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Single Cell Proteins: As Nutritional Enhancer.

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ABSTRACT

To meet the protein need of our growing population, it is important to include non-conventional protein sources in our diet. Important non – conventional sources are oil seed proteins, leaf protein concentrate, (LPC) fish protein concentrate (FPC) and single cell proteins (SCP) or biomass protein (BMP). Single cell protein recently attracted attention and holds a major potential for increasing protein supply. Proteins not only provide a nutritional component in a food system but also perform a number of other functions).The protein obtained from microbial source is designed as "Single Cell Protein" (SCP). Bacteria, Moulds, Yeasts, Green and Blue-green algae are widely used as source of single cell protein. However, blue-green algae, where cell wall lacks cellulose, are easily digestible and are the most frequently used organism. Microbial protein or SCP has various benefits over animal and plant proteins in that its requirement for growth are neither seasonal or climate dependent; it can be produced all round the year .Does not require a large expanse of land as in plant or animal protein production. It has high protein content with wide amino acid spectrum, low fat content, higher protein-carbohydrate ratio than forages, can be grown on waste and it is environmental friendly as it helps in recycling waste. Various forms of organic waste such as cellulose hemicelluloses, hydrocarbon and different types of agricultural waste are used in the production of SCP. Besides nutritional value, a protein should have desirable functional properties also for its incorporation in food. Functional properties of proteins vary with the source, composition, method of preparation/extraction, prevailing environment etc. SCP has been found to meet all the requirements for its inclusion as diet supplement for both human and livestock especially in the developing countries of Africa and the world at large. This paper is therefore aimed at reviewing the in production, processing and consumption of SCP for food and feed.

Key words: Single Cell Protein(SCP), Non conventional protein sources, Bacteria, Moulds, Yeasts, Algae.

INTRODUCTION

Microorganisms have been employed for many years in the production of high protein food content such as cheese and fermented soybean products . Protein being the main nutritional component in both types (Haider, AL-Barhawi, & Hassan, 1989). Since a large proportion of cell dry weight is accounted for protein, the nutritional value of a microbial derived food source is determined by the levels of protein produced (Patel, 1995).The development of microbial systems for use in food industry has Firstly, growth of microorganism is very much faster than of animals . Secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen. The two chief strategies with regard to substrate to consider low grade waste material or to use relatively simple carbohydrate source to produce microbial material containing very high quality of protein (Reed & Nagodawithana, 1995). The protein obtained from microbial source is designed as "Single Cell Protein" (SCP) (Vincent, 1969; Becker and Venktaraman, 1982). Microorganisms have the ability to upgrade low protein organic material to high protein food, and this has exploited on by industry. This phenomenon was employed in Germany during the First World War when the growth of *Saccharomyces cerevisiae* was exploited for human consumption. Moreover, *Candida arborea* and *C. utilis* were used during the Second World War and about 60% of the country prewar food input was replaced (Litchfield, 1983; Arora et al., 1991). In the 1960s, researchers at British Petroleum developed what they called "proteins-from-oil process": a technology for producing single cell protein by yeast fed by waxy n-paraffins, a product produced by oil refineries. Initial research work was done by Alfred Champagnat at BP's Lavera Oil Refinery in France; a small pilot plant there started operations in March in 1963, and the same construction of the second pilot plant, at Grangemouth Oil Refinery in Britain, was authorized(Bamberg,2000). The use of natural cheap substrates and waste industrial products for cultivating microorganisms appear to be a general trend in studies of applied nature,(Grewal et al., 1990; Osho, 1995) . In dealing with microbial protein production several natural products have been tested to this end, (Kuzmanova et al., 1989), tried to use grape juice byproduct as a carbon source. For the same purposes (Haider & EL-Hassy, 2000) tested date extract supplemented with nitrogen source as a suitable substrate, whereas, cashew apple juice was assayed by (Osho, 1995). Several investigations were carried out using cellulose and hemicelluloses waste as a suitable substrate for increasing single cell protein production (Azzam, 1992; Pessoa et al., 1997; Bozakuk, 2002; Zubi, 2005). The raw material was hydrolyzed by dilute acid or base and then supplemented with some nitrogen and phosphorous salts.

