

PATENT SPECIFICATION (11)

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 (72) Inventor DONALD OLIVER HITZMAN



(54) PROCESS FOR THE PRODUCTION OF SINGLE CELL PROTEIN

(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 invention relates to the production of single cell protein.

Efforts to relieve the impending worldwide shortages of protein have included various biosynthesis processes wherein biologically produced single cell protein (SCP) is obtained by the growth of a variety of microorganisms on a variety of carbon-containing substrates.

The carbon and energy sources used as substrates for such processes should be available widely, relatively cheap, uniform, and safe in that they do not leave harmful residues in the proteinaceous product ultimately obtained by the microbial conversions. Petroleum hydrocarbons have been employed as the carbon and energy source material, but have faced practical difficulties in the lack of water solubility, in the high consumption of oxygen to assist in the microbial conversion, and allegedly in traces of potentially carcinogenic agents from the petroleum feedstocks entering or adhering to the protein product.

Other processes have used oxygenated hydrocarbon derivatives as feedstocks due to the water solubility of such derivatives and hence ease of handling since microbial conversion processes are essentially conducted under aqueous conditions. Such feedstocks are readily available either from petroleum sources, natural gas sources, various waste/garbage processing and conversion of methane, and the like, from fermentation of various grains and the like, destructive distillation of wood, and so on. Such oxygenated hydrocarbons, whatever their source, are widely available and relatively cheap

feedstocks for fermentation processes. Advantages accrue in that these feedstocks are partially oxygenated, so that substantially reduced molecular oxygen requirements are involved for the microbial conversion-growth process itself.

However, another difficult and limiting factor in the commercialization of single cell protein processes has been the necessity to conduct the fermentation at relatively moderate temperatures of about 20 to 50°C., and preferably not over about 35°C. Microbial conversions are exothermic oxidation reactions with large quantities of heat being produced. Heat must be removed from the fermentation admixture continuously and consistently, or risk the overheating of the system and either the death of the microorganisms or at least severe limitations of growth encountered as temperatures rise, and hence loss in efficiency.

Many processes have concentrated on the employment of one or other of the many available yeasts as the microorganism. Yeast cells generally are slightly larger than a bacteria cell, and sometimes provide easier separation from the fermentation process media.

However, bacteria offer advantages, since higher crude protein contents of the cell are obtained from bacteria as compared to the product obtainable from yeasts in general, since the yeasts have higher proportions of nonprotein structural material in their cells. Bacteria usually have a significantly higher true protein content, frequently being higher in the nutritionally important sulfur amino acids and lysine.

Discovery of bacteria with the capability of rapid growth and high productivity rates at relatively high fermentation process temperature growth operation means less heat to be removed, less cooling apparatus involved, and ultimately relatively smaller amounts of heat needed for sterilization, coagulation, and separation processes. Danger of contamination with other microorganisms is greatly reduced

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when high temperature fermentation can be employed. Thus, thermophilic or thermotolerant bacteria are definitely needed for commercialization of the single cell protein process.

In accordance with the present invention, we have discovered a unique thermophilic mixed culture of bacteria, containing three separate species of bacteria, and which is useful in the preparation of single cell protein from culture media containing an oxygenated hydrocarbon as the carbon source.

The mixed thermophilic culture used in this invention exhibits highly desirable and useful properties. For example it exhibits higher growth rates at elevated temperature than at room temperature and it produces high cell yields, with reduced foaming tendencies under fermentation conditions.

In particular the culture grows effectively with high productivity on oxygenated hydrocarbon feedstocks, particularly lower alcohols,

most preferably methanol or ethanol, at temperatures wherein most other known bacteria species either are relatively unproductive, or simply cannot survive, or are unproductive and intolerant of an oxygenated hydrocarbon feedstock.

The mixed culture used in this invention has been deposited with the official depository United States Department of Agriculture, Agricultural Research Service, North Central Region, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, under NRRL Deposit No. B-8158 and will be referred to hereinafter under this deposit number.

As indicated, NRRL B-8158 is a mixed culture comprising three microorganisms, the individual properties of which, so far as we have been able to determine them are set forth below, the three component microorganisms being identified as A, B and C.

