

PATENT SPECIFICATION

(11) 1 510 499

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- (21) Application No. 45176/75 (22) Filed 31 Oct. 1975
(31) Convention Application No. 530 422
(32) Filed 6 Dec. 1974 in
(33) United States of America (US)
(44) Complete Specification published 10 May 1978
(51) INT CL² C12B 1/08, 1/16//B01F 5/10; C12C 11/10; C12D 13/06
(52) Index at acceptance
C6F 1A 1X 2A 2X
B1C 19H 1 4



(54) PROCESS FOR THE PRODUCTION OF SINGLE CELL PROTEIN BY FERMENTATION

(71) We, PHILLIPS PETROLEUM COMPANY, a corporation organised and existing under the laws of the State of Delaware, United States of America, of Bartlesville, Oklahoma, United States of America, 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:—

The present invention relates to an improved process for the propagation of alcohol assimilating microorganisms by the aerobic culturing of the microorganism in a foamed culture medium containing an alcohol as the main source of carbon.

Current world-wide food shortages have encouraged the research and development of methods of producing high quality, low cost microbial protein, i.e., single cell protein to alleviate the food shortages. Considerable development work in such fermentation processes has been directed toward the use of hydrocarbons and other carbonaceous materials which would normally be flared or otherwise disposed of in petroleum refining. The use of methanol as the main source of carbon has been particularly attractive because of the advantages offered thereby. Such advantages include: methanol is miscible with water, is easily and cheaply produced from a wide range of hydrocarbon materials, can be easily produced in virtually any area of the world having any form of fossil fuel supplies, is characterised by the absence of potentially carcinogenic polycyclic hydrocarbons.

In accordance with the present invention there is provided a process for the production of single cell protein, which comprises aerobically culturing an alcohol utilising microorganism in a foamed culture medium containing one or more C₁—C₁₆ straight chain aliphatic alcohols as a main source of assimilable carbon, a source of assimilable nitrogen and the inorganic salts and other factors necessary for the growth of the microorganism, allowing

the single cell protein to accumulate in the culture medium and recovering the accumulated single cell protein from the culture medium, wherein the culture is performed in a fermentation vessel in which the foamed culture medium is maintained in a state of substantially continuous internal circulation.

In this process it has been found that fermentation carried out in a foam-filled fermenter in which the foam is continuously circulated is highly efficient especially when carried out in a continuous process. The foamed contents of the fermenter can be described as the dispersion of the gaseous phase within the liquid phase or as an emulsified gaseous phase or simply as an emulsion of the gaseous and liquid phases wherein increased surface area contact is effected between the gas and liquid phases for enhancing the fermentation process. Specifically, it has been found that the fermentation productivity (grams of cells per liter of mixture per hour) is significantly higher when using the foam fermenter than when a conventional paddle stirred tank fermenter is employed.

Fermentation vessels suitable for the formation and maintenance of the contents in a foamed state are known in the fermentation art. Generally, such vessels are those which provide vigorous agitation to the contents with concomitant introduction of some free oxygen-containing substance such as air to the mixture and which permit or are designed to induce internal circulation of the culture medium. In carrying out the process, small amounts of surfactants can also be employed to aid in the formation and maintenance of the foam. However, this is not usually required since it is known that many microbial growth processes involve the formation of materials (cellular or extracellular) which have surfactant properties and thus induce foaming. In fact, in some fermentation processes it is often necessary to resort to the use of antifoam agents to control the degree of foaming during the fermentation process.

The process of this invention will be further described with reference to the accompanying drawings in which the single figure is a schematic representation of a fermenter used in the process of this invention.