The rapidly increasing world population generates the challenge of providing necessaryfood sources. In particular protein supply poses a problem since essential amino acids cannot be replaced. One possible solution to this problem is SCP production. Bacteria and yeast are candidates for the synthesis of SCP. Bacteria grow more rapidly and efficiently than yeast on cheap substrates, and they provide a higher content of protein. Oscar Andrés Prado-Rubio et al, 2010. Single Cell Protein or SCP is a term which means that microbial cells are grown and harvested for animals or human food due to its high protein content. It is also known as novel protein, petrocrop and minifood. It was introduced by Prof. Scrimshow of M.I.T. (Massachusets Institute of Technology) to give a better image than microbial Protein/bacterial protein. Though some of the organisms that produce SCP are multicellular, it is useful in designating a potential source of food protein that is already of some importance and may become overwhelmingly so in years to come(http://www.studentsguide.in/microbiology/industrial-microbiology/single-cell-protein-scp.html) .Single cell protein refers to the "source of mixed protein extracted from pure and mixed culture of algae, yeast, fungi and bacteria" The term was coined by carol L .wilson in 1966. The term is more appropriate as most of micro organism grow as single or filamentous individual. These are described as dried cells of microorganisms which are grown and allowed multiplying in large fermeters. Microorganisms have the ability to upgrade low protein organic material to high protein food, and this has been exploited on by industry. This phenomenon was employed in Germany during the First World War when the growth of *Saccharomyces cerevisiae* was exploited for human consumption. Moreover, *Candida arborea* and *C. utilis* were used during the Second World War and about 60% of the country prewar food input was replaced (Litchfield, 1983; Arora, Mukerji, & Marth, 1991). The rapidly increasing world population generates the challenge of providing additional necessary food sources. In particular protein supply poses a problem since essential amino acids cannot be replaced. One possible solution to this problem is SCP production, Oscar Andrés et al,. (2010). Bacteria and yeast are candidates for the synthesis of SCP. Protein contains the element Nitrogen in addition to Carbon, Hydrogen and Oxygen. Nitrogen comes from soil - only in limited supply, whereas the other elements are contained in the products of photosynthesis, which relies on Carbon vi oxide and water - reasonably easily available to plants. Intensive production (or extensive production) of protein from animal sources (meat) depends on protein from plants: grass for grazing or for production of animal feeds requires large areas of land, which may require nitrogenous fertilizers which can cause a variety of problems if not used at the correct amounts ; stored and prepared animal feeds also present a variety of economic and environmental problems.SCP is gaining popularity day by day because they require limited land area for growth. Research on SCP has been stimulated by a concern over the eventual food crisis or food shortage that will occur if the world's population is not controlled.

RESULTS

Production of Single Cell Protein

Many raw materials have been considered as substrates (carbon and energy sources) for SCP production (Nasseri, 2011). In many cases, these raw materials have been hydrolyzed by physical, chemical and enzymatic methods before use (Rhishipinal and Rosamma 1998 and Jhojaosadati et al., 1999). Various hydrocarbon, nitrogenous compounds, polysaccharides and agricultural wastes such as hemicelluloses and cellulose waste from plants and fibrous proteins such as horn, feather, nail and hair from animals are also abundant waste products for the production of SCP (Ashok et al.,2000). These waste products has been converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms and are rich in some growth factors required by microorganisms Atalo and Gashe (1993). In dealing with microbial protein production several natural products have been tested to this end, (Kuzmanova, Dimitrovski, & Doneva, 1989), tried to used grape juice byproduct as a carbon source. For the same purposes (Haider & EL-Hassy, 2000) tested date extract supplemented with nitrogen source as a suitable substrate, whereas, cashew apple juice was assayed by (Osho, 1995) . Several investigations were carried out using cellulose and hemicelluloses waste as a suitable substarte for increasing SCP production (Azzam, 1992; Pessoa, manciha, & Sato, 1997; Bozakuk, 2002; Zubi, 2005). The raw material was hydrolyzed by dilute acid or base and then supplemented with some nitrogen and phosphorous salts. Hydrocarbons such as N-alkanes, methane, methanol and ethanol have been used. Methane is cheap, abundant and without the toxicity problems of alkanes. It is a constituent of North Sea Gas and is also produced during anaerobic digestion. Methane contains the most highly reduced form of carbon and consequently gives high cell yields relative to the amount of gas consumed Israelidis, ((http://www.biopolitics.gr/HTML/PUBS/VOL1/isreali.htm).

