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Non Conventional Protein for Human Race from *Spirulina maxima*

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The dried cells of microorganisms are known as single cells proteins, which used as protein supplement in food or animal feeds. Microorganisms such as Yeast, Bacteria, Fungi, especially algae utilizing CO_2 and sun light as source of carbon and energy for growth to produce biomass. Spirulina is blue green mesophilic cyanobacteria with high protein content being largely used as single cell protein. Single cell protein used in diets for weight reduction and malnutrition. It has highest protein content, high vitamin value particularly B12 and lipid content, used as antioxidant and in medicine. The present review reports the microorganisms as source of single cell protein with special reference to *Spirulina maxima*.

Key Words: Microorganisms, Protein, Human race, *Spirulina maxima*.

Current need to expand world supply of food for increasing human population has resulted to explore new, alternative and unconventional protein. For this reason, in the year 1996, new sources mainly single cell protein (SCP) as coined to describe the protein production form biomass. It has been considered as alternative to conventional sources of food or feed.

Spirulina is blue green mesophile filamentous cyanobacteria, with high protein content 60-70%, B12 enriched and has high lipid content, used as an antioxidant and in medicine, being largely used as SCP, for humans and animals. SCP used as diets for weight reduction and malnutrition.

SCP is cellular material of microbial origin obtained for nutritious purposes. Especially *Spirulina maxima* cultured on Paolettis synthetic culture medium.

Paolettis Synthetic Culture Medium Composition

Synthetic pallotti media each 1 lit of Macroelements solution was added 1 ml of micro elements and 1 ml of the SE-EDTA solution.

Environmental factors play an important role on the growth of *S. maxima* like temperature, light etc (Maria and Paulada *et al* 2010., Oslen and Allermann 1991., Rosa *et al* 2006., Paoletti *et al*, 1975). More than 75% of the annual micro algal biomass production is used for the manufacture of powders, tablets, capsules. The annual world production of all micro algae species is estimated to about 10,000 tones\ year (Becker 1994, 2007; Richmond, 2004). The biomass is harvested from

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natural waters (or) artificial ponds (or) photo bio reactors and subsequently separated from the growth medium fallowed by drying. (Renaud *et al* 1994).

The two major species cultivated for this purposes are the unicellular algae, *Chlorella* and more recently filamentous Blue green algae (*Cyanobacteria, Spirulina*) (Raja *et al* 2008). Algal proteins are of high quality and comparable to conventional vegetable proteins, production costs are also high (Rasout - Amini *et al* 2009). Algal cells have celluloid cell walls, give nearly 10% dry weight, not digested in non-ruminants. Many authors have studied the effect of different post-harvesting treatments on the digestibility of various algal species (Becker, 2007).

Methods for Processing of SCP

Several methods have been developed to improve the digestibility of SCP products: mechanical disruption, auto lysis, and enzymatic treatment. The external manno protein layer of the yeast cell wall is probably the major barrier to digestion (Rumsey *et al* 2007; Kim and Chung, 2001, (Curren *et al* 1990). Pancreatic ribonuclease (RNase A) and a fungal ribo nuclease of strain *Aspergillus, Candidus M16* has been used as the source of exogenous nuclease for the reduction of nucleic acid in the cells of yeast species, allowing a substantial reduction of nucleic acid (Maul *et al* 1970; Kuhni and Rao 1995).

Finally safety and the protection of innovation throw up legal and controlled aspects, namely operating licenses, product authorization for particular applications and the legal protection of new process and strains of microorganisms (Stein Kraus 1986). SCP is cellular material of microbial origin obtained for nutritioal purposes. (Oslen and Allermann 1991). *Spirulina* is a source of SCP used for humans and animal feeds. (Rosa *et al* 2006).

