SPECIFICATION

Production of yeast cells at high cell densities

The invention is concerned with the production of single cell protein.

Efforts to relieve world-wide shortages of protein have included various bio-synthesis processes in which single cell protein (SCP) is obtained by the growth of one or another of a variety of micro-organisms on various carbon-containing substrates.

The carbon energy substrates should be readily available, relatively cheap, uniform, and safe. Petroleum hydrocarbons have been used as carbon energy source, but have faced practical difficulties in the lack of water-solubility and in the high consumption of molecular oxygen needed to assist in the microbial conversion. Other processes have centred on the use of oxygenated hydrocarbon derivatives as feedstocks due to their relative water-solubility and hence ease of handling in an aqueous ferment, and in the substantially reduced molecular oxygen requirements for the microbial conversion-growth process.

However, a limiting factor in the commercialization of single cell protein processes has been the necessity to control the ferment at relatively moderate cell densities, with only moderate yields of dried cells based on substrate consumed, and the consequent necessity of handling large amounts of total fermentation effluent liquor in order to recover the moderate amounts of SCP material. Handling large quantities of aqueous fermentation effluent liquor complicates concentration of the single cell protein product in such apparatus as centrifuges, as well as washing and drying steps.

Known continuous processes for the production of single cell protein materials from yeast cultures typically gave ferment effluents of relatively low yeast cell contents, such as 20 to 25 grams of cells per liter of ferment.

Some processes in the past have concentrated on the culturing of bacteria because of the slightly higher crude protein contents of the cell as compared to the content obtainable from yeast in general. However, yeasts are widely available and relatively simply cultured. Yeast cells are generally slightly larger as compared to bacteria cells, and, hence, yeast cells tend to be more easily separated from the fermentation effluent liquor.

Discovery of means and methods to increase cell yields, and particularly to operate at high cell densities, would be highly desirable. The resultant handling of substantially less fermentation liquor effluent volume, for example, would mean large savings in reduced sizes of piping and pumps, reduced makeup water requirements with reduced sterilization requirements, and reduced requirements of equipment sizing and handing for coagulation and separation processes.

This invention is based on the discovery of a method of operating a continuous aerobic fermentation process using yeast cultures at very high cell densities ordinarily unobtainable, and of producing high yields of yeast single cell protein product, by adding media containing high concentrations of mineral salts to the ferment in the fermentor. Cell density is defined as the concentration of cells by weight on a dry basis per volume of ferment. The ferment is defined as the total volume of aqueous fermentation broth or liquor, including cells. Cell density is usually expressed as grams/liter. Yield is defined as the amount of cells by weight produced for a given consumption by weight of carbon energy source or substrate fed to the ferment, and is usually expressed in grams/gram.

The present invention provides a method of producing a single cell protein material which comprises continuously culturing under aerobic fermentation conditions in aqueous mineral salts ferment at least one yeast species using effective yeast-culturing fermentation conditions, including a pH in the range of 3 to 7 and the presence of amounts of carbon energy substrate selected from carbohydrates, alcohols, ketones, aldehydes, acids, C_{1-20} esters, and C_{10-20} normal paraffins, assimilable nitrogen source, make-up water, oxygen and an aqueous medium comprising mineral salts and trace elements, and recovering the resulting micro-organisms as a single cell protein material, in which the mineral salts are fed to the aqueous ferment at such a rate as to maintain in the aqueous ferment the following elements in at least the designated weights per liter of aqueous ferment: P—1.9 g, K—1 g, Mg—0.15 g, Ca—0.06 g, S—0.1 g, Fe—6 mg, Zn—2 mg, Cu—0.6 mg, and Mn—0.6 mg, thereby maintaining a cell density in the aqueous ferment of at least 60 and up to 160 grams, on a dried basis, per liter of aqueous ferment.

The invention thus involves in almost force-feeding of the cells by adding highly concentrated (relatively) mineral salts into the ferment, resulting in high growth rates. The mineral salts concentration in the liquid supernatant of the ferment (that is, the ferment excluding cells) itself remains, of course, at relatively low levels, since the salts are consumed by the cells in growth and reproduction. The salts concentration in the cells plus liquid supernatant, thus, is very high.

The high cell densities obtainable by this process significantly streamline and reduce the cost of single cell protein production. The fermentation produces yeast cells at relatively very high levels, such as above 100 grams per liter of ferment. Such high cell densities in the ferment, coupled with high yields of yeast cells, mean more efficient effective production, particularly under continuous production conditions. Concentrating the single cell protein product, e.g. by centrifuge means, is sharply reduced or even eliminated in many instances. The cellular product can if desired be washed to

remove residual unconsumed salts, and sent directly to a drier such as a spray drier. The washings contain most of the remaining mineral salts not incorporated into the cells, and can be recycled to the fermentor. Requirements for water to the fermentation step are thus reduced considerably, and, importantly, there is little or no waste water requiring disposal. Alternatively, if desired, the total ferment including residual salts can be dried.

Culturing is accomplished in a growth medium comprising an aqueous mineral salts medium, the carbon energy source material, molecular oxygen, assimilable nitrogen, and of course, a starting inoculum of one or more particular species of yeast micro-organisms to be used.

In this invention, high concentrations of mineral salts are fed to the ferment and high concentrations are maintained in the ferment. It is necessary to supply suitable amounts in proper proportions of selected mineral nutrients in the feed media, in order to assure proper micro-organism growth, to maximize assimilation of the carbon and energy source by the cells in the microbial conversion process, and to achieve maximum cellular yields with maximum cell density in the fermentation media.

Although the composition of the ferment can vary over a wide range, depending in part on the yeast and substrate employed, the minerals content in the ferment (that is, liquid plus cells) in accordance with this invention is relatively high, higher than previously considered suitable or practiced by the prior art. Set forth in the table below are the minimum, broad, and preferred ranges of concentrations of various elements in the ferment, the concentration being expressed as of the element, though it is recognized that all or part of each can be present in the form of a soluble ion, or in cases such as P are present in a combined form of some type such as phosphate. The amount of each element is expressed in grams or milligrams per liter of ferment (aqueous phase, including cells):

Weight of Element per Liter of Ferment

Element	*Minimum*	*Broad Range*	*Preferred Range*
P	1.9 g	1.9—20 g	2.2—10 g
K	1 g	1—20 g	1.5—10 g
Mg	0.15 g	0.15—3 g	0.3—1.2 g
Ca	0.06 g	0.06—1.6 g	0.08—0.8 g
S	0.1 g	0.1—8 g	0.2—5 g
Fe	6 mg	6—140 mg	9—80 mg
Zn	2 mg	2—100 mg	3—40 mg
Cu	0.6 mg	0.6—16 mg	1—10 mg
Mn	0.6 mg	0.6——20 mg	0.9—8 mg

Sulfur is desirably in the form of sulfate. Some of the metals required are advantageously added in the form of a sulfate, so that the minimum concentrations of sulfur are normally exceeded. Any or all of the metals listed can be used or present as the sulfate. Preferably, the magnesium, calcium, iron, zinc, copper, and manganese are in the form of a sulfate or chloride, or in the form of a compound converted *in situ* to a sulfate or chloride. The potassium is preferably a sulfate, chloride, or phosphate or in the form of a compound converted in situ to a sulfate, chloride, or phosphate. The phosphorus is preferably in the form of phosphoric acid or in the form of a phosphate, monohydrogen phosphate, or dihydrogen phosphate, e.g., as a potassium or ammonium salt, or as a compound which is converted in situ to such a salt.