Referring more in detail to the drawing:

The single figure shows a typical fermentation reactor, as is known in the art, which is comprised of a housing 1 having a hollow interior. A draft tube 2 is positioned within the housing 1 and provides a flow path for the medium contained within the housing 1 to help induce circulation. At the lower end of the draft tube 2 there is a pump such as a turbine 3 which helps induce flow downwardly through the draft tube 2 and through emulsifying sieves 4 to the exterior of the draft tube 2 and upwardly therefrom. Positioned adjacent to the top of the housing 1 there is provided a foam breaker 5 which is operable to break foam which accumulates in the upper portion of the housing 1. An outlet 7 is provided adjacent to the lower portion of the housing 1 to draw off a portion of the contents for further processing. The outlet 7 preferably is a conduit which connects the lower portion of the housing 1 to secondary processing equipment (not shown). An inlet 8 is provided adjacent to the upper portion of the housing 1 and is adapted for the delivery of portions of the medium used in the fermentation process. Power means such as motors 9 and 10 are operably connected to the turbine 3 and foam breaker 5, respectively, for power operation thereof. A conduit 11 is in communication with the interior of the housing 1 and is adapted for the introduction of a source of oxygen, such as air, into the medium.

The fermentation is carried out with a straight chain alcohol having from 1 to 16 carbon atoms per molecule. This is referred to as the feedstock and is assimilable by the microorganism and supplies the carbon and energy for the microbial growth. Preferably the alcohol has from 1 to 6 carbon atoms per molecule and more preferably the alcohol will be either ethanol or methanol and most preferably, methanol. Examples of suitable alcohols include methanol, ethanol, 1-propanol, 1-butanol, 1-octanol, 1-dodecanol, 1-hexadecanol, 2-propanol, 2-butanol and 2-hexanol. Mixtures of alcohols can also be employed if desired.

The microorganism used in the fermentation process is capable of assimilating one or more of the above alcohols as the source of carbon and energy in the growth or propagation of the microorganism. Suitable microorganisms can be selected from bacteria, yeast and fungi.

Suitable yeasts includes species from the genera *Candida*, *Hansenula*, *Torulopsis*, *Saccharomyces*, *Pichia*, *Debaryomyces*, *Lipomyces*, *Cryptococcus*, *Nematospora*, and *Brettanomyces*. The preferred genera include

Candida, *Hansenula*, *Torulopsis*, *Pichia*, and *Saccharomyces*. Examples of suitable species include:

Candida boidinii
Candida mycoderma
Candida utilis
Candida stellatoidea 70
Candida robusta
Candida clausenii
Candida rugosa

Brettanomyces petrophilum

Hansenula minuta 75
Hansenula saturnus
Hansenula californica
Hansenula mrakii
Hansenula silvicola
Hansenula polymorpha 80
Hansenula wickerhamii
Hansenula capsulata
Hansenula glucosyza
Hansenula henricii
Hansenula nonfermentans 85
Hansenula philodendra

Torulopsis candida
Torulopsis holmii
Torulopsis versatilis
Torulopsis glabrata 90
Torulopsis molishiana
Torulopsis nemadendra
Torulopsis nitratophila
Torulopsis pinus

Pichia farinosa 95
Pichia polymorpha
Pichia membranaefaciens
Pichia pinus
Pichia pastoris
Pichia trehalophila 100
Saccharomyces cerevisiae
Saccharomyces frangilis
Saccharomyces rosei
Saccharomyces acidifaciens
Saccharomyces elegans 105
Saccharomyces rouxii
Saccharomyces lactis
Saccharomyces fructum

Suitable bacteria include species from the genera *Bacillus*, *Mycobacterium*, *Actinomyces*, *Nocardia*, *Pseudomonas*, *Methanomonas*, *Protaminobacter*, *Methylococcus*, *Arthrobacter*, *Methylomonas*, *Brevibacterium*, *Acetobacter*, *Micrococcus*, *Rhodopseudomonas*, *Corynebacterium*, *Microbacterium*, *Achromobacter*, *Methylobacter*, *Methylosinus* and *Methylocystis*. Preferred genera include *Bacillus*, *Pseudomonas*, *Protaminobacter*, *Micrococcus*, *Arthrobacter* and *Corynebacterium*.