Nutritional value of SCP of *S. maxima*

Spirulina contain phenolic acids, tocopherols and beta carotene, Omega 3, 6 poly unsaturated fatty acids, pro vitamins which are known to exhibit antioxidant properties (Miranda *et al 1998)*. Antioxidants prevent oxidative deterioration of food and to minimize oxidative damage to living cells (Pratt *et al* 1992). The free radicals in tissue damage and pathological processes, like cardiovascular disease and cancer (Halli well and Kok 1989& 1990).

Spirulina proteins are complete, since all the essential amino acids are present, forming 47% of total protein weight (Bujard *et al* 1970), but poorly represented are the sulphur containing amino acids, Methionine and Cysteine (AFAA & Clement *et al* 1967). Net protein utilization of *Spirulina* is nearly 83-90% in ordinarily dried *Spirulina*, as against 95% for pure casein (Santillan *et al* 1975; Dillon 1993, because *Spirulina* has fragile envelop of murein that's why only it is digested in humans (AFAA, Challem *et al* 1981; Bujard *et al* 1970, Frust *et al* 1978). The protein efficiency ratio value for Spirulina determined in growing rats is estimated between 1.8 and 2.6 (Frust 1978; Santillian 1974; Sautier and Tremolieves 1975) as against a Protein efficiency ratio value of casein is 2.5%. *Spirulina maxima* has total lipid content of 11% by using a better extraction system (Hudson and Karis 1974). These lipids can be separated in to a saponificable fraction (83%) and a non saponificable portion (17%) containing essentially paraffin, pigments, terpene, alcohol, and sterols (Bujard *et al* 1974; Clement 1975; Santilla 1974). Essential fatty acids has an influence on the immune systems both on humoral and cell mediated. (Hwang 1989). Essential fatty acids are Omega3 and Omega6 given by Hudson and Karis 1974.

In general carbohydrates constitute 15-25%, of the dry weight of *Spirulina*. Simple Carbohydrates, Glucose, fructose, and Sucrose are present only in small quantities, such as Glycerol, Mannitol, and Sorbitol also occur. (Quillet 1975).

The nucleic acid content of *Spirulina maxima* is about 4-6% only, and the portion of DNA is estimated between a quarter and a third of that is RNA.(AFAA 1982; Ciferri 1985; Sanytillan 1974).

Beta carotene accounts for 80% of the carotenoids present in the *Spirulina*, the remainder consisting mainly of Phycoxanthin and Cryptoxanthin (Palla and Busson 1969). For adults Vitamin, A requirements are estimated at less than 1mg\ day (Evets *et al* 1994).

Bioavailability of *Spirulina* carotenoids has been demonstrated in both the rat and chiken (Kappor and Metha 1993; Mitchell *et al* 1990; Rose and Dominy 1990). Clinical studies have also shown excellent utilization of *Spirulina* carotenoids in

humans (Annapurna *et al* 1991). 1 gram *Spirulina* dose given to school child's up to 5,000 number, they are suffering from chronic vitamin A deficiency, after 5 months, they recovered from Bitots spots on the conjunctiva of the eye ball from 80% to 10% (Seshadri 1993). Dry Spirulina contains 50-190mg\ Kg of vitamin E. (Nippon Ink 1997; Challem *et al* 1981, Earth raise farms Spirulina,

Table 1. Show average different biomolecules composition of the main group of Microorganisms (% dry weight).

Composition	Fungi	Algae	Yeast	Bacteria
Protein%	30-45	40-60	45-55	50-65
Fat%	2-8	7-20	2-6	1-3
Ash%	9-14	8-10	5-10	3-7
Nucleic acid%	7-10	3-8	6-12	8-12

(Millev and Litsky 1976).

Table 2. Microorganisms can utilize a variety of substrates like agricultural wastes, effluents, industrial wastes, natural gases like methane, gas oil, methanol, and n- alkanes are of interest (Huang& Kinsella 1986).