NRRL B-8158

Culture	A	B	C	
Genus	Bacillus	Bacillus	Unknown (probably pseudomonas)	
Spore Forming	Yes	Yes	No	
Aerobic	Yes	Yes	Yes	
Approximate size (μ)	0.5x1-2.5	0.6x1-5	0.5x1	
Motility	Yes	Yes	Yes	
Optimum temp. °C	55°	55°	55°	
Pref. temp range (°C)	50-65°	50-65°	45-60°	
pH range	5-9	5-9	5-9	
Optimum pH	6.2-6.8	6.2-6.5	6-7	
Growth factors	none	none	±	
		(Culture C seems to require presence of other two organisms for growth to occur when it is in a methanol substrate)		
Pigments in IM ₂	tan; H ₂ O sol;	Yellow; H ₂ O	pink; H ₂ O soluble	
medium + 1.5% CH ₃ OH	insoluble in organic solvents	sol; insol. in organic solvents		
Cell appearance	large curved rods to long chains	large rods	small rods, no chains	
Colony appearance in IM ₂ medium + 1.5% CH ₃ OH	Circular raised; pale tan	Circular raised; off white	small, pin point	

NRRL B-8158 (continued)

5	Culture	A	B	C	70
	Growth at 55°C on:				
10	Nutrient broth (IM ₂)	—	—	±	75
15	Nutrient broth (IM ₂) + 1.5% CH ₃ OH	±	+	±	80
	IM ₂ +0.5% CH ₃ OH	+	+	±	
	IM ₂ +1.5% CH ₃ OH	+	+	±	
20	IM ₂ +1.5% glucose	—	—	±	85
	IM ₂ +1.5% C ₂ H ₅ OH	+	+	±	
25	IM ₂ +1.5% HCHO	+	+	NR	90
	BHM+1.5% CH ₃ OH + 0.1% NaCl	+	+	NR	
30	BHM+5% CH ₃ OH	+	+	NR	95
	Plate count + 1.5% CH ₃ OH	translucent spreading edges	translucent glistening small	—	
35	Footnotes:	1) IM ₂ medium, see Example II hereinafter			100
		2) BHM medium, see US Patent 3 981 774 column 10			
		3) NR = not recorded			
40		4) ± indicates that the culture maintains itself but without visible signs of growth			105
45	The thermophilic mixed culture described above is compositionally stable. For example, the mixed culture has been isolated from a fermentation run and lyophilized under usual conditions which comprise rapidly freezing the microbial cells at a very low temperature followed by rapid dehydration under high vacuum, and storage at room temperature. To determine viability, some of the lyophilized samples were subsequently reactivated and subjected to growth of the lyophilized mixed culture under the same conditions utilized previously. The subsequent fermentations employing the reactivated culture gave essentially the same results in terms of high cell yield, and apparent composition of the fermentation culture, as did the earlier runs. Microscopic examination of the reactivated culture indicated the same three organisms in the same form and relationship existing in the reconstituted culture as in the source fermentation.				110
50	The mixed culture used in this invention was discovered in a soil sample taken two feet below the surface of earth covering a steam line at the Bartlesville, Oklahoma, Research Center of Phillips Petroleum Company. Conventional enrichment techniques in the presence of methanol were employed to isolate several separate or distinct cultures. During the course of the work in which several pure thermophilic cultures were isolated, it became apparent that a most unusual compositionally stable mixed culture of thermophilic organisms also had been obtained.				115
55	Repeated attempts at separation were made on this culture in order to isolate the pure microorganisms. For example, streak plates gave isolated colonies of the three organisms on a mineral agar media containing methanol. However, when the isolated colonies were transferred to aqueous media for further growth with methanol as the carbon and energy sources				120
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material, surprisingly and unexpectedly no growth took place. Repeated efforts of this type were carried out, but without success.

The cooperative growth exhibited by this mixed culture appears to indicate a symbiotic relationship between the three microorganisms. Theorizing, and without intending to be bound by such theorizing, but rather in an effort to help explain the relationship observed, it appears possible that a metabolic product or products of at least one of the microorganisms serves as a necessary substrate for the growth of one or other of the other microorganisms. While the exact nature of such a metabolite is not known at present, it appears possible that such metabolite may be toxic in nature to the microorganism which produces it, thereby explaining the need for the presence of the additional microorganism to consume this toxic metabolite in order for the first microorganism to continue to grow adequately.

A further indication of this symbiotic relationship that appears to exist between the three microorganisms making up this culture is the fact that fermentations employing NRRL B-8158 produce significantly less quantities of foam under typical aerobic fermentation conditions as compared to the quantities of foam normally observed under equivalent typical aerobic fermentation conditions but employing pure thermophilic bacteria *Bacillus* genus.

The carbon source used in the process of this invention is an oxygenated hydrocarbon. The term oxygenated hydrocarbon is a generic term descriptive of the compounds employable, and not necessarily a limiting term referring to the source of the substrate. The oxygenated hydrocarbons include alcohols, ketones, esters, ethers, acids, and aldehydes, which are carbon-oxygen-hydrogen-containing water-soluble compounds and are substantially water-soluble in character. The oxygenated hydrocarbons preferably should be of up to about 10 carbon atoms per molecule for better water solubility, since higher molecular weights tend to reduce water solubility level.