Other elements which may be present, at least in trace amounts, include such as sodium and cobalt, e.g., as a halide or sulfate; molybdenum, e.g., as molybdate; boron, e.g., as borate; selenium, e.g., as selenite or selenate; or iodine, e.g., as iodide.

In typical high cell density fermentation, the ferment will comprise about one-half supernatant medium and one-half cells, by volume. These one-half by volume cells, however, will contain at least about two-thirds of the mineral salts content of the ferment.

According to this process, a culture of yeast is used which is suitable for growth on carbon-containing substrates under aqueous fermentation conditions. Suitable yeasts include species from the genera *Candida, Hansenula, Torulopsis, Saccharomyces, Pichia, Debaryomyces,* and *Brettanomyces.* The preferred genera include *Candida, Hansenula, Torulopsis, Pichia,* and *Saccharomyces.* Examples of suitable species include:

Brettanomyces petrophilium
Canida boidinii
Candida lipolytica
Candida mycoderma
Candida utilis
Candida stellatoidea
Candida robusta
Candida claussenii
Pichia farinosa
Pichia polymorpha
Pichia membranaefaciens
Pichia pinus
Pichia pastoris
Pichia trehalophila
Saccharomyces cerevisiae
Saccharomyces fragilis

Candida rugosa
Candida tropicalis
Debaryomyces hansenii
Hansenula minuta
Hansenula saturnus
Hansenula californica
Hansenula mrakii
Hansenula silvicola
Hansenula polymorpha
Hansenula wickerhamii
Hansenula capsulata
Hansenula glucozyma
Hansenula henricii
Hansenula nonfermentans
Hansenula philodendra
Saccharomyces rosei
Saccharomyces acidifaciens
Saccharomyces elegans
Saccharomyces rouxii
Saccharomyces lactis
Torulopsis sonorensis
Torulopsis candida
Torulopsis bolmii
Torulopsis versatilis
Torulopsis glabrata
Torulopsis molishiana
Torulopsis nemodendra
Torulopsis nitratophila, and
Torulopsis pinus.

If desired, mixtures of two or more species of yeasts can be employed. The particular yeast employed depends in part on the carbon-containing substrate to be used since it is well known that different yeasts often require somewhat different substrates for best growth. For example, it is recognized that some particular strains of species listed above, such as of *Pichia pastoris*, do not grow on methanol.

Preferred are those *Pichia pastoris* and *Hansenula polymorpha* which do grow suitably on oxygenated-hydrocarbon feedstocks, particularly a C_1—C_9 alcohol such as methanol. Preferred are the particular strains designated as, or which are derived from, the strains deposited as *Pichia pastoris* (Culture 21—2) NRRL Y-11431, *Pichia pastoris* (Culture 21—1) NRRL Y-11430, *Hansenula polymorpha* NRRL Y-11170, and *Hansenula polymorpha* (Culture 21—3) NRRL Y-11432, since these strains are particularly suitable for use in producing SCP protein materials at high cell densities with high yields. This feature of these strains of *Pichia pastoris* (Culture 21—2) NRRL Y-11431 and *Pichia pastoris* (Culture 21—1) NRRL Y-11430 is considered particularly unusual for these species since out of four *Pichia pastoris* cultures tested, only two would grow on methanol at all. *Pichia pastoris* (Culture 21—2) NRRL Y-114321, *Pichia pastoris* (Culture 21—1) NRRL Y-11430, and *Hansenula polymorpha* (Culture 21—3) NRRL Y-11432, are novel and unique.

The carbon energy substrate can be any carbon energy source, such as hydrocarbons or oxygenated hydrocarbons, including various carbohydrates, suitable as yeast substrates. It is recognized that particular yeasts do vary in their preference for various substrates.

The preferred substrates for aqueous fermentation conditions are the carbon-oxygen-hydrogen significantly water-soluble compounds. The term "oxygenated hydrocarbon" is intended to be generic term in this disclosure descriptive of suitable compounds, and not necessarily a limiting term referring to the source of the substrate. For this disclosure, the oxygenated hydrocarbons include the water-soluble carbohydrates, as well as those alcohols, ketones, esters, acids and aldehydes, and mixtures of them, which are reasonably significantly water-soluble in character and usually of 1 to 20 carbon atoms per molecule. The more suitable oxygenated hydrocarbons usually are those of substantially greater water-solubility of up to 10 carbon atoms per molecular, or are the water-soluble carbohydrates generally.

Exemplary carbohydrates include glucose, fructose, galactose, lactose, sucrose, starch, and dextrin, alone or in admixture. Of the other types of oxygenated hydrocarbons, examples include methanol, ethanol, ethylene glycol, propylene glycol, 1-propanol, 2-propanol, glycerol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 1-pentanol, 2-hexanol, 1,7-heptanediol, 1-octanol, 2-decanol, 1-hexadecanol, 1-eicosanol, acetone, 2-butanone, 4-methyl-2-pentanone, 2-decanone, 3-pentadecanone, 2-eicosanone, formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, hexanal, 7-methyloctanal, tetradecanal, eicosanal, acetic acid, propionic acid, butyric acid, glutaric acid, 5-methylhexanoic acid, azelaic acid, dodecanoic acid, eicosanoic acid, methyl formate, methyl acetate, ethyl acetate, propyl butyrate, isopropyl hexanoate, hexyl 5-methyloctanoate, and octyl dodecanoate, as well as mixtures thereof.

It also is possible to employ in accordance with this process, though much less preferred because of the sometimes difficulty in removing residual substrate from the single cell protein cells, normal paraffins, e.g. of such as 10 to 20 carbon atoms per molecule. Yeasts generally do not assimilate paraffins of less than 10 carbon atoms per molecule. These typically include such as decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane, eicosane, and the like, and mixtures thereof.

Preferred are the water-soluble alcohols of 1 to 4 carbon atoms, water-soluble acids of 2 to 4 carbon atoms, and the water-soluble carbohydrates. Preferred are the water-soluble monohydric aliphatic hydrocarbyl alcohols. It should be noted that 2-methyl-1-propanol is inhibitory to some yeasts, and in fermentations with such yeasts this alcohol should be avoided. Particularly preferred are the alcohols of 1 to 4 carbon atoms (other than 2-methyl-1-propanol); of these methanol and ethanol are

preferred to the others; and methanol is especially preferred, due to its low relative cost.

Petroleum gases can be oxidized, and the water-soluble materials employed, such as oxidation of methane, and ethane, to provide mixtures predominantly of the corresponding alcohol as well as various aldehydes, ketones, and acids, and similarly suitable hydrocarbon fractions from various petroleum refinery sources produced within the integrated refining and chemical processing complex, sometimes termed a petrocomplex, can be utilized for fermentation purposes.