Micro organism	Substrate
Bacteria	
Aeromonas hydrophila	Lactose
Acromobacter delvacuate	n-alkanes
Acinotobacter calcoaceticus	Ethanol
Bacillus megaterium	Non-protein
nitrogen sources	
B.subtilis, Cellulomonas Sp, Flavobacterium Sp, Thermomonospora fusca, Lactobacillus Sp	Cellulose, Hemi cellulose, Glucose, Amylose, Maltose
Methylomonas methylotrophous, M.clara	Methanol
Pseudomonas florescence	Uric acid& other non-protein N2 compounds
Rhodopseudomonas capsulate	Glucose
FUNGI	
Aspergillus fumigates	Maltose, Glucose
A.niger, A.oryzae, Cephalosporium , Chaetomium cellulolyticum	Cellulose, Hemi cellulose
Penecelium cyclopium	Glucose, Lactose, Galactose
Rhizopus chinensis	Glucose, Maltose
Scytalidium aciduphilum, T.viridae, T. alba	Cellulose, Pentose
YEAST	
Amocoo torula	Ethanol
Candida tropicalis	Maltose, Glucose
Candida utilis	Glucose
Candida novellas	n-alkanes
Candida intermedia	Lactose
S.cerviceae	Lactose, Pentose, Maltose
Algae	
Chlorella pyrenoidosa, Chlorella sorokiana Chondrus crispus, Scenedesmus, Spirulina, Porphyrium sp.	CO_2 through Photosynthesis,

(Bhalla *et al* 2007)

1986). *Spirulina* is four times as rich in vitamin B12 compounds as raw liver (Hau 1995). Spirulina strains are capable of fix Iodine (Singh and Kumar 1994).

Theraputic value of *Spirulina* are as follows:

Iron deficiency anemia.
Pernicious anemia.
Vitamin A deficiency.
Inhibition of mother-child transmission of HIV.
Inhibition of infection of T4 Helper cells by HIV.
Protein energy disorders.
Cancer prevention through provision of carotenoids.
Strengthening immune system.

Selection of microorganisms and methods to improve quality of SCP

1 The wide variety of methodologies, raw materials and micro organisms that can be used for this purpose.
2 High efficiency in substrate conversion.
3 High productivity derived from the fast growth of microorganisms.
4 Independence of seasonal factors (Roth 1980; Pajaro *et al*, 1995).

Yeast is the first prime alternative animal feed with good protein, and has Vitamins, Minerals and essential Lipids but have high nucleic acids. As constituents of Nucleic acids, Purine compounds in human diet mostly metabolized to yield uric acid, whose high concentration may lead to Gout or Renal stones (Alvarez and Enriquez, 1988). High nucleic acid contents are reduced to below 2% achieved by chemical and enzymatic methods (Pajaro *et al* 1995). Various microorganisms used for the production of SCP are Bacteria (*Cellulomonas, Alcaligens sp*); Algae(*Spirulina, Chlorella sp*); Moulds*(Trichoderma, Fusarium, Rhizopus sp*); and Yeast (*Candida, Saccharomyces sp).*

Bacteria have small size, and low density, which makes harvesting form the fermented medium difficult and cost effective. Bacterial cells have high nucleic acid content relative to yeast and fungus. To decrease the nucleic acid level additional processing is needed.

Yeast are larger in size, low nucleic acid content, high lysine content and ability to grow at acidic pH, long history of it's use in traditional fermentations

Disadvantages

Low growth rate, low protein content 45-65%, low methionine content than bacteria.

Algae have cellulosic cell walls, which are not digested by human beings, they also has concentrated heavy metals, but *Spirulina* has fragile envelop of murine, that's why it is easily digested in humans.