Illustrative examples include: methanol, ethanol, propanol, butanol, pentanol, hexanol, 1,7-heptanediol, 2-heptanol, 2-methyl-4-pentanol, pentanoic acid, 2-methylbutanoic acid, 2-pentanol, 2-methyl-4-butanol, 2-methyl-3-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 2-propanol, formic acid, acetic acid, propanoic acid, formaldehyde, acetaldehyde, propanal, butanal, 2-methylpropanal, butanoic acid, 2-methylpropanoic acid, pentanoic acid, glutaric acid, hexanoic acid, 2-methylpentanoic acid, heptanedioic acid, heptanoic acid, 4-heptanone, 2-heptanone, octanoic acid, 2-ethylhexanoic acid, glycerol, ethylene glycol, propylene glycol, 2-propanone, 2-butanone, diethyl ether, methyl ethyl ether, dimethyl ether, di-n-propyl ether and n-propyl isopropyl ether, including mixtures of any two or more.

Petroleum gases, such as natural gas, such as methane, or other low carbon gases such as ethane and the like, can be oxidized to provide mixtures of predominantly the corresponding alcohols, as well as miscellaneous minor amounts of miscellaneous ketones, aldehydes, ethers, acids, and the like.

Among the oxygenated hydrocarbons, particularly preferred are the water-soluble alcohols of 1 to 7 carbon atoms per molecule. Such alcohols include both linear and branched alcohols, primary, secondary, as well as tertiary. Such alcohols can be monohydroxy, as well as polyhydroxy.

Exemplary alcohol species include such as methanol, ethanol, propanol, butanol, pentanol, hexanol, 1,7-heptanediol, 2-heptanol, 2-methyl-4-pentanol, 2-pentanol, 2-methanol-4-butanol, 2-methyl-3-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 2-propanol, glycerol, ethylene glycol and propylene glycol, including mixtures of any two or more.

Most preferred alcohols are those of 1 to 4 carbon atoms per molecule, and especially methanol because of its relatively low cost, wide availability, water solubility, and since governmental regulations on use are less cumbersome than with ethanol. Methanol also is readily obtainable by simple oxidative conversion of natural gas, which is primarily methane, and thus is or can be made readily available in many areas of the world which presently have surplus stocks of methane and little market for same, and correspondingly also sometimes have large populations of hungry people in this needlessly protein-deficit world.

A presently commercially available material sometimes termed "Methyl Fuel" Chemical and Engineering News, September 17, 1973, page 23) is exemplary of a commercially available mixture of methanol and controlled percentages of higher alcohols containing up to about 4 carbon atoms per molecule, and could be employed as a suitable substrate.

Since the fermentation process of this invention is a process of aerobic fermentation, there must also be supplied adequate oxygen for the fermentation admixture. Aerobic fermentation processes basically are well known in the art, and means of supplying oxygen to fermentation admixtures are also well known. Generally, the supply of molecular oxygen to the aqueous fermentation reaction admixture can be provided by passing adequate volumes of air of ordinary oxygen content, or oxygen-enriched air if desired, or air and an augmented supply of such as pure oxygen separately, through the fermentation vessel. Offgases can be recovered recycled if desired, for maximum utilization of oxygen, such as by stripping carbon dioxide from the offgases and recycling. In effect, using the oxygenated hydrocarbon as carbon and energy source substrate, a part of the oxygen-demand needed for growth of the microorganism is supplied by the oxygen

content of the substrate. Nevertheless, additional quantities of molecular oxygen must be supplied for suitable growth, since the assimilation of the substrate and corresponding growth of the microorganism is, in a sense, a combustion process. Usually from 0.1 to 10, more usually from 0.7 to 2.5, actual volumes per minute of air are supplied to the fermentation admixture per actual volume of aqueous liquid in the fermentor, equivalent to from 0.02 to 2.1, preferably from 0.14 to 0.55 volumes of oxygen.

The pressures employed can vary over a wide range e.g. from 0.1 to 100 atmospheres (10.13-10, 132 kPa), more usually from 1 to 30 atmospheres (101.3-3, 039 kPa), and preferably from 1 to 5 atmospheres (101.3-506.5 kPa). Pressures greater than atmospheric pressure are advantageous in the process since such higher pressures tend to increase the dissolved oxygen content in the aqueous fermentation medium, which in turn tends to promote more rapid microbial growth.

The fermentation process of this invention is advantageously carried out at a temperature in the range of 45 to 65°C, more preferably from 50 to 60°C.

High concentrations of some of the described carbon and energy source substrates, such as methanol, may be inhibitory to satisfactory microbial growth or even toxic to the microorganisms in the fermentations employing the mixed culture, and should be avoided.

The fermentation process can be carried out in a batch or in a continuous fashion, the preferred for economical SCP production is the growing of microbial cells in large quantities in a continuous process. A continuous process is particularly suitable when a carbon and energy source material is a lower alcohol, such as methanol or ethanol, which lends itself readily to controllable feed. The use of such microorganisms and the use of such feedstocks in a batch fermentation process is considered to be generally uneconomical on a practical basis. However, such substrates are conveniently utilized in a continuous fermentation process with overall high efficiencies and economies. In the fermentation, after the fermentor has been properly inoculated with the mixed culture species, the oxygenated hydrocarbon can be added as a separate stream, or admixed with water as an aqueous stream to sterilize same, or with mineral media to sterilize same, or any or all of these. Usually, it is fed separately for ease of control in a concentration in the feed stream to the fermentor broadly in the range of 2.5 to 35 weight percent, more usually and conveniently 10 to 15 weight percent.