The salts in the supernatant are at a relatively low concentration, since there is a high take-up by the growing reproducing cells. The mineral salts in the cells may not be as fed or applied since some may be in a bound organic form. Mineral analysis of the ferment, of course, would reflect a total mineral content.

In addition to the mineral salts, vitamins (organic growth factors) can be employed in the ferment as is known in the art, where their presence is desirable for the propagation of the particular yeast chosen. For example, many yeasts for their proper propagation, seem to require the presence of one or both of the vitamins biotin and thiamine, or other medium constituents which contain these vitamins, e.g., yeast extract. Thus, for example, with a yeast such as a *Hansenula polymorpha*, it is desirable to employ biotin in an amount of 0.04 to 0.8 milligram per liter of aqueous mineral medium and thiamine hydrochloride in an amount of 4 to 80 milligrams per liter of aqueous mineral medium. Alternatively, all or part of the biotin and thiamine can be provided by use of, for example, yeast extract.

Regarding the water used in the fermentation medium, it has previously been shown that residual amounts of chlorine remaining in the water after purification tend to render ineffective the growth factors in fermentation media, particularly vitamins such as biotin or thiamine. Thus, it has been suggested that in fermentation systems employing an aqueous mineral medium prepared from water containing residual chlorine, the removal of the chlorine before adding the growth factors avoids the loss or deactivation of the growth factors. Thus, it is preferred to treat the residual chlorine-containing water so as to effectively eliminate the traces of residual chlorine and thus avoid the vitamin loss.

It has now been found that water containing amounts of residual chlorine previously thought to be objectionable can nevertheless be utilized in fermentation processes employing vitamins yet without inactivation of the vitamins by the chlorine if the vitamins are added to the fermentation zone as a separate stream from the aqueous nutrient medium. Thus, the mineral nutrient medium can now include water containing trace amounts of chlorine. This arrangement thus avoids the need for pretreating, by expensive and/or time-consuming methods, water that contains residual trace amounts of chlorine.

The above described separate addition of the vitamins to the fermentation zone is preferably and conveniently accomplished by admixing the vitamins with at least a portion of, but preferably the whole of, the carbon energy substrate stream prior to charging these materials to the fermentation zone. If an aqueous admixture of vitamins and carbon energy substrate is employed, the water used for initial dilution of the vitamins should preferably be free of traces of residual chlorine, such as deionized water, to avoid any premature loss before mixing with the aqueous carbon substrate stream such as methanol-in-water.

In a preferred modification which can be carried out if desired, an admixture can be made of water and a water-soluble carbon substrate such as methanol, such as about 20 volume percent methanol in water, and then the vitamins can be dissolved in the methanol-in-water solution, and then fed to the fermentor. By this mode, residual chlorine need not be first removed, but yet the vitamins are fully preserved.

In a preferred embodiment, the separate addition of vitamins to the fermentation zone is accomplished as follows: the vitamin is admixed with an aqueous dilution of said methanol or ethanol; a medium containing the trace minerals Fe, Zn, Mn and Cu is added and the resulting mixture is fed to the fermentation zone separately from a mineral medium containing P, K, Mg, S and Ca. The trace mineral salts may also comprise elements such as cobalt, molybdenum, boron selenium, and iodine, as well as manganese, copper, zinc and iron. The use of this preferred embodiment not only avoids the vitamin inactivation problem caused by traces of chlorine in the water used for the aqueous mineral salts medium, but also avoids another problem that is often encountered in the fermentation processes. This problem is the formation of precipitates in the heat sterilization zone employed to treat the aqueous mineral salts medium, requiring frequent cleaning. The presence of the trace mineral salts in its usual admixture with the primary mineral nutrient salts apparently promotes the formation of troublesome precipitates in the heat sterilization zone. Thus, by not including the trace mineral salts in the aqueous mineral salts medium stream, but instead charging the trace mineral salts in admixture with the vitamins and at least a portion of the carbon energy substrate solves two very troublesome problems. As noted above, the water used to prepare the admixture of trace mineral salts, at least a portion of the carbon energy substrate, and the vitamins should preferably be free of residual traces of chlorine.

The stream comprised of vitamins, a portion of the carbon energy substrate, and trace minerals can be sterilized by filtration if desired. However, it is preferable and convenient to combine said stream with the major carbon energy substrate stream prior to charging to the fermentation zone and filtering the entire combined streams just prior to charging to the fermentation zone.

The fermentation itself is an aerobic process requiring molecular oxygen, which is supplied by a molecular-oxygen-containing gas such as air, oxygen-enriched air, or even substantially pure molecular oxygen, to maintain the ferment with an oxygen partial pressure effective to assist the micro-organism species in growing in a thriving fashion. By using an oxygenated hydrocarbon substrate, the total oxygen requirements for growth of the micro-organism are reduced from the requirements when a paraffin is used. Even so, adequate quantities of molecular oxygen must be supplied for growth, since the assimilation of the substrate and corresponding growth of the micro-organisms is, in part, a combustion process.

The rate at which molecular oxygen is supplied to the ferment should be such that the growth of the yeast is not limited by lack of oxygen. Fermentor designs vary widely in their ability to transfer oxygen to the culture. Although the overall aeration rates can vary over a considerable range, with fermentors that are very efficient is oxygen transfer aeration generally is conducted at a rate of about 0.5 to 8, preferably about 1 to 6, volumes (at the pressure employed and at 25°C) of molecular oxygen-containing gas per liquid volume in the fermentor per minute. This amount is based on air of normal oxygen content being supplied to the reactor, and in terms of pure molecular oxygen the respective ranges would be about 0.1 to 1.7, or preferably about 0.2 to 1.3, volumes (at the pressure employed and at 25°C) of molecular oxygen per liquid volume in the fermentor per minute.

The pressure employed for the microbial fermentation step can range widely. Typical pressures are from 0 to 150 psig, (0 to 1034 MPa), preferably from 0 to 60 psig (0 to 414 MPa) in excess of atmospheric) in particular at least slightly over atmospheric pressure, as a balance of equipment and operating costs versus oxygen solubility achieved. Greater than atmospheric pressures are advantageous in that such pressures do tend to increase the dissolved oxygen concentration in the aqueous ferment, which in turn can help increase cellular growth rates. At the same time this is counterbalanced by the fact that high pressures do increase equipment and operating costs.

The fermentation temperature can vary somewhat, but generally will be 25°C to 65°C, generally preferably 28°C to 50°C. The yeast cultures *Pichia pastoris* Culture 21—2 deposited as NRRL Y-11431 and *Pichia pastoris* Culture 21—1 deposited as NRRL Y-11430 generally prefer a ferment temperature of about 30°C. The *Hansenula polymorpha* NRRL Y-11170 and *Hansenula polymorpha* Culture 21—3 deposited as NRRL Y-11432 appear to prefer a ferment temperature on the order of about 38°C to 40°C.

Yeasts require a source of assimilable nitrogen. The assimilable nitrogen can be supplied by any nitrogen-containing compound or compounds capable of releasing nitrogen in a form suitable for metabolic utilization by the yeast micro-organism. While a variety of organic nitrogen source compounds, such as protein hydrolysates, theoretically can be employed, usually cheaper nitrogen-containing compounds such as ammonia, ammonium hydroxide, urea, and various ammonium salts such as ammonium phosphate, ammonium sulfate, ammonium pyrophosphate, and ammonium chloride can be utilized. Ammonia gas itself is convenient for large scale operations, and can be employed by bubbling through the aqueous microbial ferment in suitable amounts. At the same time, such ammonia also assists in pH control.