REFERENCES

1. Olsen, J and Allermann, K , La biomasa microbiana como fuente de protein. *In Biotecnologia basica. Eds J. Bu"lock and B. Kristiansen. Acribia, Zaragoza.*1911.
2. Rosa, P.C,; Carvalho, L.S.; Goldbeck, L,; Costa, J.A.V; Schossenke, D.; Touey, *J. and Soares, L.A.S* . 2006.
3. Bhalla, T.C., N.N. Sharma and M.Sharma, Production of metabolites, Industrial enzymes, Amino acids, Organic acids, Antibiotics, Vitamins and single cell proteins. National science digital library, India. 2007
4. Becker, E.W., Micro algae, Biotechnology and Microbiology. Canbridge university press, Cambridge, Uk. 1994.
5. Becker, E.W., Micro algae as source of protein. *Biotechnol. Adv.,* 2007; **25**:207-210.
6. Curran, D.A., B.J. Tepper and T.J. Montville, . Use of bicarbonates for microbisl control and improved water binding capacity in codd filters. *J. Food .Sci.,* 1990; **55**: 1564-1566.
7. Huang, T.Y and J.E Kinsella, Functional properties of phosphorylated yeast protein: solubility, water- holding capacity and viscocity. *J.Agri. Food Chem.,* 1986; **344**: 640-674.
8. Kim, J.K and H.Y. Chung, Preservation of manipulated yeast diet. *Aquac. Int.,* 2001; **9**:171-181.
9. Kunhi, A.A. and .R.R,Rao, The utility of a fungal ribo nuclease for reducing the nucleic acid content of permealized yeast cells. *Food Biotechnol.*, 1955; **9**:13-28.
10. Maul, S.B., A.J. Sinskey and S.R. Tannebaum, New process for reducing the nucleic acid content of yeast. *Nature,* 1970; **228**: 181-181.
11. Miller, B.M. and W.Litskey,Single cell Protein in industrial microbiology. *Mc Graw Hill book Co.,* New York. 1976
12. Parajo, J.C., V. Santos, H. Dominguez and M. Vazquez, Nh4OH based pretreatment for improving the nutritional quality of single cell protein. *Applied BIochem. Biotechnol.,* 1995; **55**:133-149.
13. Raja.R., S.Hemaiswarya, N.A. Kumar, S. Sridhar and R.Rengaswamy, A perspective on the

biotechnological potential of micro algae. Cr. *Rev. Microbiol.,* 2008; **34**:77-88.