Examples of suitable species include: 120

	<i>Bacillus subtilis</i>	<i>Microbacterium ammoniophilum</i>	
	<i>Bacillus cereus</i>	<i>Archromobacter coagulans</i>	
	<i>Bacillus aureus</i>	<i>Brevibacterium butanicum</i>	
5	<i>Bacillus acidi</i>	<i>Brevibacterium roseum</i>	
	<i>Bacillus urici</i>	<i>Brevibacterium flavum</i>	60
	<i>Bacillus coagulans</i>	<i>Brevibacterium lactofermentum</i>	
	<i>Bacillus mycoides</i>	<i>Brevibacterium paraffinolyticum</i>	
	<i>Bacillus circulans</i>	<i>Brevibacterium ketoglutamicum</i>	
10	<i>Bacillus megaterium</i>	<i>Brevibacterium insectiphilum</i>	
	<i>Bacillus licheniformis</i>		
	<i>Pseudomonas methanolica</i>	Suitable fungi include species from the genera <i>Aspergillus</i> , <i>Monilia</i> , <i>Rhizopus</i> , <i>Penicillium</i> , <i>Mucor</i> , <i>Alternaria</i> and <i>Helminthosporium</i> .	65
	<i>Pseudomonas ligustri</i>	Examples of suitable species of fungi include:	70
15	<i>Pseudomonas oryzae</i>	<i>Aspergillus niger</i>	
	<i>Pseudomonas methanica</i>	<i>Aspergillus glaucus</i>	
	<i>Pseudomonas fluorescens</i>	<i>Aspergillus flavus</i>	
	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus terreus</i>	
	<i>Pseudomonas oleovorans</i>	<i>Aspergillus itaconicus</i>	75
	<i>Pseudomonas putida</i>	<i>Penicillium notatum</i>	
20	<i>Pseudomonas boreopolis</i>	<i>Penicillium chrysogenum</i>	
	<i>Pseudomonas pyocyanea</i>	<i>Penicillium glaucum</i>	
	<i>Pseudomonas methylphilus</i>	<i>Penicillium griseofulvum</i>	80
	<i>Pseudomonas brevis</i>	<i>Penicillium expansum</i>	
	<i>Pseudomonas acidovorans</i>	<i>Penicillium digitatum</i>	
25	<i>Pseudomonas methanooxidans</i>	<i>Penicillium italicum</i>	
	<i>Pseudomonas aerogenes</i>	<i>Rhizopus nigricans</i>	
	<i>Protaminobacter ruber</i>	<i>Rhizopus oryzae</i>	85
	<i>Corynebacterium simplex</i>	<i>Rhizopus delemar</i>	
	<i>Corynebacterium hydrocarboxydans</i>	<i>Rhizopus arrhizus</i>	
30	<i>Corynebacterium alkanum</i>	<i>Rhizopus stolonifer</i>	
	<i>Corynebacterium oleophilus</i>	<i>Mucor mucedo</i>	
	<i>Corynebacterium hydrocarboclastus</i>	<i>Mucor genevensis</i>	
	<i>Corynebacterium glutamicum</i>		
	<i>Corynebacterium viscosus</i>		
35	<i>Corynebacterium dioxydans</i>		
	<i>Corynebacterium alkanum</i>		
	<i>Micrococcus cerificans</i>		
	<i>Micrococcus rhodius</i>		
	<i>Arthrobacter rufescens</i>		
40	<i>Arthrobacter parafficum</i>		
	<i>Arthrobacter simplex</i>		
	<i>Arthrobacter citreus</i>		
	<i>Methanomonas methanica</i>		
	<i>Methanomonas methanooxidans</i>		
	<i>Methylomonas agile</i>		
45	<i>Methylomonas albus</i>		
	<i>Methylomonas rubrum</i>		
	<i>Methylomonas methanolica</i>		
	<i>Mycobacterium rhodochrous</i>		
50	<i>Mycobacterium phlei</i>		
	<i>Mycobacterium brevicale</i>		
	<i>Nocardia salmonicolor</i>		
	<i>Nocardia minimus</i>		
	<i>Nocardia corallina</i>		
	<i>Nocardia butanica</i>		
55	<i>Rhodopseudomonas capsulatus</i>		
		The growth of the microorganism is sensitive to the operating temperature of the fermenter and each particular microorganism has an optimum temperature for growth. The broad temperature range employed for the fermentation process of this invention would be from about 30°C to 65°C and more preferably between 35°C and 60°C. The temperature selected will generally depend upon the microorganism employed in the process since they will have a somewhat different temperature/growth rate relationship.	90
		In the practice of the present invention, a suitable nutrient medium is supplied to the fermenter to provide nutrients such as an assimilable source of nitrogen, phosphorus, magnesium, calcium, potassium, sulfur and sodium as well as trace quantities of copper, manganese, molybdenum, zinc, iron, boron, iodine and selenium. As is well known in the art of fermentation, the relative amounts of the above nutrients can vary depending on the microorganism selected for the process. In addition, the nutrient medium can also contain vitamins as is known in the art when their	95
			100
			105
			110