The pH range in the aqueous microbial ferment should be in the range 3 to 7, more preferably and usually 3.5 to 5.5. Preferences of certain micro-organisms for a pH range are dependent to some extent on the medium employed, as well as on the particular micro-organism, and thus may change somewhat with change in medium as can be readily determined by those skilled in the art.

The average retention time of the ferment in the fermentor can vary considerably, depending in part on the fermentation temperature and yeast culture employed. Generally, the retention time will be about 2 to 30 hours, preferably 4 to 14 hours, based on average retention.

High concentrations of some of the described carbon and energy substrates, particularly those such as methanol or formaldehyde, may be inhibitory to satisfactory microbial growth or even toxic to the micro-organisms in the fermentation. It is therefore desirable to avoid excessively high concentrations of substrates and to maintain the substrate concentration in the ferment at a maximum tolerable level. With some of the lower alcohols, this level in the ferment is preferably kept at 0.001 to 5 volume percent, particularly 0.01 to 0.05 volume percent, while with the aldehydes the level is preferably kept at one-tenth of these figures due to the toxicity of aldehydes, so as to neither starve nor inhibit the growth rates of the micro-organisms chosen.

When the carbon and energy source material contains an aldehyde in amounts potentially deleterious to the micro-organism, the deleterious aldehyde effects can be alleviated by first treating the substrate with a suitable amount of a nitrogen-containing compound, preferably ammonia, ammonium hydroxide, or other active ammonium compound, in a ratio of about 0.01 to 10 mol equivalents of such nitrogen-containing compounds per mol of aldehyde. Such a treated substrate then is not only the carbon energy source, but also contains at least a portion of the necessary assimilable nitrogen.

Conveniently, the fermentation is conducted in such a manner that the carbon-containing substrate can be controlled as a limiting factor, thereby providing good conversion of the carbon-containing substrate to yeast cells and avoiding potential contamination of the yeast cells with a substantial amount of unconverted substrate. The latter is not a problem with water-soluble substrates,

since any remaining traces are readily washed off. It may be a problem, however, in the case of non-water-soluble substrates such as the higher n-paraffins, requiring added product treatment steps such as removal of residual hydrocarbon by suitable solvent washing steps.

Continuous operation is much to be preferred for ease of control, production of uniform quantities of uniform products, and most economical uses of all equipment. In a continuous process, the carbon and energy source material as substrate, aqueous mineral medium, assimilable nitrogen source, and molecular oxygen-containing gases, are added continuously to the ferment in the fermentor combined with continuous withdrawal of ferment. Although the volume ratio of added carbon energy substrate:added aqueous mineral medium can vary over a wide range, depending in part on the nature of the carbon-containing substrate, generally it will be in the range 1:9 to 6:4, preferably in the range 2:8 to 5:5.

If desired, part or all of the carbon energy source material and/or part of the assimilable nitrogen source such as ammonia can be added to the aqueous mineral medium prior to passing the aqueous mineral medium to the fermentor. Most convenient in my work in high cell density fermentations has been the use of a feed ratio of about 40 volume percent alcohol to 60 volume percent mineral salts medium.

Each of the streams introduced into the reactor is preferably controlled at a predetermined rate, or in response to a need determinable by monitoring, such as concentration of the carbon and energy substrate, pH, dissolved oxygen, oxygen or carbon dioxide in the off-gases from the fermentor, or cell density measurable by light transmittancy. The feed rates of the various materials can be varied so as to obtain as rapid a cell growth rate as possible, consistent with efficient utilization of the carbon and energy source, to obtain as high a yield of yeast cells relative to substrate charge as possible. Thus, by the process of the invention, yeast cells can be obtained in yields of about 30 to 110 grams per 100 grams substrate charged, depending in part on the particular substrate used.

All equipment, reactor, or fermentation means, vessel or container, piping, attendant circulating or cooling devices, and the like, most preferably are sterilized, usually by employing steam such as at about 250°F (121°C) for at least about 15 minutes. The sterilized reactor is inoculated with a culture of the specified micro-organism in the presence of all the required nutrients, including molecular oxygen, and the carbon-containing substrate.

The type of fermentor is not critical in the practice of the fermentation process of the invention, though operation in a foam-filled fermentor is preferred. A fermentor designed to encourage and maintain the produced foam is beneficial to the process of achieving the increased oxygen transfer necessary to maintain desired high cell densities and rapid growth rates.

In starting out a fermentation, the aqueous mineral medium, suitable concentration of carbon source, assimilable nitrogen, trace components where desired, and the starting innoculum of yeast are placed in a sterilized fermentor, and suitable flows of oxygen and the various feeds are gradually commenced. If desired, the initial fermentation substrate can be such as glucose, with gradual change to such as methanol as cell density builds up. It is possible to begin at low mineral salts levels in the aqueous ferment and build up to a high mineral salts level by feeding an aqueous mineral medium having a high concentration of mineral salts to the ferment, though the usual procedure is simply to add medium of high salt concentration to the fermentor initially to commence immediate operation. A brief lag time will usually occur at start up before the innoculum builds up enough cells for full input of salts and substrate to be utilized.

The yeast cells produced in accordance with the present process can be recovered from the fermentation admixture effluent by conventional means, such as by centrifugation or filtration. If desired, extracellular products can be recovered from the substantially cell-free remaining supernatant liquid by conventional means. The substantially cell-free effluent can be treated, for example, with acetone or a C_1—C_8 alcohol such as methanol or ethanol to precipitate any polymeric material produced extra-cellularly. The cell-free effluent also can be treated by solvent extraction and/or base extraction to recover, if desired, other extra-cellular products such as pigments, vitamins, or organic acids produced during the culturing process. The cell-free effluent, with or without such intervening treatment, can be returned to the fermentor as a part of the aqueous makeup, or as a substantial or almost total part of the aqueous makeup, to avoid waste disposal problems insofar as possible.

The microbial cells usually are killed by heat or chemical means, and this can be done before or after the separation of the cells from the fermentor effluent. The yeast cells are a valuable source of protein for man as well as beast. For human consumption, the cells can be treated as necessary to reduce the nucleic acid, but for animal feed purposes such treatment does not appear presently necessary.

In accordance with this invention, employing high cell density operation, e.g., a cell density within the range of 60 to 160, preferably 70 to 150, grams of yeast cells, on a dried basis, per liter of fermentation admixture, can be obtained in high yield. If desired, the cells can be recovered from the fermentation admixture by centrifugation or other separation means. Also, if desired, the concentrated cells then can be washed, e.g. by mixing with water, and separated, e.g. by recentrifuging, or by adding water prior to or during centrifugation to substantially free the cells of mineral medium, and the washings including the separated mineral medium can then be returned to the fermentor as water and

mineral medium makeup, thus substantially reducing or avoiding waste disposal problems. The recovered cells can then be simply dried to produce a dried product for future use. If desired, the high cell density fermentor effluent in total can be dried to produce a whole dried product of dried cells and residual water soluble substances including salts, and this whole-dried product used as a very useful animal feed of high protein-high salts character.