Microorganisms for single cell protein production:
Various bacteria, mold, yeast and algae have been employed for the production of single cell proteins. The bacteria include *Brevibacterium* , *Methylophilus methylitropous* , *Acromobacter delvaevate* , *Acinetobacter calcoacenticu,s Aeromonas hydrophilla, Bacillus megaterium, Bacillus subtilis, Lactobacillus species,Cellulomonas species, Methylomonas methylotrophus, Pseudomonas fluorescens, Rhodopseudomonas capsulata, Flavobacterium species, Thermomonospora fusca* and others,some of the algae used are *Chlorella pyrenoidosa, Chlorella sorokiana, Chondrus crispus,, Scenedesmus acutus, Porphyrium sp* and *Sprulina maxima* .The filamentous fungi that have been used include *Chaetomium celluloliticum* , *Fusarium graminearum* , *Aspergillus fumigates, A. niger, A.oryzae, Cephalosporium cichhorniae, Penicillium cyclopium, Rhizopus chinensis,Scytalidium aciduphlium, Tricoderma viridae,* and *Tricoderma alba Paecilomyces varioti.* Yeasts such as *Candida utilis* (Torula yeast), Candida lipolytica, *Candida tropicalis Candida novellas, Candida intermedia* and *Saccharomyces cerevisiae* are all aamong the various organisms that have been used for the production of SCP Bhalla et al,.(2007). The desired microorganisms are cultured on the medium under sterile condition. Organisms to be cultured must have the following properties which are: it should be non pathogenic to plants human and animals, it must have good nutritional values, it must be usable as food and feed, should not contain toxic compounds and production cost should be low.

Table 1: **Microorganisms and substrates for SCP production.**

Organism	**Substrate**	**Crude protein (%)**
M. clara	Methanol	-
Yeasts		
Candida sp.	n-Alkenes	65
Candida utilis (Torula)	Ethanol, Sulphite waste liquor	50-55
Kluyveromyces fragilis	Cheese whey (lactose)	45-54
Saccharomyces cerevisiae	Molasses	53
Fungi		
Fusarium graminearum	Glucose	-
Cephalosporium eichhorniae	Cassava starch	48-50
Chaetomium cellulolyticum	Agriculture and forestry wastes	45
Paecilomyces varioti	Sulphite waste liquor	55
Penicillium eye/opium	Cheese whey (Iactose)	47
Scytalidium acidophthlum	Acid, hydrolyzed waste paper	44-47

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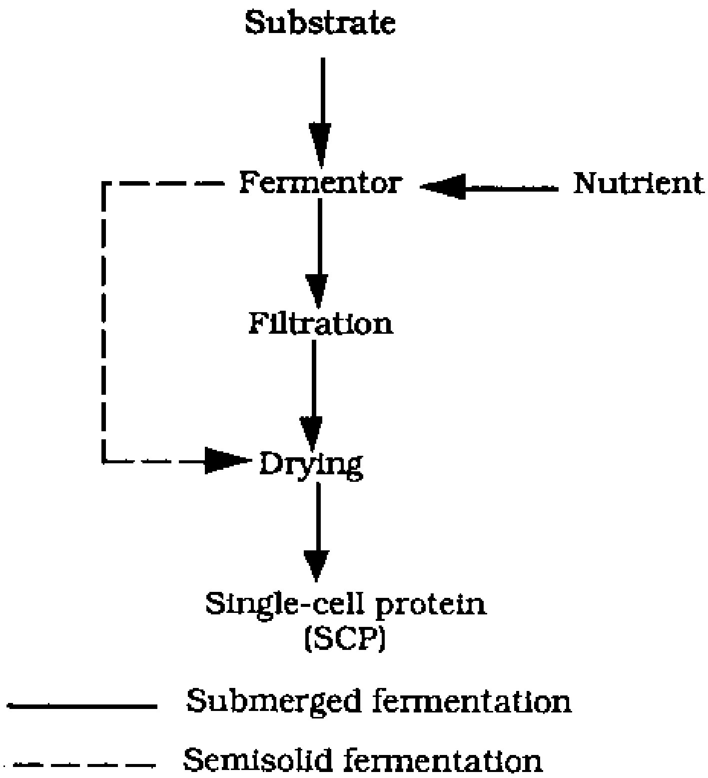
Cultivation of SCP

Single cell protein can be produced by two types of fermentation processes, namely submerged fermentation and semisolid state fermentation (figure 3) (Varavinit et al., 1996). In the submerged process, the substrate to be fermented is always in a liquid which contains the nutrient needed for growth. The substrate is held in the fermentor which is operated continuously while the product biomass is continuously harvested. The product is filtered or centrifuged and then dried. For semisolid fermentation, the preparation of the substrate is not as elaborate; it is also more conducive to a solid substrate such as cassava waste. Submerged culture fermentations are more capital intensive and have a higher operating cost when compared with semisolid fermentations which, however, have a lower protein yield (Oguntimehin).