Culturing is accomplished in an aqueous growth medium comprising an aqueous mineral salt medium, the carbon and energy source material, molecular oxygen, and, of course, a starting inoculum of the mixed culture.

The fermentation growth rates can be adjusted by controlling the feed of oxygenated hydrocarbon. The feed rate of the carbon and energy source material should be adjusted so that the amounts being fed to the fermentor substantially are the same as the rate of consumption by the organism to avoid a significant buildup in the fermentor, particularly of any toxic materials which might inhibit the growth or even kill the microorganisms. A satisfactory condition usually can be exhibited by observation of little or no carbon and every feed material in the effluent being withdrawn from the fermentor, though a satisfactory check also can be obtained by watching the feed source material in the fermentor effluent so as to maintain at a desirable low level of from 0 to 0.2 weight percent.

Generally the retention time of microbial cells in the fermentor means in a continuous process is of the order on the average of from 2 to 4 hours under such conditions, though this is not critical and can vary widely.

As is customary, the fermentation medium will contain mineral nutrients and a source of assimilable nitrogen, in addition to the molecular oxygen, and the carbon and energy sources as described. The source of nitrogen can be any nitrogen-containing compound capable of releasing nitrogen in a form suitable for metabolic utilization by the organism. While a variety of organic nitrogen source compounds such as proteins or urea, or the like, can be employed, usually inorganic nitrogen source materials are more economical and practical. Preferred nitrogen sources are ammonia or ammonium hydroxide, as well as various other ammonium salts such as ammonium carbonate, ammonium citrate, ammonium phosphate, ammonium sulfate and ammonium pyrophosphate. Ammonia gas is convenient and can be employed by simply bubbling such through the aqueous fermentation media in suitable amounts.

The pH of the aqueous microbial fermentation admixture should preferably be in the range, 5.5 to 7.5, more preferably from 6 to 7. Feed of the ammonia assists in controlling the pH desired, since otherwise the aqueous media tends to be slightly acidic. Of course, pH range preferences for microorganisms generally are dependent to some extent on the media employed, and thus the pH preference may change at least slightly with a change in mineral media, for example.

In addition to the oxygen, nitrogen, and carbon and energy source, it is necessary to supply selected mineral nutrients in necessary amounts and proportions to the feed media in order to assure proper microorganism growth, and to maximize the assimilation of the oxygenated hydrocarbon by the cells in the microbial conversion process.

A source of phosphate or other phosphorus, magnesium, calcium, sodium, manganese,

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molybdenum, and copper ions appear to provide the essential minerals. A typical mineral nutrient medium useful in the fermentation process of this invention is as follows:

<u>FM-12 Medium</u>		
	<u>Component</u>	<u>Amount</u>
10	H ₃ PO ₄ (85%)	2 ml
	KCl	1 g
15	MgSO ₄ · 7H ₂ O	1.5 g
	CaCl ₂ · 2H ₂ O	0.2 g
	NaCl	0.1 g
	Trace Mineral Solution	5 ml
20	Distilled Water	To make 1 liter
<u>Trace Mineral Solution</u>		
25	CuSO ₄ · 5H ₂ O	0.06 g
	KI	0.08 g
	FeCl ₃ · 6H ₂ O	4.80 g
	MnSO ₄ · H ₂ O	0.30 g
30	Na ₂ MoO ₄ · 2H ₂ O	0.20 g
	ZnSO ₄ · 7H ₂ O	2.00 g
	H ₃ BO ₃	0.02 g
	H ₂ SO ₄ (conc.)	3 ml
35	Distilled Water	To make 1 liter

Other mineral medium concentrations also can be employed, examples of which are provided in this disclosure in the Examples section.

In either a batch, or in the preferred continuous operation, all equipment, reactor or fermentation means, vessel or other container, piping and attendant circulating or cooling devices, should be sterilized, such as by the employment of steam at about 250°F. for several minutes, e.g. about 15 minutes, prior to actual startup. The sterilized reactor then is inoculated with a culture from our mixed culture in the presence of all the required nutrients, and including the molecular oxygen and the oxygenated hydrocarbon feed.

In the fermentation process, as the culture begins to grow, the introduction of air or other molecular oxygen, nutrient medium, nitrogen source if added separately, and the alcohol or other oxygenated hydrocarbon, are maintained. The addition rate of the feed stream or streams can be varied so as to obtain as rapid a cell growth as possible consistent with the utilization of the carbon and energy source input, so that the objective of a maximized high yield cell weight per weight of feed charged is obtained. Of course, any of the feed streams

can be added either incrementally or continuously as desired or convenient.