The following are descriptive runs employing the process of this invention. Particular amounts of materials, particular types of feedstocks, and particular species or strains of yeast should be considered as illustrative and not as limitative of this invention.

Example I

In a run conducted under continuous aerobic fermentation conditions, methanol and an aqueous mineral salts medium in a volume ratio of 30.15:69.85, respectively, were fed individually to a fermentor inoculated with the yeast *Pichia pastoris* Culture 21—2 deposited as NRRL Y-11431. No pre-conditioning medium or substrate was employed. The fermentor was a 4-liter fermentor with a 2-liter liquid volume, with automatic pH, temperature, and level control. Agitation was provided by two impellers rotating at 1000—1200 rpm. The aeration rate was 1—1.5 volumes (at about atmospheric pressure and about 25°C) per volume of ferment per minute of air supplemented with and including sufficient oxygen to maintain in the fermentation mixture an amount of dissolved oxygen equal to about 20 percent of that which would be dissolved in the fermentation mixture saturated with air at atmospheric pressure and about 30°C. Aqueous ammonium hydroxide (from 2 parts concentrated ammonium hydroxide and 1 part deionized water, by volume) was added at such a rate as to maintain the pH of the fermentation mixture at about 3.5.

The aqueous mineral salts medium employed was prepared by mixing, for each liter of solution, 12.5 ml 85 percent H_3PO_4, 2.5 g 85 percent KOH, 8.5 g KCl, 7.0 g $MgSO_4 . 7H_2O$, 1.5 g $CaCl_2 . 2H_2O$, 25 ml of trace mineral solution A, 25 ml of trace mineral solution B, 10 ml of a biotin-thiamine hydrochloride solution, about 0.08 ml of antifoam agent (Mazu DF-37C), and sufficient deionized water to make 1 liter of solution.

Trace mineral solution A was prepared by mixing, for each liter of solution, 4.8 g $FeCl_3 . 6H_2O$, 2.0 g $ZnSO_4 . 7H_2O$, 0.02 g H_3BO_3, 0.20 g $Na_2MoO_4 . 2H_2O$, 0.30 g $MnSO_4 . H_2O$, 0.08 g KI, 0.06 g $CuSO_4 . 5H_2O$, 3 ml conc. H_2SO_4, and sufficient deionized water to make 1 liter of solution.

Trace mineral solution B was prepared by mixing, for each liter of solution, 2.0 g $FeCl_3 . 6H_2O$, 2.0 g $ZnSO_4 . 7H_2O$, 0.3 g $MnSO_4 . H_2O$, 0.6 g $CuSO_4 . 5H_2O$, 2 ml conc. H_2SO_4, and sufficient deionized water to make 1 liter of solution.

The biotin-thiamine hydrochloride solution was prepared by mixing 2 mg biotin, 200 mg thiamine hydrochloride, and 50 ml deionized water.

The fermentation was conducted at about 30°C and about atmospheric pressure, with a retention time of 7.0 hours.

Yeast cells were separated from the fermentation effluent by centrifugation, washed by suspension in water and recentrifugation, dried overnight at 100°C, and weighed. On a dried basis, the yeast cells were produced in a yield of 42.3 g per 100 g of methanol fed, the cell density being at the high level of 100.7 g of cells per liter of effluent.

Example II

A further fermentation run was conducted using essentially the procedure described in Example I except that the composition of the aqueous mineral salts medium was somewhat different, the volume ratio of methanol to the aqueous mineral salts medium was 40.8 to 59.2, the aqueous ammonium hydroxide for pH control was prepared from 3 parts concentrated ammonium hydroxide and 1 part deionized water, by volume, and the fermentation retention time was 8.35 hours.

The aqueous mineral salts medium for use in this run was prepared by mixing, for each liter of solution, 20.0 ml 85 percent H_3PO_4, 4.0 g 85 percent KOH, 12.0 g KCl, 10.4 g $MgSO_4 . 7H_2O$, 2.4 g $CaCl_2 . 2H_2O$, 40 ml of the trace mineral solution A as described in Example I, 40 ml of the trace mineral solution B as described in Example I, 16 ml of the biotin-thiamine hydrochloride solution as described in Example I, about 0.08 ml of antifoam agent, and sufficient deionized water to make 1 liter of solution.

Yeast cells were separated from the fermentation effluent, washed, and dried as in Example I. On a dried basis, the yeast cells were produced in a yield of 41.4 g per 100 g of methanol fed, the cell density being at the very desirably high level of 133.3 g of cells per liter of effluent.

Example III

A continuous aerobic fermentation process was conducted in the fermentor as described in Example I, this time inoculated with the yeast species *Hansenula polymorpha* Culture 21—3 deposited as NRRL Y-11432. To the fermentor was fed a mixture of methanol and aqueous mineral salts medium containing 300 ml methanol per liter total solution. The stirred fermentation mixture was aerated by passing into the fermentor 2 volumes (at about atmospheric pressure and about 25°C) per volume of ferment per minute of air supplemented with and including sufficient oxygen to maintain in the

fermentation mixture an amount of dissolved oxygen equal to about 20 percent of that which would be dissolved in the fermentation mixture saturated with air at atmospheric pressure and about 38°C.

Aqueous ammonium hydroxide (from 2 parts concentrated ammonium hydroxide and 1 part deionized water, by volume) was added at a rate to maintain the pH of the fermentation mixture at 3.7 to 4.1.

The mixture of methanol and aqueous mineral salts medium was prepared by mixing, for each liter of solution, 300 ml methanol, 6 ml 85 percent H_3PO_4, 3 g KCl, 4.5 g $MgSO_4 . 7H_2O$, 0.6 g $CaCl_2 . 2H_2O$, 0.3 g NaCl, 10 ml of trace mineral solution A as described in Example I, 10 ml of trace mineral solution B as described in Example I, 4 ml of the biotin-thiamine hydrochloride solution described in Example I, 4 drops of antifoam agent, and sufficient deionized water to make 1 liter of solution.

The fermentation was conducted at about 38°C and about atmospheric pressure, with a retention time of 5.66 hours.

Yeast cells were separated from the fermentation effluent, washed, and dried as in Example I. On a dried basis, the yeast cells were produced in a yield of 31.0 g per 100 g of methanol fed, the cell density being at 73.3 g of cells per liter of effluent.

Example IV

In a continuous aerobic fermentation process, methanol and an aqueous mineral medium in a volume ratio of 36.9 to 63.1, respectively, were fed individually to a fermentor inoculated with the yeast species *Pichia pastoris* Culture 21—1 deposited as NRRL Y-11430. The fermentor was a 1500-liter foam-filled fermentor with a liquid volume of about 600 liters, with automatic pH, temperature, and level control, and equipped with a draft tube. Agitation was provided by a turbine below the draft tube, driven at 750 rpm. The aeration rate was about 1.6 volumes of air (at about 38 psig (262 MPa over atmospheric pressure) and about 25°C) per volume of ferment in the fermentor per minute. Anhydrous ammonia was added at a rate to maintain the pH of the ferment mixture at about 3.5.