The cultivation involves several basic process engineering operations, such as stirring and mixing of a multiphase system (gas-liquid-solid), transport of oxygen from the gas bubbles through the liquid phase to the microorganisms, and heat transfer from the liquid phase to the surroundings. The U-loop fermenter is a special bioreactor type designed for intensifying mass and energy transport phenomena by enhancing the mixing of the multiphase system and favoring heat transfer in SCP production[Andersen, 2005 and , the production of SCP involves the following basic steps which are: preparation of suitable medium with suitable carbon source, prevention of contamination of medium and the fermentor, production of the desired microorganisms and separation of microbial biomass and its processing.

Figure 1: Flow chart for single-cell protein production



(Oguntimehin)

The medium must contain a carbon source for cultivating the heterotrophic organisms such as fossil carbon sources like n-alkanes, gaseous hydrocarbons, methanol and ethanol, renewable sources like carbon iv oxide molasses, whey, polysaccharides, effluent of breweries, distilleries, confectioneries and canning industries or other solid substrates such as salts of Potassium, Manganese, Zinc, Iron and gaseous ammonia are also added for cultivating many microorganisms.(Nasseri, 2011)

Aeration is an important operation in the cultivation, heat is generated during cultivation and it is removed by using a cooling device. The microbial biomass can be harvested by various methods. Single cell organisms like yeast and bacteria are recovered by centrifugation while filamentous fungi are recovered by filtration. It is important to recover as much water as possible prior to final drying done under clean and hygienic conditions.

NUTRITIONAL BENEFITS OF SINGLE CELL PROTEINS

For the assessment of the nutritional value of SCP, factors such as nutrient composition, amino acid profile, vitamin and nucleic acid content as well as palatability, allergies and gastrointestinal effects should be taken into consideration (Lichtfield, (1968).). Also long term feeding trials should be undertaken for toxicological effects and carcinogenesis (Israelidis,).Nutritive and food values of SCP vary with the microorganisms used. The method of harvesting, drying and processing has an effect on the nutritive value of the finished product. Single cell protein basically comprises proteins, fats carbohydrates, ash ingredients, water, and other elements such as phosphorus and Potassium. The composition depends upon the organism and the substrate upon which it grows. Proteins not only provide a nutritional component in a food system but also perform a number of other functions (Mahajan and Dua, 1995).Some typical compositions which are compared with soymeal and fish meal. It has high protein and low fat content and It is a good source of vitamins particularly B-complex, with good amino acid composition and it is furnished with thiamine, riboflavin, glutathione, folic acid and other amino acids but less in sulphur containing amino acids. SCP from yeast and fungi has up to about 50 – 55 **%** protein and it has high protein –carbohydrate ratio than forages. It is rich in lysine but poor in methionine and cysteine. It has also been noted for having good balance of amino acids and rich in B – complex vitamins and more suitable as poultry feed. SCP produced from bacteria has more than 80% protein although they are poor in sulphur containing amino acids and it has high nucleic acid content. (Kurbanoulu, 2011). Yeast single-cell proteins (SCPs) are playing a greater role in the evolution of aquaculture diets. With excellent nutrient profiles and capacity to be mass produced economically, SCPs have been added to aquaculture diets as partial replacement for fishmeal (Coutteau and Lavens, 1989,Olvera-Novoa et al.,2002, Li and Gatlin,2003) and for HUFA-fortification of rotifer and *Artemia* (McEvoy et al., 1996). Some yeast strains with probiotic properties, such as *Saccharomyces cerevisiae* (SC) (Oliva-Teles and Goncalves, 2001) and *Debaryomyces hansenii* (Tofar et al., 2002), boost larval survival either by colonizing the gut of fish larvae, thus triggering the early maturation of the pancreas, or via the immuno-stimulating glucans derived from the yeast cell wall (Campa-Cordova et al., 2002, Burgents et al., 2004). However, many of these yeast supplements are deficient in sulfated amino acids, particularly methionine (Oliva-Teles and Goncalves, 2001), which restricts their extensive use as the sole protein source.