14. Rasoul-Amini, S.Y., Ghasemi, M.H. Marowvat and A.Mohagheghzadek, PCR amplification of 18s r-RNA, single cell protein production and fatty acid evaluation of some naturally isolated microalgae, *Food Chem.,* 2009; **116**:129-136.
15. Renaud, S.M., D.L. Parry and V.L. Thinh, Microalgae for use in tropical aqua culture I: Gross chemical and fatty acid composition of 12 species of Microalga from the Northen territory, *Australia. J. Applied Phycol*.,1994; **6**:337-345.
16. Richmond, A., Hand book of Microalgal culture: *Biotechnology and applied Phycology. Black well, Oxford,* Uk. 2004.
17. Roth, F.X., Microorg., anisms as a source of protein for animal nutrition. *Anim. Res. Dev.*1980; **12**: 7-19.
18. Rumsey, G.L., S.G. Hughes and J.L. Kinsella, (2007., 1990). Use of dietary yeast Saccharomyces cereviceae nitrogen by the lake trout. *J. world Aquac. Soc.,* ; **21**:205-209.
19. M.S.Miranda, R.G. Cintra, S.B.M Barros and J. Mancini- Filho., *Brazilian journal of Medical and Biological Research.* 1998; **31**: 1075-1079.
20. Pratt, DE. Natural anti oxidents from plant material. In: Huang MT, Ho, CT& Lee CY (Editors), Phenolic copounds in food and their effects on health. 11. *American chemical society,* Washington, 1992; 54-71
21. Halliwell B & Gutteridge JMC free radicals in biology and medicine. 2nd edn. *Clarendon press, Oxford,* 1989; 299-357.
22. KoK, J.F, Van Poppel G, Melse J, erhuel E, Schouten EG, Kruyssen EH& Hoffan A(1990). Antioxident and poly unsaturated acids have a combined association with *coronary atherosclerosis*, 1990; **86**: 85-90.
23. Annapurna V., *et al* Bioavailability of Spirulina carotenes in pre-school children.*" J. Clin. Biochem Nutrition.* 1991; **10**: 145-151.
24. Association francaise pour l'al gologie apliquee(*AFAA*). Actes du premier symposium sur la spiruline Spirulina platensis(Gom) Geitler de l' *AFAA.* 1982.
25. Bujard. E; U. Braco, auron.J, Mottu.F, Nabholz. A., Wuhrmann.J.J & Clement.G (1970). " composition and nutritive value of BGA (Spirulina) and their possible use in food formulations". *3rd international congress of food science and technology*, Washington . 1970.
26. Challem.J.J., Passwater, R.A and Mindell.E.m . Spirulina keats publishing, Inc . New canaan, Conn*ecticut.* 1981.
27. Ciferri, O& Tiboni O 195" Spirulina, the edible microorganism*" Microbiol. Rev*. **47**: 551-578.
28. Clement. G production et constituents characteristiques des algues Spirulina platensis et maxima" *Ann. Nutr. Aliment.* 1975; 29: 477-487.
29. Dillon, J.C and Phan P.A. Spirulina as a source of proteins in human nutrition" *Bull. Inst. Oceno, Monaco, Special.* 1993; **12**: 103-107.
30. Earthrise farms Spirulina, product typical analysis*", San Rafel*, USA. 1986.
31. Frust, P.T Spirulina, *Human nature,* 1978; **1**(3): 60-65.
32. Hau, R vitamin B12 in der microalgae Spirulina platensis" *Fit furs LEBEN1*, 1995; 29.
33. Hudson, B.J.F& Karis.I.G . " The lipids of the Alga spirulina", *J. Sci. Fd. Agric*. 1974; **25**: 759-763.
34. Hwang D. " Essential fatty acids and immune response", *FASEB J.* 1989; **3**: 2052-2061.
35. Kapoor. R& Metha.U "utilization of beta carotene from Spirulina platensis by rats," *Plant foods (Nutr. Jan.* 1993; **43**(1): 1-7.
36. Mitchell.G.V; Grundel.E; Jenkins.M; Blakey. S.R. " effects of graded dietary levels of Spirulina maxima on vitamin A and E in male rats*". J-Nutr.* 1990; **120**(10): 1235-40.
37. Nippon Ink& Chemicals, *" Spirulina"*. 1977.
38. Palla. J. C & Busson. F. Etude des carotenoids de Spirulina platensis (Gom) Geilter (cyanophycess*)" . C.R Acad. Sc.* Paris, 1969; **269***:* 1704-1707.
39. Quillet M " Recherches sur les substances glucidiques elaboress par les spirulines", *Ann. Nutr. Aliment.* 1975; **29**: 553-561.
40. Ross.E& Dominy.W (1990)" The nutritional value of dehydrated blue green algae(Spirulina platensis) for poultry.*" Poult. Sci* . 1990; **69**(5): 794-800.
41. Santillan.C cultivation of Spirulina for human consumption and for animal feed" International congress of food science and technology. *Madrib,* 1974.
42. Seshadri. C.V large scale nutritional supplementation with Spirulina alga". All india coordinated project on Spirulina. *Shri Am Murugappa Chettiar research cnter,* Madras India. 1993.
43. Alvarez, R and Eriquez . Nucleic acid reduction in yeast. *Applied Microbiol. Biotechnol.,* 1988; **29**: 208-210.
44. Maria Caula.costamonceiro *et al.*, Aassays of 3 different types of cultural conditions on Spirulina maxima growth. *Brajilian archis od biology and technology* . 2010; **53**(2): 369-373.