Instrumentation can be maintained to measure cell density, pH, dissolved oxygen content, oxygenated hydrocarbon concentration in the fermentor admixture, temperature, feed rates of input and output streams, and the like. It is preferred that materials fed to the fermentor be sterilized prior to introduction into the fermentor. When the oxygenated hydrocarbon is a material capable of sterilizing other materials, such as ethanol, or methanol, in some instances it may be convenient to add this component to other streams, such as the mineral media, in sterilizing amounts, and thus accomplish several purposes without the necessity for separate heat sterilization of the mineral media, for example, thus providing as maximum and economical an operation as possible. Heat added to any stream ultimately generally must be taken out by cooling means in the fermentor, since the aerobic fermentation is one in which heat is being produced.

The type of fermentor employed appears not to be critical. High productivity of the mixed culture with alcohol appears to be best achieved in a continuous process. Of course, watch must be maintained to control growth rates to avoid foam-out of the fermentor which could lower the effective liquid volume and cause some loss of fermentor contents. In this respect the process of this invention has a distinct advantage in that only low levels of foam are produced and liquid levels can be more readily maintained in the fermentor without danger of foam-out. Particularly, addition of antifoam to the fermentation admixture is to be avoided, if at all possible, since antifoams such as the silicones may be detrimental to the dissolved oxygen content at the recommended high fermentation temperatures, and may cause organisms to grow at a slower rate, or even to die. The foam produced with the mixed culture is not harmful to growth, and definitely is beneficial in maintaining the organisms in a system of high dissolved oxygen content. Foam helps provide relatively large areas of gas/liquid contacting interfaces. Thus, fermentation can be improved and heat transfer improved as to control, uniformity, and avoidance of hot spots.

Of course, a foam-inducing substance such as some of the detergents, preferably non-ionic, could be employed, if desired, to assist or induce additional foaming, though normally this is not necessary, or even desirable.

The recovery of microbial cells from the fermentation process can be accomplished by the usual techniques, such as acidification of fermentation effluent to a pH of such as about 4, and heating the acidified effluent to a temperature suitable to kill the microorganisms, such as about 80°C., though low enough not to damage the protein product. The effluent then can be centrifuged, washed, and recentrifuged

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to recover the microbial cells from the fermentor effluent. The cells can be treated to cause lysis to expedite recovery of protein and other materials from the cell. The fermentation also produces a number of desirable by-products which can be recovered from the fermentor effluent. These extracellular products can be economically helpful in the overall process, since they include valuable products such as polysaccharides, amino acids, such as glutamic acid, enzymes, vitamins, and the like.

The single cell protein produced in accordance with this invention is a valuable source of protein for humans as well as for animals. For human consumption, the cells can be treated to reduce the nucleic acid content, if desired, though for animal feed purposes such treatment does not appear necessary.

The following Examples are illustrative.

20 EXAMPLE I

A continuous fermentation run utilizing the thermophilic mixed culture of the instant invention was carried out. A 7 liter fermentor equipped with an aerator, stirrer, dissolved oxygen monitor, and means for measuring the controlling temperature and pH of the fermentation mixture, was charged with about 500 ml of fermentation reaction mixture from a previous fermentation run utilizing the thermophilic mixed culture NRRL - B8158, and 10 ml of methanol as the inoculant. The reactor also was charged with 2 liters of fermentation mineral medium FM-12.

The stirring rate was maintained at 1,000 rpm throughout the course of the run and the pH was controlled at from 6.2 to 6.35 by addition as necessary of ammonium hydroxide solution.

Air was introduced into the fermentor at a rate of 2 liters per minute throughout the course of the run. At 6 hours into the run essentially pure oxygen also was introduced to the fermentor at a rate of 0.5 liters per minute, then was increased to 0.75 liters per minute at 118 hours, to 1.5 liters per minute at 174 hours and to 2 liters per minute at 190 hours during the run.

The medium continuously charged initially was the FM-12 medium previously described. At 22 hours, the mineral medium was changed to an aqueous composition of 7.5% by volume methanol in addition to the FM-12 medium plus 0.75 grams of potassium chloride, twice the normal trace mineral content, three times the normal manganese component content (all on a per liter basis), and with deletion of sodium chloride. At 166 hours, the feed was changed to an aqueous composition of 10% by volume methanol, 2.5 ml phosphoric acid (85%) per liter, 2 grams per liter potassium chloride, 1.75 grams per liter magnesium sulfate $\cdot 7H_2O$, 0.25 grams per liter of calcium chloride $\cdot 2H_2O$, 20 ml per liter of a manganese sulfate $\cdot H_2O$ aqueous solution (0.3 g/l), and 35 ml per liter of the trace mineral solution previously described.