presence is known to be desirable for the propagation of certain microorganisms. For example, many yeasts appear to require the presence of one or both of the vitamins, biotin and thiamin for their proper propagation. A typical example of a suitable nutrient medium is as follows:

One Liter Aqueous Solution

Component	Amount
H_2PO_4 (85%)	2.0 ml
KCl	1.0 g
$MgSO_4 \cdot 7H_2O$	1.5 g
$CaCl_2 \cdot 2H_2O$	0.2 g
NaCl	0.1 g
Trace Mineral Solution	5.0 ml

The trace mineral solution as listed in the above recipe is formulated as given in the recipe below:

One Liter Aqueous Solution (Trace Mineral Solution)

Component	Amount
$CuSO_4 \cdot 5H_2O$	0.06 g
KI	0.08 g
$FeCl_3 \cdot 6H_2O$	4.80 g
$MnSO_4 \cdot H_2O$	0.30 g
$Na_2MoO_4 \cdot 2H_2O$	0.20 g
$ZnSO_4 \cdot 7H_2O$	2.00 g
H_3BO_3	0.02 g

When using the nutrient medium described above the source of assimilable nitrogen is supplied by the separate addition of aqueous ammonia (NH_4OH) to the fermentation vessel. The amount of NH_4OH added will depend upon the pH desired for the reaction mixture. Without any added NH_4OH the pH will be about 2, for the nutrient medium. Preferably for the utilization of yeasts or fungi in the fermentation process the pH is preferably in the range of approximately 3—5 and for the utilization of bacteria the pH should preferably be in the range of approximately 6—7.5.

The fermentation reaction is an aerobic process wherein the oxygen needed for the process can be supplied from a free oxygen-containing source such as air which is suitably supplied to the fermentation vessel at a pressure of from approximately 1—100 atmospheres and preferably from 1—10 atmospheres. One good source of oxygen is oxygen enriched air. The fermentation reaction is often favorably affected by use of pressure within the above-described broad and preferred ranges.

Preferably the fermentation process of the instant invention is a continuous type but it is to be noted that it can be conducted as a batch process. In the continuous or batch process modes of operation the fermentation reactor is

first sterilized and subsequently inoculated with a culture of the desired microorganism in the presence of all the required nutrients including oxygen and the carbon source. In the continuous method of operation the oxygen source or air is continuously introduced along with continuous introduction of nutrient medium, nitrogen source (if added separately) and alcohol at a rate which is either predetermined or in response to need which can be determined by monitoring such things as alcohol concentration, dissolved oxygen, and oxygen or carbon dioxide in the gaseous effluent from the fermenter. The feed rate of the various materials can be varied so as to obtain as rapid a cell growth as possible consistent with efficient utilization of the alcohol feed, i.e., a high yield of cell weight per weight of alcohol feed charged.

