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FERMENTATION PROCESS FOR CONVERTING HYDROCARBONS TO PROTEINACEOUS MATERIALS

Gordon Homer Evans, Kingswood, England, and Jean Lindsay Shennan, Falkirk, Scotland, assignors to The British Petroleum Company Limited, London, England

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ABSTRACT OF THE DISCLOSURE

A process for the production of yeast biomass from a hydrocarbon substrate wherein the new yeast strain, *Candida lipolytica* strain CBS number 6331 is continuously cultivated in the presence of a straight chain hydrocarbon having at least 10 carbon atoms per molecule, an aqueous nutrient medium and a gas containing free oxygen.

The present invention is concerned with improvements in and relating to a continuous process for converting hydrocarbons to proteinaceous material. In particular the invention relates to a continuous process for the production of single cell protein by cultivating a new yeast strain on a hydrocarbon as the carbon substrate.

In recent years a number of processes have been proposed for the production of single cell protein by cultivating a yeast on a hydrocarbon substrate, in the presence of an aqueous nutrient medium and a gas containing free oxygen. The proposed processes can be either batch or continuous. Many hydrocarbon assimilating strains of yeast have been disclosed as suitable for use in such processes.

We now provide a new strain of yeast which is particularly suitable for use in continuous industrial scale processes of the foregoing type. The new strain remains stable over prolonged periods of continuous cultivation and the yeast produced has a high crude protein content.

Accordingly the present invention is a process for the conversion of a hydrocarbon into a proteinaceous material which comprises continuously cultivating *Candida lipolytica* strain C.B.S. number 6331 in the presence of a straight chain hydrocarbon having at least 10 carbon atoms per molecule, an aqueous nutrient medium and a gas containing free oxygen.

Preferred hydrocarbons are normal paraffins recovered from petroleum fractions in the kerosine or gas oil boiling ranges. In particular these hydrocarbons are gas oil boiling range normal paraffins containing 11 to 23 and mainly 14 to 21 carbon atoms per molecule and kerosine boiling range normal paraffins containing 10 to 13 carbon atoms per molecule. Straight chain hydrocarbons obtained from petroleum feedstocks by molecular sieve treatment are most suitable.

Crude protein contents in the range 63 to 65 percent by weight in relation to the dry weight of whole cells can be obtained by cultivating the new strain on the preferred kerosine normal paraffins.

Production rates of about 5 grams per litre per hour with a minimum of about 2.5 grams per litre per hour can be obtained by the use of the new strain. The strain has a high yield factor of about 1, i.e. ratio of the weight of yeast produced in relation to the weight of hydrocarbon utilised by the yeast. It has a high growth rate, particularly on the preferred hydrocarbons, thus permitting operation at relatively high dilution rates, for example growth rates (division times) of less than 4 hours giving a D max. of more than $0.15h^{-1}$. It can be cultivated at commercially acceptable growth rates and yield factors under non aseptic conditions in the presence of bacterial contamination. When cultivated in a pressure vessel under an elevated over pressure, for example in the range 1.5 to 5.0 kilograms per square centimeter absolute, preferably under aseptic conditions of operation the strain exhibits a marked increase in yield factor. This feature makes a valuable contribution to the overall economics of the process since yield factor is a measure of the efficiency with which the assimilable hydrocarbons are converted into cellular materials.

The process can be carried out using any of the known cultivation techniques. Preferred temperature ranges are from 27 to 33° C. and preferred pH ranges are 4.0 to 5.5. Most suitably cultivation is caried out in a stirred, aerated pressure vessel. Where over pressure is applied it can be in the range up to 5 kilograms per square centimeter absolute. It is a further feature of the new strain that high growth rates can be maintained over a wide range of temperature and pH, such as for example a pH range of 3 to 5.7 and a temperature range of 20 to 35° C. This facilitates, in non aseptic operation, the selection of conditions of pH and temperature which cause "wash-out" of microbial contamination or at least suppresses the contaminant to levels which do not substantially affect the production of yeast biomass. For example bacterial contamination can be suppressed by operation at a pH in the range 4 to 4.8 and conveniently the temperature can be in the range of about 27 to 33° C.

The new strain is a mutant derived from a wild yeast which we have isolated and identified as *Candida lipolytica* in accordance with the taxonomic criteria of Lodder. The new strain is lodged at the Centraalbureau Voor Schimmelcultures, Baarn, Holland, where it has the C.B.S. number 6331.

In addition to the features previously described the new strain has the following characteristics.

The morphological characteristics of the new strain are the same as for *Candida lipolytica* C.B.S. strain number 2078 and C.M.I. strain 93743. These characteristics correspond with standard description of *C. lipolytica* var. *lipolytica* given by J. Lodder, "The Yeasts. A Taxonomic Study." 2 ed. 1970 pp. 991–993, except that when plated on glucose/yeast extract/peptone agar or Dalmau plate cultures on corn meal agar (Lodder 2 ed. 1970 p. 992), the colonies are cream coloured, smooth, have a matt surface with no folding and no pseudomycelium is formed.