The media feed rate during the run ranged from 700 ml per hour at 22 hours to 817 ml per hour at 118 hours, 781 ml per hour at 166 hours, and 763 ml per hour at 382 hours.

Samples of the fermentation effluent were withdrawn from time to time to recover the cells therefrom. Values obtained for cell content in terms of dry weight of cells in grams per liter and the yields calculated are presented below in Table I. Also shown in the table are values

Table I

40 Time, Hours	46	118	166	190	286	358	105
Retention Time, Hours	2.36	2.27	2.38	2.83	2.33	2.43	
45 Cells(a) g/l	26.86	27.13	27.04	35.21	35.53	35.57	110
Solids(b) g/l	26.82	27.66	27.87	35.5	36.66	36.76	115
50 Cell Yield(c) %	44.7	46.1	46.47	44.4	45.8	45.9	
55 Productivity(d) g/l/hr	11.4	12.2	11.7	12.5	15.7	15.1	120

(a) Value obtained by evaporating a 10 ml sample of fermenter effluent overnight at 100°C and subtracting weight of mineral solids contained in 10 ml of medium.

60 (b) Value obtained by centrifuging 100 ml sample of fermenter effluent, resuspending solids in distilled water and centrifuging again to recover solids which are dried overnight at 100°C. 125

(c) Value obtained by dividing recovered solids (g/l) by methanol charged (g/l) $\times$ 100.

65 (d) Value obtained by dividing recovered solids (g/l) by retention time (hr). 130

calculated for productivity in terms of grams of cells per liter per hour for the fermentation process.

At about 126 hours into the run, samples of the fermentation admixture were withdrawn and prepared for lyophilization of the microbial cells according to procedures known in the art. These lyophilized samples then were stored for subsequent use in a later fermentation run.

Periodic samples also were taken from the fermentation reaction mixture for microscopic examination of the microbial cells in terms of their gross morphology. Such microscopic examination showed that the culture was composed of a large Gram positive curved rod, a large Gram negative rod, and a small Gram negative rod. Occasionally, a large Gram positive rod (not curved) also was observed, but this was believed to be a transitory variant of the large curved Gram positive rod.

EXAMPLE II

After about one month, a single tube of the lyophilized microbial culture from the fermentation run of Example I above was opened aseptically by conventional procedures and added to 100 ml of a fermentation medium designated IM2 which also contained 0.5% methanol. The composition of medium IM2 is shown below.

IM2 Medium	
Component	Amount, g
KH ₂ PO ₄	2.0
K ₂ HPO ₄	3.0
MgSO ₄ · 7H ₂ O	0.4
CaCl ₂ · 2H ₂ O	0.04
NaCl	0.1
(NH ₄) ₂ SO ₄	2.0
Trace Mineral Solution	0.5 ml
Distilled Water	To make 1 liter

The flask charged with the revived lyophilized culture was incubated with shaking at 55°C. After 24 hours, the shake flask showed good growth of the culture and 5 ml of the mixture was transferred to 100 ml of medium IM2 also containing 1.5% by volume methanol. Good growth developed within 24 hours and a third transfer was made to the same medium in two flasks, each containing 500 ml of IM2 medium plus 1.5% by volume methanol. This third transfer involved 100 ml of the culture being added to each of the two flasks. The culture was allowed to grow for 32 hours and then was utilized as the inoculant for a continuous fermentation run in the apparatus described above in Example I. The fermentor was charged with 1,000 ml of FM-12 medium, and 1,000 ml of the inoculant to which was added 10 ml of methanol. The temperature was maintained at 55°C, and the pH was controlled at from 6.25 to 6.4 by the continuous addition of ammonium hydroxide solution as before. Initially the stirrer was operated at 300 rpm, and air was introduced at a rate of 0.5 liters per minute, while the culture was permitted to establish itself in the fermentor. After 7 hours, a continuous introduction of feed media having the same composition as the last named feed media shown in Example I above was introduced. In addition, the air rate was increased to 2 liters per minute while the rpm was set at 1,000 for the stirrer. After 30 hours, the air rate was reduced to 1.75 liters per minute, while oxygen was introduced at 0.75 liters per minute, later increased to 1 liter per minute at 54 hours, and to 1.5 liters per minute at 198 hours. Again the fermentation effluent was sampled from time to time to provide data on the cell content in terms of grams per liter based on a dry weight and in terms of the yield and productivity of the fermentation. The data obtained during the run are presented below in Table II.

Table II(a)

Time, Hours	70	166	190	214
Retention Time, Hours	2.60	2.70	2.63	2.64
Cells g/l	34.33	33.56	33.87	34.84
Solids g/l	34.44	35.4	34.55	35.52
Cell Yield %	43.6	44.2	43.2	44.2
Productivity g/l/hr	13.2	13.1	13.1	13.5

(a) See footnotes for Table I above.