Table 2: Amino acid composition of SCP from Candida utilis.

Amino acid	mg g-1	FAO (mg g-1)
Aspartic acid	66.5	-
Threonine	34	40
Serine	36	-
Glutamic acid	90.5	-
Glycine	28	-
Alanine	46	-
Cysteine	24	20
Valine	40.5	42
Methionine	15.5	22
Isoleucine	32	42
Leucine	44	48
Tyrosine	26	-
Phenylalanine	30	28
Histidine	16	-
Lysine	76	42
Arginine	38	-
Proline	24	-

Kurbanoglu, (2001).

Table 3. The chemical composition of SCP from *C. utilis* on a dry weight basis.

Components	g/ g-100
Total protein	49.8
Total lipids	5.4
Ash	9.7
RNA	5.94
DNA	1.53

Kurbanoglu, (2001).

Table 4: Average Composition Of The Main Group Of Microoranisms

Nutrients	Fungi	Algae	Yeast	Bacteria (% dry weight)
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1.5-3.0
Ash	9-14	8-10	5-9.5	3-7
Nucleic Acid	7-10	3-8	6-12	8-12

Miller and Litsky, (1976)

Aside from the nutritional values of SCP, it also has the benefits of the possibility of its production through-out the year since it is independent of seasonal as well as climatic conditions, (Ndihi, 2010). Waste materials are used as substrate for the production of these proteins therefore it reduces the environmental pollution and helps in recycling of materials. SCP organisms grow faster and produce large quantities of protein from relatively small area of land and time. These have proteins with required amino acids that can be easily selected by genetic engineering and finally during the production of SCP biomass, some organisms produce useful bye products such as organic acids and fats.

Problems With SCP

Despite the very attractive features of SCP as a nutrient for humans there are many problems that deter its adoption on a global basis. Such problems include high concentration of nucleic acids (6-10) % which elevates serum uric acid levels and results in kidney stone formation. About 70 to 80% of the total Nitrogen is represented by amino acids while the rest occur in nucleic acids. This concentration of nucleic acid is higher than other conventional protein and it is characteristics of all fast growing organisms. The problem which occurs from the consumption of protein with high nucleic acid concentration (18-25g/100g protein dry weight) is the production of high concentration of uric acid in the blood causing health disorders such as gout and kidney stone (Nasseri et al., 2011). It has also been noted that the cell wall of the microorganisms may be non digestible, there may be unacceptable color and flavors (especially in algae and yeast), their cells should be killed before consumption, there may also be possible skin reactions from consumption of foreign protein and gastrointestinal reactions may occur resulting in nausea and vomiting. SCP from algae may not be suitable for human consumption because they are rich in chlorophyll, (except Spirulina), also it has low density i.e. 1-2 gm dry weight/litre of substrate and there is lot of risk of contamination during growth. SCP from yeast and fungi has high nucleic acid content, the filamentous fungi show slow growth rate than yeasts and bacteria there is high contamination risk and some strains produce mycotoxins and hence they should be well screened before consumption. SCP from bacteria has also been found to be associated with these pitfalls which include: high ribonucleic acid content, high risk of contamination during the production process and recovering the cells is a bit problematic. All these detrimental factors affect the acceptability of SCP as global food.

DISCUSSION AND CONCLUSION

SCP is gaining popularity day by day because they require limited land area for growth and also help in recycling of waste. According to Ashok, et al., (2000), application of agro-industrial residues in bioprocesses such as cultivation of SCP on the one hand provides alternative substrates, and on the other hand helps in solving pollution problems, which their disposal may otherwise cause. . Research on SCP has been stimulated by a concern over the eventual food crisis or food shortage that will occur if the world's population is not controlled. SCP has 60-70% protein content. The fact that they are very rich in protein with very high amino acid spectrum, high concentration of vitamins essentially B complex and low concentration of fat (Table 2-4) makes them a suitable protein food for human and animal consumption. The high nucleic acid in SCP could be removed or reduced with one or all of the following treatments: chemical treatment with sodium hydroxide, treatment of cells with 10% sodium chloride, activation of endogenous nucleases during final stage of microbial biomass production and thermal shock. These methods are aimed at reducing the ribonucleic acid content from about 7% to 1% which is considered to be within the acceptable level (Zee and Smart, 1974). Huei-hsiung Yang in his study developed a simple method for reduction of nucleic acid in Brevibacterium JM98A by incubation of non-proliferating cells at pH 10. 3 and 55°C for 3 hour. For future success of SCP, first, food technology problems have to be solved in order to make it similar to familiar foods and second, the production should compare favorably with other protein sources. the idea that the single cell protein could help the less developed countries in future food shortages was gaining research interest among scientists in universities and industry, particularly in oil. The result was the development of SCP technology either for livestock or for human consumption. Considering the fact that microorganisms have high rate of multiplication, high content of protein, utilize a variety of carbon sources as energy source, help in waste recycling by utilizing them as growth medium for SCP production, microbial strains with high yield and no toxic by-products as well as good composition can be selected and microbial biomass production as single cell protein is independent of seasonal as well as climatic conditions. The use of SCP as food ingredient is still in its stages of development, there are lots of prospects concerning the improvement of using SCP as food, methods of using genetic engineering procedures for mass production of these protein containing microorganisms are been employed. Attempt to improve the acceptability of SCP products should be intensified. Further research and development will ensure usage of microbial biomass as single cell protein or as diet in supplement in development countries.