This contrasts with the pseudomycelium formation in the typical strains of *Candida lipolytica* e.g. C.B.S. Strain 2078 and C.M.I. Strain 93743.

The physiological characteristics are identical with those of the *C. lipolytica* C.B.S. Strain No. 2078 and C.M.I. strain 93743 previously mentioned.

(a) Morphological observations when cultivated in glucose yeast extract peptone water

When cultivated for 3 days at 25° C. in glucose yeast extract peptone water the cells are short ovoid to long ovoid and measure 3 to 6 by 5 to 11 microns. Rarely some elongated cells are observed which measure up to about 20 microns in length. No pellicle is formed.

(b) Ascospore or Ballistospore formation

No ascospores or ballistospores are formed when the strain is grown on the standard sporulation media.

(c) Assimilation of nitrate and decomposition of urea

The strain does not assimilate potassium nitrate. Urea is decomposed.

(d) Utilisation of sugars

The strain will not ferment sugars but the following compounds are assimilated: glucose, ethanol, glycerol, erythritol and succinic acid.

The following compounds are not assimilated: galactose, sorbose, d-ribose, L-rhamnose, sucrose, maltose, cellobiose, trehalose, lactose, mellibiose, raffinose, melezitose, inulin, soluble starch, d-xylose, L-arabinose, d-arabinose, ribitol, galactitol, d-mmanitol, d-glucitol, α-methyl d-glucoside, salicin and inositol. Gelatin is liquified and litmus milk is peptonised.

Growth in vitamin free media is weak and growth is stimulated by the presence of thiamine.

The following examples illustrate the invention without limiting its scope.

EXAMPLE 1

Candida lipolytica strain C.B.S. Number 6331, was cultivated continuously under aseptic conditions in a stirred, aerated pressure vessel having a working volume of 1800 litres. The carbon substrate was a mixture of kerosine range normal paraffins having C_{10} to C_{13} carbon atoms per molecule obtained by subjecting a petroleum feedstock to molecular sieve treatment. The aqueous nutrient medium had the following composition:

	Grams
H_3PO_4	1.594
KCl	0.916
$MgSO_47H_2O$	0.521
$MnSO_44H_2O$	0.035
$FeSO_47H_2O$	0.052
$ZnSO_47H_2O$	0.153
$CuSO_47H_2O$	4.36×10^{-4}
H_2SO_4	0.172

Thiamine hydrochloride—220 milligrams.
Tap water to 1 litre.

After an initial start-up period of about 16 hours during which the culture reached a concentration of 16 grams per litre, continuous operation was commenced at a dilution rate of 0.16 volumes/volume per hour, a temperature of 32° C. and a pH of 5.5. The pH was maintained by addition of ammonia as required. The hydrocarbon was fed at a rate of 10 litres per hour and the aqueous nutrient medium was fed at a rate of 260 litres per hour. The fermentation was aerated at 1.50 volumes/volume per minute and the pressure inside the fermenter maintained at 2.50 kilograms per square centimeter absolute.

The yield factor was 0.88 (Yield factor=Wt. of yeast product/wt. of hydrocarbon utilized by the yeast produced). The broth had a dry cell weight of 23.6 grams per litre.

The run was continued for 1000 hours. During this period the broth was sampled at intervals of 24 hours and the culture examined for strain variation. The samples were plated on Sabouraud's Dextrose Agar (Oxoid) and Malt Exract Agar (Oxoid). (Oxoid is a registered trademark.) The plates were incubated for 3 days at 30° C. The resulting colonies were cream coloured, smooth, with a matt surface and no folding.

Throughout the fermentation no signs of culture variation were observed on plating. No pseudomycelium was observed by microscopic examination.

The crude protein content was assessed at regular intervals by the procedure given in "Fertiliser and Feeding Stuffs Regulations." Statutory Instrument No. 218, 1968.

The yeast had a crude protein content in the range 63 to 66 percent by weight in relation to the dry weight of the yeast.

EXPERIMENT

By way of comparison a known hydrocarbon utilising strain of *Candida lipolytica,* namely, C.M.I. number 93743 was subjected to identical conditions of cultivation to those previously described for the new strain C.B.S. number 6331.

At the end of the 16-hour start up period the cell concentration was about 1.5 grams/litre. Owing to the slow growth rate the start up period was extended until a cell concentration of 16 grams/litre was reached (i.e. after about 40 hours). Continuous operation aws commenced at a dilution rate of 0.15 volumes/volume per hour and the culture washed out. It was not possible to achieve steady operation because the growth rate was too slow.

