an aqueous phase containing the surfactant product which is recycled to the mixer 26 through the pipes 28 and 27, and (c) a yeast cream phase through the pipe 33, containing one part of yeast which may still be slightly contaminated by the oil with 4 parts of aqueous liquid containing the surfactant. The aqueous washing liquid containing the surfactant is continuously employed and recycled through the circuit 26, 30, 3, 28, 27. The rate of flow may be regulated as desired, for example between 20 and 100 parts, and its concentration of surfactant product is also regulated, for example between 1 per mil and 1 percent, so as to ensure the best possible separation of the oil by the separator 3.

However, this recycling cannot be carried out completely without the addition of surfactant because the pipe 33 carried away from the circuit 4 parts of washing solution, and consequently some surfactant and the pipe 14 brings in 4 parts of aqueous nutritive medium containing mineral salts. It is therefore necessary to send to waste through the pipe 34 a small proportion of the washing liquor in order to avoid its enrichment in mineral salts. It is also necessary to compensate for the loss of washing liquor and surfactant by a makeup which is carried out by the pipe 29.

The yeast cream of the pipe **33** must now be freed from the surfactant and any traces of oil which it may contain.

If abundant and cheap salt water is available whilst soft water is expensive, it is advantageous to carry out a bulk washing with 100 parts of salt water, which arrive through the pipe **35** into the mixer **36**. The effluent from the mixer **36** is fed by the pipe **37** to the centrifugal separator **4**, which is of the same type as the separators **2** and **3**. Three phases leave this in increasing order of density:

A not very abundant phase of oil, sometimes wet is evacuated by the pipes **39** and **31** to the oil tank **32**. This tank must be purged periodically for the purpose of discharging through the pipe **44** any water which may have been carried along with the oil. A salt water phase containing the surfactant leaves via the pipe **38** and passes to waste. A yeast cream phase, in the main freed from surfactant and residual traces of oil, leaves through the pipe **40**. This phase consists of one part of yeast and 4 parts of salt water. It must undergo a final washing with soft water for the purpose of separating from it most of the salt water. This final washing is carried out in the mixer **41**, fed by the pipe **42** with, for example 50 parts of soft water. The pipe **43** feeds the separator **5** with the effluent from the mixer **41**.

The centrifugal separator **5** may be more simple than the preceding ones because it only separates two phases: 50 parts of a soft water washing phase through the pipe **19**, this phase containing a low proportion of salts from the salt water of the preceding washing operation, and a yeast cream phase consisting of one part of yeast and 4 parts at least of slightly salt water through the pipe **45**. The centrifuging is regulated in such a way that this yeast cream is as thick as possible, so as to have a minimum amount of water to evaporate during the final drying. In order to permit its flow to the dryer **6** along the pipe **47**, the thick cream is slightly heated by a heat exchanger **46**. The steam roller dryer **6** produces the final dry yeast, with a maximum of 10% of water. This yeast is sent to the packing department via the unit **7**: screw conveyor and storage silo.

The 50 parts of washing water of the pipe **19** are used in the installation for all the requirements of practically soft water: through the pipe **16** makeup of water for the nutritive medium, through the pipe **49** makeup of water for the washing circuit with the liquid containing the surfactant. The pipe **48** carries the whole of the excess of this washing water to the pipe **35** so that it can be used with the salt water and in this way reduce its salt content.

Considerable simplifications may be made in the installation described above. If abundant and cheap soft water is available, it is possible to do away with the separator **5** and to replace the salt water in the circuit of mixer **36** by soft water. The use of an edible surfactant product, such as saccharose palmitate, would make it possible to minimise the extent of the washing of the separator **4**, to use in it soft water even if expensive, to the exclusion of salt water, and to do away with the separator **5**.

The unmetabolised petroleum oil stored in the tank **32** differs from the petroleum oil used as the raw material by virtue of the fact that the yeasts have metabolised its paraffinic constituents. Its pour point is thus considerably lowered, and the installation described above may therefore be regarded as a dewaxing installation, with the production of yeasts in the place of paraffin wax.

The installation may obviously be used by employing as raw material paraffinic hydrocarbons manufactured by extraction of petroleum fractions by means of molecular sieves. In this case the whole of the hydrocarbons may be metabolised, which simplifies the purification stages for the yeast.

We claim:

1. A process for the removal, at least in part, of straight chain hydrocarbons from a petroleum fraction, with production of edible yeast, which comprises the steps of continuously cultivating, in a fermenter, a strain of straight chain paraffinic hydrocarbon-consuming yeast which is adapted to grow on straight chain paraffinic hydrocarbons, 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 continuously separating from the mixture a fraction comprising yeast, a fraction comprising the major part of the aqueous phase and a petroleum fraction having a reduced proportion of straight chain hydrocarbons, the aqueous phase fraction being recycled to constitute part of the aqueous nutrient medium.

2. A process according to claim **1** in which the feedstock is selected from the group consisting of kerosine, gas oil and a lubricating oil.

3. A process for the removal, at least in part, of waxes from a wax-containing petroleum gas oil, with production of edible yeast, which comprises the steps of continuously cultivating, in a fermenter, a strain of straight chain paraffinic hydrocarbon-consuming yeast which is adapted to grow on straight chain paraffinic hydrocarbons, in the presence of a wax-containing petroleum gas oil; and in the presence of an aqueous nutrient medium; and in the presence of a gas containing free oxygen and continuously separating from the mixture a fraction comprising yeast, a fraction comprising the major part of the aqueous phase and a fraction comprising gas oils of reduced content of wax, the aqueous phase fraction being recycled, to constitute part of the aqueous nutrient medium.

4. A process according to claim **1** in which the petroleum fraction which is treated contains 3–45% by wt. of straight chain paraffinic hydrocarbons.

5. A process according to claim **1** in which the yeast is of the family Cryptococcaceae.

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

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

8. A process according to claim **6** in which the yeast is of the genus Candida.

9. A process according to claim **6** in which the yeast is *Candida lipolytica*.

10. A process according to claim **1** in which the yeast is grown in the presence of a nutrient medium containing an agent selected from the group consisting of vitamins of group B and biotin.

11. A process according to claim **1** in which the yeast is grown in the presence of a nutrient medium containing extract of yeast.

12. A process according to claim **1** in which the pH of the nutrient medium lies in the range 4–5.

13. A process according to claim **12** in which the pH of the nutrient medium is maintained in the range 4–5 during growth of the yeast by the stepwise or continuous adidtion of an aqueous material of high pH.

14. A process according to claim **13** in which the pH is controlled by the addition of an ammoniacal solution.

15. A process according to claim **1** in which the product obtained from the fermenter is separated into the three fractions by centrifuging.

16. A process according to claim **15** in which the yeast and aqueous phase containing fractions are blended, treated with aqueous solution of a surfactant and centrifuged to yield an oil phase, an aqueous phase and a fraction comprising yeast.

17. A process according to claim **16** in which the fraction comprising yeast is subjected to successive alternate washing and centrifuging stages, wherein washing is carried out by means of an aqueous solution of a surfactant, and thereafter the pasty phase is washed with water and centrifuged.

18. A process according to claim **1** in which the free-oxygen-containing gas is air.

19. A process according to claim **1**, wherein the fraction is substantially free of straight chain hydrocarbons.

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