REFERENCES

[1] Andersen, B.R. Jorgensen, J.B. and Jorgensen, S.B. U-loop reactor modeling for Optimization, part 1: Estimation of heat loss. CAPEC, Technical University of Denmark, **2005**.
[2] Ågren, G. Stenram, U. Nordgren, H. Eklund, A. Glas, J.E.(**1974**). *Annals of Nutrition and Metabolism* Vol. 17, No. 1
[3] Arora, D., Mukerji, K. & Marth, E. (**1991**)(*vol*. 3). Single cell protein in Hand book of applied mycology (pp. 499-539). India: Banaras Hind University.
[4] Ashok Pandey Carlos R. Soccol , Poonam Nigam , Vanete T. Soccol ,Luciana P.S. Vandenberghe , Radjiskumar Mohan ((**2000**). *Bio resource Technology* 74 (2000) 81±87
[5] Atalo K and Gashe BA, *Biotechnology Letters* 15: 1151-1156 **1993**.
[6] Azzam, A. M. (**1992**). *Journal Environmental Science and Engineering*, 27, 16431653.
[7] Bamberg, J. H. (**2000**). *British Petroleum and global oil, 1950-1975: the challenge of nationalism. Volume 3 of British Petroleum and Global Oil 1950-1975: The Challenge of Nationalism, J. H. Bamberg British Petroleum series*. Cambridge University Press. pp. 426–428. ISBN 0521785154. http://books.google.com/books?id=LVC0VlPOJxEC.
[8] Becker, E.W., Venktaraman, L.V. (**1982**). Biotechnology and exploitation of algae-The Indian approach. German Agency for technical Cooperation.E schborn, FRG p. 216.
[9] Bhalla, T.C., Sharma, N.N. and Sharma M. (**2007**). Production of Metabolites, Industrial Enzymes, Amino Acids, Organic Acids, Antibiotics, Vitamins and Single Cell Proteins. National Science Digital Library, India.
[10] Bozakouk,A. H. (**2002**). *Acid hydrolysis of Phragmites austral; is powder for production of single cell protein by Candida utilis*. M.Sc. Thesis, Benghazi: Garyounis University.
[11] Burgents, J. E., Burnett, K. G. & Burnett, L. E. (**2004**) Disease resistance of Pacific white shrimp, *Litopenaeus vannamei*, following the dietary administration of a yeast culture food supplement. Aquaculture 231:1-8. .
[12] Campa-Córdova, A. I., Hernández-Saavedra, N. Y., De Philippis, R. & Ascencio, F. (**2002**) *Fish Shellfish Immunol*. 12:353-366.
[13] Grewal, H., Kalra, &. Kahlom, S. (**1990**). *Journal of Resarch*,1,90-96.
[14] Haider, M., AL-Barhawi, R. & Hassan, H. (**1989**). *Edu. and Sci.*, 7,123-135.
[15] Haider, M. & EL-Hassy, M. (**2000**). *Garyounis journal of Science*,1, 7-23.
[16] Huei-hsiung yang (**1976**). Reduction of Nucleic Acid in Single Cell Protein by an endogenous polynucleotide phosphorylase. A dissertation in microbiology. PhD theses submitted to the Graduate Faculty of Texas Tech. University.