As is known in the art, the feed rate of the alcohol is an important variable to control since in high concentration this material can actually inhibit cell growth and may even kill the microorganism. Therefore, the feed rate of the alcohol is adjusted such that the alcohol is consumed by the microorganism at essentially the same rate as it is being fed to the fermenter. When this condition is attained there will be, of course, little or no alcohol in the effluent which is continuously withdrawn from the fermenter in a continuous type of process. However, satisfactory operation can be achieved with up to about 0.5 percent by volume alcohol concentration in the effluent. For high cell productivity or growth rate, the concentration of alcohol in the feed to the fermenter should be from about 7 percent up to about 30 percent by volume.

For batch or continuous operation of the process of this invention, the concentration of feedstock, e.g., methanol, in the culture medium should be within the range of from 0.001 up to 5 percent (v/v) and preferably from 0.005 up to 0.5 percent (v/v). It is possible, of course, and may in some instances be desirable, to add the feedstock incrementally to an otherwise typical batch fermentation process.

It is well known in the art that instrumentation is available to measure cell density, pH, dissolved oxygen and alcohol concentration in the fermenter as well as the feed and effluent streams so as to provide a rather complete monitoring of the fermentation process with the instrumentation being adapted to control the input rates so as to optimize the process. The materials fed to the fermenter are preferably subjected to sterilization as is normally done in the art in order to prevent contamination of the desired fermentation mixture by unwanted viable microorganisms.

The effluent removed from the fermentation vessel is suitably treated for separating the microbial cells, containing single cell protein, therefrom. The usual method of treatment is

well known to those in the art and employs the use of heat and/or chemical reagents, e.g., acids, to kill the microbial cells and aid in their separation from the aqueous phase by coagulation or flocculation of the cells. After this treatment the mixture is next centrifuged to remove most of the liquid phase and then the separated cells are further dried such as by drum dryers or spray dryers. If yeast is used as the culture the above sequence of steps can be modified by first centrifuging the effluent to separate the cells which are then killed by heat prior to or during a later drying step. After separation and drying, the cells which contain a high amount of protein are then ready or available for use as a food source by animals and/or humans.

The single cell protein produced by the above process has a particularly important utility in the world today. As has been increasingly emphasized in recent years, the supply or abundant and inexpensive protein available for human or animal consumption such as fishmeal and soya bean meal is being strained by an ever-increasing world population and recent reduction in production of certain types of protein as, for example, fishmeal based on anchovy fishing harvests. The production of single cell protein (SCP) offers a way to alleviate this situation by providing a source of protein suitable for inclusion in the diets of poultry, swine, and cattle which directly or indirectly provide protein for humans. The

microbial cells produced according to the above process are suitable single cell protein sources and can thus be employed for food purposes. It is known that the protein produced by this process can be employed in other areas such as the production of proteinaceous adhesive compositions and the like. The following are typical examples of the above process.

EXAMPLE I.

Three fermentation runs were conducted with methanol as the carbon and energy source in a fermenter operating under essentially foam-filled conditions. Said fermenter was of the general type described above. The volume of said fermenter was about 1500 liters. In each run the temperature was maintained at 39°C and the pH at 6.6. In each run essentially no methanol was detected in the fermenter effluent and the methanol concentration in the feed was 10 percent by volume. The nutrient medium employed in these runs was that previously described. The micro-organism employed in each of these runs was a bacterium characterized as a *Pseudomonas* species and was *Pseudomonas methanica* as identified by the depository number NRRL B3449. The data presented in Table I below were taken after each run had reached essentially steady state operation (after about 12 hours continuous operation). The runs were carried out at 3 different pressures as shown in the table.