Steady state operation was achieved at a lower dilution rate of 0.07 volumes/volume per hour using an over pressure of 1.5 kilograms per square centimeter absolute. The crude protein content of the yeast product was about 54 percent by weight in relation to the dry weight of the yeast. The plated colonies were cream coloured, wrinkled, hirsute, dull and coarsely folded. Microscopic examination showed abundant pseudomycelium; septate mycelium was also found.

The yield factor was 0.58.

EXAMPLE 2

C. lipolytica C.B.S. Strain 6331 was cultivated under the same conditions and at the same dilution rate 0.07 volumes/volume per hour and an over pressure of 1.5 kilograms per square centimenter absolute, as for the C.M.I. 93743 strain of *C. lipolytic*a previously described by way of comparison in the experiment following Example 1.

The yield factor was 0.73 and the crude protein content was about 59 percent by weight in relation to the dry weight of yeast.

EXAMPLE 3

Candida lipolytica strain C.B.S. 6331 was inoculated into an aqueous nutrient medium and a hydrocarbon as the source of utilisable carbon contained in a stirred, aerated, pressure vessel having a working volume of 55 litres. The hydrocarbon was a mixture of gas-oil range normal paraffins having 11 to 18 and mainly 14 to 17 carbon atoms per molecule which was obtained by subjecting a gas oil petroleum feedstock to a molecular sieve treatment. The aqueous nutrient media had the following composition:

	Grams
H_3PO_4	1.455
K_2SO_4	0.969
$MgSO_47H_2O$	0.470
$ZnSO_47H_2O$	0.138
$FeSO_47HsO$	0.047
$MnSO_44H_2O$	0.041
$(NH_4)_2SO_4$	0.500

$CuSO_45H_2O$—0.413 milligrams.
Thiamin HCl—203 milligrams.
Tap water to 1 litre.

After a period of batch growth, continuous operation was commenced at a dilution rate of 0.14 to 0.15 volumes per volume per hour, a temperature of 32° C. and a pH of 4.5, maintained by ammonia addition as required. The hydrocarbon was fed at a rate of 220.5 ml./h. and the aqueous nutrient medium was fed at a rate of 7.42 litres/h. The fermentation was mechanically agitated, aerated at 0.75 volumes/volume per minute and the pressure inside the fermenter was maintained at 1.5 kilograms per square centimeter absolute.

About 240 hours after the initial inoculation with the culture yeast, an inoculum containing five bacteria belonging to the genera Acinetobacter, Escherichia, Paracolobactrum and Pseudomonas was added to the fermenter broth. Bacterial counts carried out at intervals over the following one hundred and eighty hours on samples of fermenter broth indicated that the number of viable bacterial cells present varied between 300,000 to 2,500,000 per millilitre of broth.

After operation for from 330 to 402 hours the cell density of the yeast (*Candida lipolytica* strain C.B.S. 6331) was 23.5 grams per litre and the yield factor was 0.99. The yeast product had a crude protein content of 59 percent by weight in relation to the dry weight of the whole cells.

The pH of the broth was then raised from 4.5 to 4.8 during a period of operation from 520 to 680 hours. Bacterial counts carried out on the broth between 800 to 1200 hours of operation were of the order of 10^9 to 3×10^9 cells per millilitre. The number of *Candida lipolytica* cells present during the same period were of a similar order to the numbers of bacterial cells.

Between 1122 to 1218 hours of operation the dry cell weight of the yeast was 21.3 grams per litre and the yield factor was 0.96. The crude protein content of the yeast was about 59 percent by weight in relation to the dry weight of the whole cells.

After 1218 hours of operation the pH was increased further to 5.0 whereupon operation of the fermenter became unsteady, difficult to control and gave a variable yeast factor. At the same time the bacterial count increased until it exceeded the yeast count.

This example demonstrates satisfactory continuous operation of a fermentation for the production of yeast biomass using the new strain *Candida lipolytica* strain C.B.S. 6331 in the presence of a substantial level of bacterial contamination.

It also demonstrates that commercial production of yeast biomass is not satisfactory when the fermentation is operated under nonaseptic conditions at pH values of about 5 or higher.

We claim:

1. A process for the conversion of a hydrocarbon into a proteinaceous material which comprises continuously cultivating *Candida lipolytica* strain C.B.S. number 6331 in the presence of a straight chain hydrocarbon having at least 10 carbon atoms per molecule, an aqueous nutrient medium and a gas containing free oxygen.

2. A process as claimed in claim **1** wherein cultivation is carried out in a pressure vessel at an over pressure in the range 1.5 to 5.0 kilograms per square centimeter absolute.

3. A process as claimed in claim **1** when carried out under nonaseptic conditions of operation and at a pH in the range of 4 to 4.8.

References Cited

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A. LOUIS MONACELL, Primary Examiner

R. B. PENLAND, Assistant Examiner