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Samples of the microbial culture were also obtained during the fermentation run for microscopic examination as described in Example I. In this instance, there was also observed the large Gram positive curved rods and the large and small Gram negative staining rods. At 238 hours into the run, the culture was lost while attempting to change the feed to a higher alcohol concentration.

10 EXAMPLE III

Presented below in Table III are analytical

Table III
Chemical Analysis of Microbial

15 Cells Obtained in A Run With NRRL-8158

Crude Protein(a), wt. %	85.63
Ash, wt. %	9.19
Amino nitrogen, wt. %	13.4
20 Carbon, wt. %	44.7
Hydrogen, wt. %	6.79
Nitrogen, wt. %	13.7
Phosphorous, wt. %	1.71
Potassium, wt. %	0.91
25 Magnesium, wt. %	0.26
Calcium, wt. %	0.1
Sodium, wt. %	<0.01
Iron, ppm	1300
30 Zinc, ppm	55.8
Manganese, ppm	126
Copper, ppm	20

(a) Nitrogen content (13.7) x 6.25.

data characterizing the microbial culture obtained in the run of Example I in terms of a chemical analysis of the microbial cells recovered. In Table IV there is also presented an amino acid content analysis of microbial cells recovered from another fermentation run utilizing the mixed thermophilic culture NRRL-B8158. For purposes of comparison, an amino acid content analysis of a pure thermophilic microorganism (HTB-7) obtained during the course of the isolation of the thermophiles from the initial soil sample previously described is also presented in Table IV.

It can be noted that the percentage of total amino acids which are essential amino acids is slightly higher for the mixed culture product (47%) than for the pure culture product (46%). Furthermore, if the essential sulfur-containing amino acids are supplied by addition of synthetic methionine, which is very likely since essentially all SCP's have been found to be low in these amino acids, the Chemical Score values show that the mixed culture product is twice as good from a nutritional standpoint as the pure culture product based on the next lowest essential amino acid Chemical Score Value.

WHAT WE CLAIM IS:-

1. A process for the production of single cell protein which comprises aerobically culturing a thermophilic bacterium in an aqueous culture medium at a temperature in the range 45 to 60°C, said medium containing one or more oxygenated hydrocarbons as a source of assimilable carbon, a nitrogen source and the

Table IV
Amino Acid Content of Thermophilic
Cultures Grown on Methanol: Grams
Per 100 Grams Product

	Pure (HTB-7)	Chem. Score(1) (2) Values	Mixed (NRRL-B-8158)	Chem. Score(1) Values	
45 <u>Essential Amino Acids</u>					110
leucine	5.37	75	5.76	104	
isoleucine	4.56	83	4.90	118	
50 lysine	5.54	96	5.67	141	115
methionine	1.50	{ 34	1.22	{ 36	
55 cystine	*		*		120
threonine	2.95	90	2.79	88	
phenylalanine	2.68	{ 80	2.78	{ 88	
60 tyrosine	2.55		2.72		125
tryptophan	0.60	43	0.89	90	
65 valine	5.13	91	5.46	121	130



Samples of the microbial culture were also obtained during the fermentation run for microscopic examination as described in Example I. In this instance, there was also observed the large Gram positive curved rods and the large and small Gram negative staining rods. At 238 hours into the run, the culture was lost while attempting to change the feed to a higher alcohol concentration.

EXAMPLE III

Presented below in Table III are analytical

Table III
Chemical Analysis of Microbial
Cells Obtained in A Run With NRRL-8158

Crude Protein ^(a) , wt. %	85.63
Ash, wt. %	9.19
Amino nitrogen, wt. %	13.4
Carbon, wt. %	44.7
Hydrogen, wt. %	6.79
Nitrogen, wt. %	13.7
Phosphorous, wt. %	1.71
Potassium, wt. %	0.91
Magnesium, wt. %	0.26
Calcium, wt. %	0.1
Sodium, wt. %	<0.01
Iron, ppm	1300
Zinc, ppm	55.8
Manganese, ppm	126
Copper, ppm	20

(a) Nitrogen content (13.7) x 6.25.

data characterizing the microbial culture obtained in the run of Example I in terms of a chemical analysis of the microbial cells recovered. In Table IV there is also presented an amino acid content analysis of microbial cells recovered from another fermentation run utilizing the mixed thermophilic culture NRRL-B8158. For purposes of comparison, an amino acid content analysis of a pure thermophilic microorganism (HTB-7) obtained during the course of the isolation of the thermophiles from the initial soil sample previously described is also presented in Table IV.