TABLE I

	Run No.		
	1	2	3
Pressure atmospheres	1	1.97	2.6
Fermenter charge, kg	830	810	750
Air flow, m ³ /hr	164	125(a)	68.5(b)
Dissolved O ₂ in fermenter, %(c)	48	55	15
O ₂ Level in exhaust air, %(d)	78	67	40
Medium feed rate, l/hr	145	235	270
NH ₄ OH (25%) feed rate (e) kl/hr	0.8	2.3	4
Dry cell wt, g/l	22.7(f)	30.6(g)	24.6(f)
Fermenter stirrer, rpm	1110	950	940
<u>Calculated Values</u>			
Dilution rate, hr ⁻¹	0.175	0.29	0.36
Retention time, hr.	5.7	3.44	2.8
Aeration rate/V/V/min	3.3	2.5	1.5
O ₂ Consumed, kg/kg cells	3.6	2.3	3.3
Cell yield, kgCH ₃ OH (h)/kg cells	3.48	2.58	3.21
Crude protein, % (i)	75	75	75
Productivity, g cells/l/hr	4.0	8.9	8.8

(a) At 2 atmospheres inlet pressure.

(b) At 2.75 atmospheres inlet pressure.

(c) Based on dissolved O₂ content with no cells present.(d) Based on normal O₂ content of air.(e) Approximate values of NH₄OH (25% by wt NH₃) consumption.

(f) Cells isolated by filtration of a sample through a Millipore filter.

"Millipore" is a Registered Trade Mark.

(g) Cells isolated by centrifuging a 10 cc sample, washing cells, recentrifuging, drying, and weighing cells.

(h) Based on methanol consumed.

(i) Nitrogen content of cells by Kjeldahl analysis $\times 6.25$.

The results of these runs demonstrate the excellent productivity results of the continuous fermentation process using an essentially foam filled fermenter with oxygen transfer capabilities of about 1000 mmole O₂ per liter per hour of liquid fermentation reaction mixture.

EXAMPLE II. (Control)

A continuous fermentation run (4) was also carried out using the same bacterial culture, nutrient medium, and methanol concentration in the feed as the runs of Example I. The temperature (40°C) and pH (6.3) were also very close to the same values used in the runs of Example I. However, this run employed a conventional large tank equipped with a simple blade stirrer as the fermentation vessel operated at atmospheric pressure. Volume of the fermentation mixture was about 1125 liters. No

methanol was detected in the fermenter effluent. A dry cell weight of 19.1 g/l was obtained in this run. Other calculated results are presented below for this run:

Dilution rate, hr ⁻¹	0.12	25
Retention time, hr	8.3	
Yield, kg CH ₃ OH/kg cells	4.0	
Crude protein, %	75	
Productivity, g/l/hr	2.3	

The productivity results from this run are clearly inferior to those of Run 1 of Example I, a comparable run using the foam-filled fermenter.

EXAMPLE III.

Two other continuous methanol fermentation runs were carried out using the foam-filled fermenter employed in Example I and using the same nutrient medium as in Example I but

with a yeast culture identified as *Hansenula polymorpha*.

5 These runs employed a 10 percent by volume methanol concentration in the feed and no methanol was detected in the effluent from the fermenter. Each run was conducted at atmospheric pressure.

10 During the course of Run 5 it was discovered that the fermentation mixture had become contaminated with a filamentous fungus.

This contamination was not believed to have had a significant effect on the operating data for the run but the reactor system was sterilized before Run 6 which ran with no apparent contamination.

Data from Runs 5 and 6 are presented in Table II below. The data shown are considered typical for the continuous fermentation of methanol under the conditions shown.

TABLE II

	Run No.	
	5	6
Fermenter charge, kg	800	725
Temperature, °C	38	39
Air flow, m ³ /hr	101	121
Dissolved O ₂ in fermenter, % (a)	24	— (b)
O ₂ Level in exhaust air, %	— (b)	84
pH	3.5	3.6
Medium feed rate, l/hr	112	86
NH ₄ OH(25%) feed rate (c), l/hr	— (b)	1
Dry cell wt, g/l	26	24
Fermenter stirrer, rpm	1000	980
<u>Calculated Values</u>		
Dilution rate, hr ⁻¹	0.14	0.12
Retention time, hr	7.2	8.4
Aeration rate, V/V/min	2.1	2.8
O ₂ Consumed, kg/kg cells	3	3.4
Cell yield, kg CH ₃ OH (d)/kg cells	3.04	3.29
Crude protein, % (e)	54	54
Productivity, g/l/hr	3.6	2.9

(a) See footnote (c) Table I.