[17] Jhojaosadati SA, Rasoul K, Abbas J and Hamid RS, *Resources Conservation and Recycling* 27 (1-2): 125-138 **1999**.
[18] Karger, S. and Bassel, A.S.(**2011**).Institute of Medical Chemistry and Institute of Pathology, University of Uppsala, Uppsala, and Karolinska Institutet, Stockholm copyright 2011
[19] Kurbanoglu Esabi Basaran (**2001**). *Turk J Biol*. 25(2001). 371-377 Copyright TUBITAK
[20] Kuzmanova, S., Dimitrovski, A. & Doneva. D. (**1989**). *Microbiology Skopj*, 26,159-164.
[21] Li, P. and Gatlin, D. M., III (**2003**) *Aquaculture* 219:681-692.
[22] Litchfield, H. (**1983**), *Science*, 219,740-746.
[23] Lichtfield, J.H. (**1968**). "The production of fungi. Single-cell Protein I",
[24] Mahajan, A.and Dua, S. (**1995**). *J. Food Sci. Technol*. 32: 162-165.
[25] Mahajan, A. Neetu and Ahluwalia, A.S.(**2010**). *African Journal of Microbiology Research* Vol. 4 (1), pp. 055-060.
[26] Mateles and Tannenbaum, eds. The M.I.T. Press, Cambridge, MA.
[27] McEvoy, L. A., Navarro, J. C., Hontoria, F., Amat, F. & Sargent, J. R. (**1996**) *Aquaculture* 134:339-352.
[28] Microbiologyprocedure.com
[29] Miller, B.M. and Litsky, W. (**1976**). Single Cell Protein in Microbiology. McGraw-Hill Book Company. P 408
[30] Nasseri, A.T., Rasoul-Amini, S., Moromvat, M.H. and Ghasemi, Y.(**2011**). *American Journal of Food Technology*. 6(2): 103-116.
[31] Ndihi Uppangala (**2010**.)http://www.biotecharticle.com/
[32] "Nutrition - Single Cell Protein, Twenty Years Later". http://www.biopolitics.gr/HTML/PUBS/VOL1/isreali.htm.
[33] Oliva-Teles, A. & Gonçalves, P. (**2001**) *Aquaculture* 202:269-278.
[34] Olvera-Novoa, M. A., Martínez-Palacios, C. A. & Olivera-Castillo, L. (**2002**) *Aquacult. Nutr*. 8:257-264.
[35] Oscar A.P. Jorgensen, J.B., and Jørgensen, S.B. (**2010**).Systematic Model Analysis for Single Cell Protein (SCP) Production in a U-Loop Reactor 20th European Symposium on Computer Aided Process Engineering – ESCAPE20.
[36] Osho, A. (**1995**). *Nahruny*, pp,521-529.
[37] Piper, S. (**2004**). Continuous Cultures of *Methylococcus capsulatus*. Center of Microbial Biotechnology (Biocentrum) - Technical University of Denmark, 2004. Master's thesis.
[38] Patel, G. S. (**1995**). *Indian Journal of Agricultural Research*, 3, 114-134.
[39] Pessoa, A., manciha, I. & Sato, S. (**1997**). *Industrial Microbial. and Biotechnol.*6,360-363.
[40] Reed, G. & Nagodawithana, T. (**1995**). Biotechnology enzymes, biomass, food and feed, Bibliographic Citation.9, 168-215.
[41] "Single cell protein (SCP)". http://www.studentsguide.in/microbiology/industrial-microbiology/single-cell-protein-scp.html
[42] Soland, L.(**2005**). Characterization of Liquid Mixing and Dispersion in a U-loop Fermentor. Technical University of Denmark, 2005. Master's Thesis.
[43] Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F. J., Vázquez-Juárez, R. & Lésel, R. (**2002**) *Aquaculture* 204:113-123.
[44] Vincent WA (**1969**). *Algae for food and feed Proc. Biochem*. 4: 45-47.

[45] Varavinit, S., Srithongkum, P., De-Eknamkul, C., Assavanig, A. and Charoensiri, K. (**1996**), Production of Single Cell Protein from Cassava Starch in Air-lift Fermenter by *Cephalosporium eichhorniae*. Starch - Stärke, 48: 379–382. doi: 10.1002/star.19960481007
[46] Zee, J.A. and Simard, R.E. (**1974**). *Applied Microbiology*, 29(1):59-62.
[47] Zubi, W. (**2005**). Production of single cell protein from base hydrolyzed of date extract by-product by the fungus *Fusarium graminearum.* M. Sc.Thesis, Benghazi: Garyounis University.