The primary aqueous mineral salts medium was prepared by mixing, for each liter of solution, 12.2 ml 75 percent H_3PO_4, 6.0 g KCl, 6.0 g $MgSO_4 . 7H_2O$, 0.8 g $CaCl_2 . 2H_2O$, 2.0 g 85 percent KOH, 2.0 ml of trace mineral solution C, 0.8 ml of a biotin-thiamine hydrochloride solution, and sufficient tap water to make 1 liter of solution, the tap water first having been treated with enough sodium thiosulfate to react with the free chlorine present therein.

Trace mineral solution C was prepared by mixing, for each liter of solution, 65 g $FeCl_3 . 6H_2O$, 18 g $ZnSO_4 . 7H_2O$, 5.0 g $MnSO_4 . H_2O$, 6.0 g $CuSO_4 . 5H_2O$, 2.0 ml conc. H_2SO_4, and sufficient deionized water to make 1 liter of solution.

The biotin-thiamine hydrochloride solution was made by mixing the components in the ratio of 0.4 g biotin to 40 g thiamine hydrochloride to 1 liter deionized water.

The fermentation was conducted at 30°C and 38 psig (262 MPa over atmospheric) pressure, with a retention time of 12.7 hours.

Yeast cells were separated from the fermentation effluent by centrifugation, washed by suspension in water and recentrifugation, dried overnight at 100°C, and weighed. On a dried basis, the yeast cells were produced in a yield of 37 g per 100 g of methanol fed, the cell density being a very desirable 110.3 g of cells per liter of effluent.

Example V

In a continuous aerobic fermentation process, methanol and an aqueous mineral salts medium in a volume ratio of 40 to 60, respectively, were fed individually to a fermentor inoculated with the yeast species *Pichia pastoris* Culture 21—1 deposited as NRRL Y-11430. The fermentor was a 1500-liter foam-filled fermentor with a liquid volume of about 610 liters, with automatic pH, temperature, and level control. Agitation was provided by two conventional paddle-type turbines driven at 1000 rpm. The aeration rate was about 4 volumes of air (at about 38 psig (262 MPa over atmospheric pressure) and about 25°C) per volume of ferment in the fermentor per minute. Anhydrous ammonia was added at such a rate as to maintain the pH of the fermentation mixture at about 3.5.

The aqueous mineral salts medium was prepared by mixing, with each liter of tap water, 15.86 ml 75 percent H_3PO_4, 9.53 g K_2SO_4, 7.8 g $MgSO_4 . 7H_2O$, 0.6 g $CaCl_2 . 2H_2O$, and 2.6 g 85 percent KOH. The trace mineral solution plus biotin was fed separately via the methanol stream at a rate of 10 ml per liter of methanol. The trace mineral solution plus biotin was prepared by mixing 780 ml trace mineral solution D, 20 ml water, 200 ml methanol and 0.032 g biotin.

Trace mineral solution D was prepared by mixing, for each liter of solution, 65 g $FeSO_4 . 7H_2O$, 20 g $ZnSO_4 . 7H_2O$, 3.0 g $MnSO_4 . H_2O$, 6.0 g $CuSO_4 . 5H_2O$, 5.0 ml conc. H_2SO_4, and sufficient deionized water to make 1 liter of solution.

The aqueous mineral salts medium was fed at a rate of 31.5 liters per hour and the methanol at a rate of 21 liters per hour.

The fermentation was conducted at 30°C and about 38 psig (262 MPa over atmospheric) pressure, with a retention time of 11.6 hours.

For analytical purposes yeast cells were separated from the fermentation effluent by

centrifugation, washed by suspension in water and recentrifugation, dried overnight at 100°C, and weighed. On a dried basis, the yeast cells were produced in a yield of 40.6 g per 100 g of methanol fed, the *cell density* being a very desirable 128.4 g of cells per liter of effluent. The total solids content of the ferment (effluent from the fermentor) was 134.7 g per liter, cells plus dissolved solids. The effluent from the fermentor was fed directly through a pasteurizer to kill the yeast and into a sprayer dryer without further concentration or treatment.

Example VI

In a continuous aerobic fermentation process, methanol and an aqueous mineral salts medium in a volume ratio of 29 to 71, respectively, were fed individually to a fermentor inoculated with the yeast species *Hansenula polymorpha* NRRL Y-11170. The fermentor was a 1500-liter foam-filled fermentor with a liquid volume of about 560 liters, with automatic pH, temperature, and level control, and equipped with a draft tube. Agitation was provided by a turbine, below the draft tube, driven at 810 rpm. The aeration rate was about 5 volumes of air (at about 38 psig (262 MPa over atmospheric pressure) and about 25°C) per volume of ferment in the fermentor per minute. Anhydrous ammonia was added at such a rate as to maintain the pH of the fermentation mixture at about 3.5.

The aqueous mineral salts medium was prepared by mixing 10.38 ml 75 percent H_3PO_4, 4.29 g KCl, 6.44 g $MgSO_4 . 7H_2O$, 0.86 g $CaCl_2 . 2H_2O$, 0.43 g NaCl, 3.0 ml of trace mineral solution C, and 0.64 ml of a biotin-thiamine hydrochloride solution in 1000 ml tap water, the tap water first having been treated with enough sodium thiosulfate to react with the free chlorine present therein.

The fermentation was conducted at 39—40°C and 38 psig (262 MPa over atmospheric) pressure, with a retention time of 7.6 hours.

For analysis yeast cells were separated from the fermentation effluent by centrifugation, washed by suspension in water and recentrifugation, dried overnight at 100°C, and weighed. On a dried basis, the yeast cells were produced in a yield of 33.3 g per 100 g of methanol fed, the cell density being a desirable 76.2 g of cells per liter of effluent.

In research leading to the present invention there were discovered three unique cultures of yeasts having the capability of rapid growth and high producitivity rates, namely, *Pichia pastoris* Culture 21—1, deposited as NRRL Y-11431; *Pichia pastoris* Culture 21—1, deposited as NRRL Y-11430; and *Hansenula polymorpha* Culture 21—3, deposited as NRRL Y-11432. These unique cultures grow particularly well at higher mineral salts levels and cell densities.

These three unique cultures of yeasts grow effectively with high productivity on oxygenated hydrocarbon feedstocks, particularly lower alcohols, most preferably methanol or ethanol. This is particularly noteworthy with regard to the new *Pichia pastoris* cultures since some cultures of the species *Pichia pastoris* simply cannot grow on methanol. These unique species are designated as follows:

Culture Name	*Strain Designation*	*Depository Designation*
Pichia pastoris	21—2	NRRL Y-11431
Pichia pastoris	21—1	NRRL Y-11430
Hansenula polymorpha	21—3	NRRL Y-11432

The designations NRRL Y-11431, NRRL Y-11430, and NRRL Y-11432 reflect the fact that the novel yeast cultures 21—2, 21—1, and 21—3 have been deposited with the official depository, United States Department of Agriculture, Agricultural Research Service, Northern Regional Research Laboratory, Peoria, Illinois 61604.