(b) Not determined.

(c) See footnote (e) Table I.

(d) See footnote (h) Table I.

(e) See footnote (i) Table I.

These results demonstrate the use of a yeast for the continuous fermentation of methanol in a foam-filled fermenter to produce single cell protein.

25 Since yeast has an inherently slower growth rate than bacteria, the productivity shown in Table II is lower than was obtained when using bacteria.

WHAT WE CLAIM IS:—

30 1. A process for the production of single cell protein, which comprises aerobically culturing an alcohol utilising microorganism in a foamed

culture medium containing one or more C₁—C₁₀ straight chain aliphatic alcohols as a main source of assimilable carbon, a source of assimilable nitrogen and the inorganic salts and other factors necessary for the growth of the microorganism, allowing the single cell protein to accumulate in the culture medium and recovering the accumulated single cell protein from the culture medium, wherein the culture is performed in a fermentation vessel in which the foamed culture medium is maintained in a state of substantially continuous internal circulation.



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with a yeast culture identified as *Hansenula polymorpha*.

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	5	6
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Temperature, °C	38	39
Air flow, m ³ /hr	101	121
Dissolved O ₂ in fermenter, % (a)	24	— (b)
O ₂ Level in exhaust air, %	— (b)	84
pH	3.5	3.6
Medium feed rate, l/hr	112	86
NH ₄ OH(25%) feed rate (c), l/hr	— (b)	1
Dry cell wt, g/l	26	24
Fermenter stirrer, rpm	1000	980
Calculated Values		
Dilution rate, hr ⁻¹	0.14	0.12
Retention time, hr	7.2	8.4
Aeration rate, V/V/min	2.1	2.8
O ₂ Consumed, kg/kg cells	3	3.4
Cell yield, kg CH ₃ OH (d)/kg cells	3.04	3.29
Crude protein, % (e)	54	54
Productivity, g/l/hr	3.6	2.9

(a) See footnote (e) Table I.

(b) Not determined.

(c) See footnote (e) Table I.

(d) See footnote (h) Table I.

(e) See footnote (i) Table I.

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1. A process for the production of single cell protein, which comprises aerobically culturing an alcohol utilising microorganism in a foamed

culture medium containing one or more C₁—C₁₀ straight chain aliphatic alcohols as a main source of assimilable carbon, a source of assimilable nitrogen and the inorganic salts and other factors necessary for the growth of the microorganism, allowing the single cell protein to accumulate in the culture medium and recovering the accumulated single cell protein from the culture medium, wherein the culture is performed in a fermentation vessel in which the foamed culture medium is maintained in a state of substantially continuous internal circulation.

(2) (14)



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- 8
2. A process according to claim 1, wherein said main source of assimilable carbon is a C_1-C_6 straight chain aliphatic alcohol.
- 5 3. A process according to claim 2, wherein the main carbon source is methanol.
4. A process according to claim 3, wherein the microorganism is *Pseudomonas methanica*.
- 10 5. A process according to claim 3, wherein the microorganism is *Hansenula polymorpha*.
6. A process according to claim 3, 4 or 5, wherein the methanol concentration in the culture medium is in the range 0.001 to 5 vol. per cent.
- 15 7. A process according to any one of the preceding claims, wherein the fermentation is conducted at superatmospheric pressure.
8. A process according to any one of the

preceding claims, wherein the fermentation is conducted at a temperature in the range 30 to 65°C. 20

9. A process according to any one of the preceding claims, wherein the fermentation is conducted on a continuous basis.

10. A process according to claim 1 substantially as hereinbefore described in Example I or III. 25

11. Single cell protein when produced by a process claimed in any one of the preceding claims.

For the Applicants,
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Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1978.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.