It can be noted that the percentage of total amino acids which are essential amino acids is slightly higher for the mixed culture product (47%) than for the pure culture product (46%). Furthermore, if the essential sulfur-containing amino acids are supplied by addition of synthetic methionine, which is very likely since essentially all SCP's have been found to be low in these amino acids, the Chemical Score values show that the mixed culture product is twice as good from a nutritional standpoint as the pure culture product based on the next lowest essential amino acid Chemical Score Value. WHAT WE CLAIM IS:—

1. A process for the production of single cell protein which comprises aerobically culturing a thermophilic bacterium in an aqueous culture medium at a temperature in the range 45 to 60°C, said medium containing one or more oxygenated hydrocarbons as a source of assimilable carbon, a nitrogen source and the

Table IV
Amino Acid Content of Thermophilic
Cultures Grown on Methanol: Grams
Per 100 Grams Product

	Pure (HTB-7)	Chem. Score ⁽¹⁾ (2) Values	Mixed (NRRL-B-8158)	Chem. Score ⁽¹⁾ Values	
Essential Amino Acids					
leucine	5.37	75	5.76	104	
isoleucine	4.56	83	4.90	118	
lysine	5.54	96	5.67	141	
methionine	1.50	{ 34	1.22	{ 36	
cystine	*		*		
threonine	2.95	90	2.79	88	
phenylalanine	2.68	{ 80	2.78	{ 88	
tyrosine	2.55		2.72		
tryptophan	0.60	43	0.89	90	
valine	5.13	91	5.46	121	



Table IV. (continued)

**Amino Acid Content of Thermophilic
Cultures Grown on Methanol: Grams
Per 100 Grams Product**

5				70
	<u>Non Essential Amino Acids</u>			
10	alanine	5.65	6.19	75
	arginine	3.39	3.01	
	aspartic acid	6.50	6.26	
15	glycine	3.71	4.19	80
	glutamic acid	10.47	10.69	
20	histidine	1.26	1.22	85
	proline	2.32	2.38	
	serine	2.09	1.75	
25	Total Essential amino Acids	30.88	32.19	90
	Total Amino Acids	66.27	67.88	
30	Crude Protein	85.63	84.4	95
	(1) Chemical Score Values: based on essential amino acid content of egg as 100 for same weight of protein.			
	(2) Based on averages from five pure thermophile runs.			
35	(* = not detected)			100

mineral salts and other growth factors essential to the growth of the said bacterium, wherein there is used a bacterial mixture having the characteristics of NRRL B-8158 as hereinbefore defined.

2. A process according to claim 1, wherein the oxygenated hydrocarbon is a water-soluble alcohol, ketone, ester, ether, acid or aldehyde containing up to 10 carbon atoms per molecule, or a mixture of two or more thereof.

3. A process according to claim 2, wherein the oxygenated hydrocarbon is methanol, ethanol, a propanol, a butanol, a pentanol, a hexanol, 1,7-heptanediol, 2-heptanol, 2-methyl-4-pentanol, pentanoic acid, 2-methylbutanoic acid, 2-pentanol, 2-methyl-4-butanol, 2-methyl-3-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 2-propanol, formic acid, acetic acid, propanoic acid, formaldehyde, acetaldehyde, propanal, butanal, 2-methylpropanal, butanoic acid, 2-methylpropanoic acid, pentanoic acid, glutaric acid, hexanoic acid, 2-methylpentanoic acid, heptanedioic acid, heptanoic acid, 4-heptanone, 2-heptanone, octanoic acid, 2-ethylhexanoic acid, glycerol, ethylene glycol, propylene glycol, 2-propanone, 2-butanone, diethyl ether, methyl ethyl ether, dimethyl ether, di-n-propyl ether, n-propyl

isopropyl ether, or a mixture of two or more thereof.

4. A process according to claim 2, wherein the oxygenated hydrocarbon is a monohydric or polyhydric alcohol of 1 to 7 carbon atoms.

5. A process according to any one of the preceding claims, wherein the culture is conducted at a temperature in the range 50° to 60°C.

6. A process according to any one of the preceding claims, wherein the culture medium is maintained at a pH in the range 5.5 to 7.5.

7. A process according to claim 6, wherein said pH is maintained in the range 6 to 7.

8. A process according to any one of the preceding claims, wherein the oxygenated hydrocarbon is methanol and the conditions are maintained so that the amount of methanol in the fermentor effluent is in the range of 0 to 0.2 weight percent.

9. A process according to any one of the preceding claims, wherein from 0.02 to 2.1 actual volumes of oxygen are supplied per minute per volume of liquid in the culturing medium, and said culture medium is maintained under a pressure in the range 0.1 to 100 atmospheres.

10. A process according to any one of

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the preceding claims, wherein the culture is conducted under foam culture fermentation conditions.

5 11. A process according to any one of the preceding claims, wherein, at the end of the culturing process the bacterial cells are subjected to lysis before recovery of the single cell protein from the culture medium.

10 12. A process according to claim 1, substantially as hereinbefore described in any of

the preceding Examples.

13. Single cell protein when produced by a process according to any one of the preceding claims.

For the Applicants,
D. YOUNG & CO.,
Chartered Patent Agents,
9 & 10 Staple Inn,
London WC1V 7RD.