This invention provides, in one aspect, processes for culturing oxygenated hydrocarbon-assimilating microbial cells belonging to three new cultures or strains of micro-organisms under aqueous aerobic culturing conditions. These strains have been classified:

	Pichia pastoris Culture 21—1 NRRL Y-11430	*Pichia pastoris Culture 21—2 NRRL Y-11431*	*Hansenula polymorpha Culture 21—3 NRRL Y-11432*
Division	Ascomytina	Ascomytina	Ascomytina
Class	Hemiascomycetes	Hemiascomycetes	Hemiascomycetes
Order	Endomycetales	Endomycetales	Endomycetales
Family	Saccharomycetaceae	Saccharomycetaceae	Saccharomycetaceae
Genus	Pichia	Pichia	Hansenula

The novel and unique microorganisms can be further characterized by properties as shown in the following tabulation:

Culture Property or Test Result[a]	*Pichia pastoris Culture 21-1 NRRL Y-11430*	*Pichia pastoris Culture 21-2 NRRL Y-11431*	*Hansenula polymorpha Culture 21-3 NRRL Y-11432*
Gram straining	+	+	+
Spore Forming	+	+	+
Aerobic	+	+	+
Approx. size, μ	3—5	3—5	3—5
Optimum temps., °C.	30	30	38—40
Optimum pH	3.5—5.5	3.5—5.5	3.5—5.5
Growth factors	Biotin	Biotin	Biotin and thiamine
Cell appearance on malt extract agar	Colonies turn deep tan with formation of hat-shaped spores	Form hat-shaped spores	Form hat-shaped spores
Colony appearance on YM agar	Cream colored; no pseudomycelium	Cream colored; no pseudomycelium	Cream colored no pseudomycelium
Assimilation of sugars			
Glucose	+	+	+
Galactose	W/L		
L-Sorbose	—		
Maltose	—		
Sucrose	—		
Cellobiose	—		
Trenalose	—		
Lactose	—		
Melibiose	—		
Raffinose	—		
Melezitose	—		
Inulin	—		
Soluble Starch	W/L		
Xylose	—		
L-arabinose	—		
D-arabinose	—		
D-Ribose	—		
L-Rhamnose	—		
Ethyl alcohol	+	+	+
Methyl alcohol	+	+	+
Glycerol	+		
Erythritol	—		
Adonitol	—		
Dulcitol	—		
Mannitol	+		
Sorbitol	+		
Methyl-d-glucoside	—		
Salicon	—		
Inositol	—		
Fermentation of sugars			
Glucose	+	+	+
Galactose	—		
Sucrose	—		
Lactose	—		
Maltose	—		
Raffinose	—		
Trehalose	—		
Nitrate Assimilation	—		
Urease Activity	—		
Arbutin split	—		

Culture Property or Test Result[a]	*Pichia pastoris Culture 21-1 NRRL Y-11430*	*Pichia pastoris Culture 21-2 NRRL Y-11431*	*Hansenula polymorpha Culture 21-3 NRRL Y-11432*
Fermentation of sugars			
Pseudomycelium or hyphae formed			
on corn meal	–		
on yeast agars	–	–	–
Guanine plus cytosine content of DNA	40%		

+=positive
–=negative
W/L=weak to latent reaction
(a)=if value not shown, means not determined

Of course, as with all micro-organisms, some of the characteristics may be subject to some variation depending on the medium and particular conditions.

The media recipes shown in the Examples can be used to culture these novel yeast species, though they will grow on other than methanol-containing substrates. These yeast cultures can be employed not only with the high salts-media feeds as herein described, but also can be employed under conventional fermentation conditions using a medium such as:

Medium IM—1

Component	*Amount*
KH_2PO_4	5.0 g
$MgSO_4.7H_2O$	0.5 g
$CaCl_2.2H_2O$	0.1 g
KCl	0.5 g
$(NH_4)_2SO_4$	3.0 g
Biotin	0.04 mg
thiamine	4.0 mg
Trace mineral solution[a]	2.5 ml
Water	1,000 ml
Sterile methanol[b] to give	0.5—1.0 vol %

(a) See recipe below.
(b) Added just prior to use.

Trace Mineral Solution

Component	*Amount*
$CuSO_4.5H_2O$	0.06 g
KI	0.08 g
$MnSO_4.H_2O$	0.3 g
$Na_2MoO_4.2H_2O$	0.2 g
H_3BO_3	0.02 g
$ZnSO_4.7H_2O$	2.0 g
$FeCl_3.6H_2O$	4.8 g
Distilled water	1,000 ml
H_2SO_4 (conc.)	3 ml

Claims

1. A method of producing a single cell protein material which comprises continuously culturing under aerobic fermentation conditions in aqueous mineral salts ferment at least one yeast species using effective yeast-culturing fermentation conditions, including a pH in the range of 3 to 7 and the presence of amounts of carbon energy substrate selected from carbohydrates, alcohols, ketones, aldehydes, acids, C_{1-20} esters, and C_{10-20} normal paraffins, assimilable nitrogen source, make-up water, oxygen and an aqueous medium comprising mineral salts and trace elements, and recovering the resulting micro-organisms as a single cell protein material, in which the mineral salts are fed to the aqueous ferment at such a rate as to maintain in the aqueous ferment the following elements in at least the designated weights per liter of aqueous ferment: P—1.9 g, K—1 g, Mg—0.15 g, Ca—0.06 g, S—0.1 g, Fe—6 mg, Zn—2 mg, Cu—0.6 mg, and Mn—0.6 mg, thereby maintaining a cell density in the aqueous ferment of at least 60 and up to 160 grams, on a dried basis, per liter of aqueous ferment.

Culture Property or Test Result[a]	*Pichia pastoris Culture 21-1 NRRL Y-11430*	*Pichia pastoris Culture 21-2 NRRL Y-11431*	*Hansenula polymorpha Culture 21-3 NRRL Y-11432*
Fermentation of sugars			
Pseudomycelium or hyphae formed			
on corn meal	—		
on yeast agars	—	—	—
Guanine plus cytosine content of DNA	40%		

+=positive
—=negative
W/L=weak to latent reaction
(a)=if value not shown, means not determined

Of course, as with all micro-organisms, some of the characteristics may be subject to some variation depending on the medium and particular conditions.

The media recipes shown in the Examples can be used to culture these novel yeast species, though they will grow on other than methanol-containing substrates. These yeast cultures can be employed not only with the high salts-media feeds as herein described, but also can be employed under conventional fermentation conditions using a medium such as:

Medium IM—1

Component	*Amount*
KH_2PO_4	5.0 g
$MgSO_4.7H_2O$	0.5 g
$CaCl_2.2H_2O$	0.1 g
KCl	0.5 g
$(NH_4)_2SO_4$	3.0 g
Biotin	0.04 mg
thiamine	4.0 mg
Trace mineral solution[a]	2.5 ml
Water	1,000 ml
Sterile methanol[b] to give	0.5—1.0 vol %

(a) See recipe below.
(b) Added just prior to use.

Trace Mineral Solution

Component	*Amount*
$CuSO_4.5H_2O$	0.06 g
KI	0.08 g
$MnSO_4.H_2O$	0.3 g
$Na_2MoO_4.2H_2O$	0.2 g
H_3BO_3	0.02 g
$ZnSO_4.7H_2O$	2.0 g
$FeCl_3.6H_2O$	4.8 g
Distilled water	1,000 ml
H_2SO_4 (conc.)	3 ml

Claims

1. A method of producing a single cell protein material which comprises continuously culturing under aerobic fermentation conditions in aqueous mineral salts ferment at least one yeast species using effective yeast-culturing fermentation conditions, including a pH in the range of 3 to 7 and the presence of amounts of carbon energy substrate selected from carbohydrates, alcohols, ketones, aldehydes, acids, C_{1-20} esters, and C_{10-20} normal paraffins, assimilable nitrogen source, make-up water, oxygen and an aqueous medium comprising mineral salts and trace elements, and recovering the resulting micro-organisms as a single cell protein material, in which the mineral salts are fed to the aqueous ferment at such a rate as to maintain in the aqueous ferment the following elements in at least the designated weights per liter of aqueous ferment: P—1.9 g, K—1 g, Mg—0.15 g, Ca—0.06 g, S—0.1 g, Fe—6 mg, Zn—2 mg, Cu—0.6 mg, and Mn—0.6 mg, thereby maintaining a cell density in the aqueous ferment of at least 60 and up to 160 grams, on a dried basis, per liter of aqueous ferment.

2. A process according to claim 1 in which the yeast is of the genus *Candida, Hansenula, Torulopsis, Saccharomyces, Pichia, Debaryomyces,* or *Brettanomyces.*

3. A process according to claim 2 in which the yeast is selected from the species

Brettanomyces petrophilium
Candida boidinii
Candida lipolytica
Candida mycoderma
Candida utilis
Candida stellatoidea
Candida robusta
Candida claussenii
Candida rugosa
Candida tropicalis
Debaryomyces hansenii
Hansenula minuta
Hansenula saturnus
Hansenula californica
Hansenula mrakii
Hansenula silvicola
Hansenula polymorpha
Hansenula wickerhamii
Hansenula capsulata
Hansenula glucozyma
Hansenula henricii
Hansenula nonfermentans
Hansenula philodendra
Pichia farinosa
Pichia polymorpha
Pichia membranaefaciens
Pichia pinus
Pichia pastoris
Pichia trehalophila
Saccharomyces cerevisiae
Saccharomyces fragilis
Saccharomyces rosei
Saccharomyces acidifaciens
Saccharomyces elegans
Saccharomyces rouxii
Saccharomyces lactis
Torulopsis sonorensis
Torulopsis candida
Torulopsis bolmii
Torulopsis versatilis
Torulopsis glabrata
Torulopsis molishiana
Torulopsis nemodendra
Torulopsis nitratophila, and
Torulopsis pinus

4. A process according to any one of claims 1 to 3 in which the carbon and energy source is at least one of glucose, fructose, galactose, lactose, sucrose, starch, and dextrin.

5. A process according to any one of claims 1 to 3 in which the carbon and energy source is decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane, eicosane, or a mixture of two or more of them.

6. A process according to any one of claims 1 to 3 in which the carbon and energy source is methanol, ethanol, ethylene glycol, propylene glycol, 1-propanol, 2-propanol, glycerol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 1-pentanol, 2-hexanol, 1,7-heptanediol, 1-octanol, 2-decanol, 1-hexadecanol, 1-eicosanol, acetone, 2-butanone, 4-methyl-2-pentanone, 2-decanone, 3-pentadecanone, 2-eicosanone, formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, hexanal, 7-methyloctanal, tetradecanal, eicosanal, acetic acid, propionic acid, butyric acid, glutaric acid, 5-methylhexanoic acid, azelaic acid, dodecanoic acid, eicosanoic acid, methyl formate, methyl acetate, ethyl acetate, propyl butyrate, isopropyl hexanoate, hexyl 5-methyloctanoate, octyl dodecanoate, or a mixture of two or more of them.

7. A process according to any one of claims 1 to 3 in which the carbon and energy source material is a C_{1-4} alcohol and each liter of the ferment contains the elements in the ranges: P—1.9 to 20 g, K—1 to 20 g, Mg—0.15 to 3 g, Ca—0.06 to 1.6 g, S—0.1 to 8 g, Fe—6 to 140 g, Zn—2 to 100 mg, Cu—0.6 to 16 mg, and Mn—0.6 to 20 mg.

8. A process according to claim 7 in which each liter of ferment contains the elements in the ranges: P—2.2 to 10 g, K—1.5 to 10 g, Mg—0.3 to 1.2 g, Ca—0.08 to 0.8 g, S—0.2 to 5 g, Fe—9 to 80 mg, Zn—3 to 40 mg, Cu—1 to 10 mg, and Mn—0.9 to 8 mg.

9. A process according to claim 8 in which the alcohol is methanol or ethanol.

10. A process according to claim 9 in which the aqueous ferment further contains at least one of sodium, cobalt, molybdenum, boron, and selenium.

11. A process according to claim 9 or 10 in which the aerobic fermentation conditions include the use of one or more vitamin(s).

12. A process according to claim 11 in which the vitamin(s) comprise(s) at least one of biotin and thiamine.

13. A process according to claim 12 in which the vitamin is admixed with an aqueous dilution of said methanol or ethanol; a medium containing the trace minerals, Fe, Zn, Mn and Cu is added and the resulting mixture is fed to the fermentation zone separately from a mineral medium containing P, K, Mg, S and Ca.

14. A process according to claim 9 in which the fermentation temperature is in the range 25°C to 65°C, the pressure is in the range 0 to 150 psig (0 to 1034 MPa over atmospheric) and the fermentation time is in the range 2 to 30 hours based on average retention.

15. A process according to claim 9 in which a cell density of 70 to 150 grams, on a dried basis, per liter of fermentation admixture, is maintained.

16. A process according to claim 14 using methanol as the alcohol and a strain designated as *Pichia pastoris* NRRL Y-11430 as the yeast.

17. The process according to claim 14 using methanol as the alcohol and a strain designated as *Pichia pastoris* NRRL Y-11431 as the yeast.

18. The process according to claim 14 using methanol as the alcohol and a strain designated as *Hansenula polymorpha* NRRL Y-11432 as the yeast.

19. A process according to any one of claims 9 to 18 in which the fermentation effluent liquor including cells is subjected to water washing, whereby the aqueous mineral medium remaining is substantially separated from the cells, leaving a wet cell mass, the wet cells are dried to produce dried cells, and the water-washings and separated mineral medium are recycled to provide at least in part makeup water and mineral medium.

20. A process according to any one of claims 9 to 18 in which the total fermentation effluent including cells and residual salts is dried, thereby producing a dried cell product containing residual water-soluble substances, including salts.

21. A process according to any one of claims 9 to 18 comprising centrifuging the fermentation effluent liquor including cells, thereby producing a stream of concentrated cells, and a lean recycle liquor; water-washing said concentrated cell material, and drying said washed cells.

22. A process according to claim 21 in which the lean recycle liquor and/or the water-washings form the water-washing step is/are used at least in part as at least a portion of the makeup medium.

23. A process according to claim 1 substantially as hereinbefore described in any one of Examples I to VI.

24. Single-cell protein material obtained by a process according to any one of the preceding claims.

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* C L M S *