J. Sci. Trans. Environ. Technov. 2009, 2(3): 126-130

Isolation and Characterizations of Single Cell Protein (SCP) from *Spirulina* and Yeast cultured in different media

V. Sivakumari*[1], G. Shanthi[2] and A. Rajendran[1]

[1]Department of Environmental and Herbal Sciences, Tamil University, Thanjavur – 613 010, Tamil Nadu, India

[2]Kunthavai Nacchiyar Government Arts College for Women, Thanjavur-613 007, Tamil Nadu, India

Abstract

Biomass of *Spirulina* and yeast as well as their biochemical constituents differed when cultured in different media. Agrobased wastes had also supported good growth of *Spirulina* and yeast indicating recycling of agro-based industrial wastes could be achieved by culturing this economically important blue green algae (*Spirulina)* and yeast (*Saccharomyces cerevisiae*).

Keywords : carbohydrates, chlorophyll, *Saccharomyces cerevisiae, Spirulina,* yeast

INTRODUCTION

The dried cells of microorganisms (algae, bacteria, actinomycetes and fungi) used as food or feed are collectively known as "Microbial Protein" or single cell protein (SCP) as per the recent nomenclature (Scrimshaw, 1968). There are a number of advantages in the production of microbial protein over the protein from of conventional crops used as food and feed, including rapid succession of generations (algae-2-6hrs; yeast-1-3 hrs; bacteria-0.5-2 hrs), genetic modifications (e.g., for composition of amino acids), high protein content of 43-85% in the dry mass, broad spectrum of original raw materials used for the production, which also include waste products, production in continuous cultures, consistent quality and not dependent on climate in determinable amount, low land requirements and being ecologically beneficial (Subba Rao, 1982).

Fermented yeast (*Saccharomyces spp*) was recovered as a leavening agent for bread as early as 2500 B.C (Ferry, 1930). From the 16th century blue green algae e.g., *Spirulina* has been consumed as a major source of protein (Clement, 1968). *Spirulina* is a good source of β-carotene (a precursor of vitamin A) and therefore, helps in maintaining healthy eyes and skin. Further, β-carotene is known to be the best anticancer substance (Schwartz *et al*., 1986). In 1973, some *Actinomycetes* and filamentous fungi were also reported to produce protein from various substrates (Tannenbaum and Wagt, 1975).

The major substrates of culturing *Spirulina* and Yeast are the raw materials, which contain sugars, starch, lingo celluloses from woody plants and herbs having residues with nitrogen and phosphorous contents and organic wastes generated by certain industries are also rich in aromatic compounds or hydrocarbons (Bull *et al*., 1983). Further *Spirulina* and *Scenedesmus* were found to utilize even swine wastewater and clean it indicating their use in waste recycling (Gantar *et al*., 1991). This paper describes the production of single cell proteins from *Spirulina* and Yeast in different culture media containing agro-based waste (Mollases) and Yeast sludge.

MATERIALS AND METHODS

About 250 ml of sample with surface algal growth was collected in sterile container from Antenna Trust, Kadahaenanthal, at Madurai in Tamilnadu, India. To isolate the algae from the sample it was inoculated into CFTRI (Venkataraman, 1983) and *Spirulina* (Scrimshaw, 1968) media (Table 1). The cultivation media was prepared by transferring aseptically about 10% v/v of the algal growth to sterile media and incubated for 7-10 days.

Table 1. Composition CFTRI and *Spirulina* media used in the present study

Nutrients	CFTRI medium (g/l)	*Spirulina* media (g/l)
$NaHCo_3$	4.50	8.00
$(NH_2)CO$	-	0.03
K_2HPO_4	0.50	-
$NaNo_3$	1.50	
K_2So_4	1.00	0.50
NaCl	1.00	5.00
$MgSo_4.7H_2O$	1.20	0.16
$CaCl_2 2H_2O$	0.04	
$FeSo_4$	0.01	0.005
H_3PO_4	-	0.08
p^H	9.0	9.0

Morphological characteristics were observed micro-

*Corresponding Author
email: *vsivamicro@gmail.com*

scopically. Harvesting of cyanobacteria i.e., *Spirulina* sp. and yeast were troublesome as their spiral filaments float on the surface of water, because of gas filled vacuoles in their cells, resulting in floating of algal mass. For harvesting the cells, the surface growth was scrapped using a scrapper and filtered. The wet cells were then spread as a thin layer on glass plates and then dried in a hot air oven at 60^0C for ½ hour to 1 hour.

Determination of Biomass in *Spirulina* and yeast

To determine the fresh weight (weight of the biomass in wet conditions) of algal biomass, sample was centrifuged at 15,000 rpm for 10 min. Then the pellet was washed twice with distilled water and was transferred onto pre-weighed aluminum foil cup and weighed. To estimate the dry weight of the algal biomass homogenized culture was centrifuged at 15,000 rpm for 10 min, the pellet was washed twice with distilled water, transferred on to pre-weighed aluminum foil cups, dried at 60^0C for ½ hour to 1 hour and weighed.

Biochemical analsyes

Amount of Protein was estimated by Lowry's method (Lowry *et al*., 1951), Carbohydrate content by Anthrone method (Hedge, 1962) and Carotenoid and chlorophyll contents by the method of Arnon, (1949).

RESULTS

After 7-10 days of incubation the media were observed for the development of algal growth. A thick green layer of algal mat floating on the surface of the media (CFTRI and *Spirulina* medium) was observed. The microscopic observation showed the presence of long, slender, spirally coiled filaments (Fig. 1). The length of filament ranged from 8-15mm. The CFTRI soft agar tubes of *Spirulina* medium had isolated green coloured mat like growth with colonies that are not prominent but are found spreading over the entire medium (Fig. 2). On the CFTRI broth, the *Spirulina* was well grown in the aerated flasks (Fig. 3).

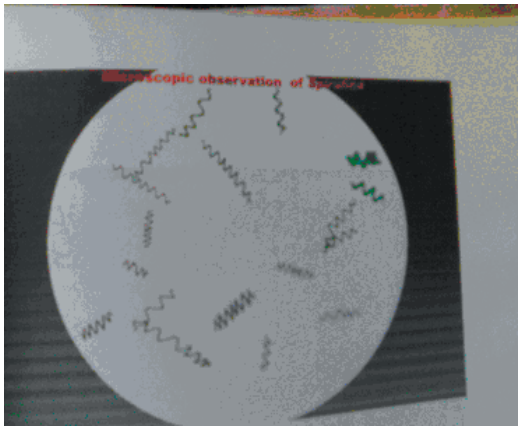

Figure 1. Microscopic view of the algal mat

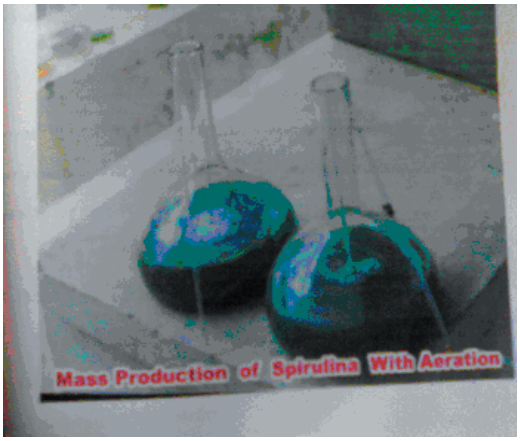

Figure 2. *Spirulina* growth in *Spirulina* medium

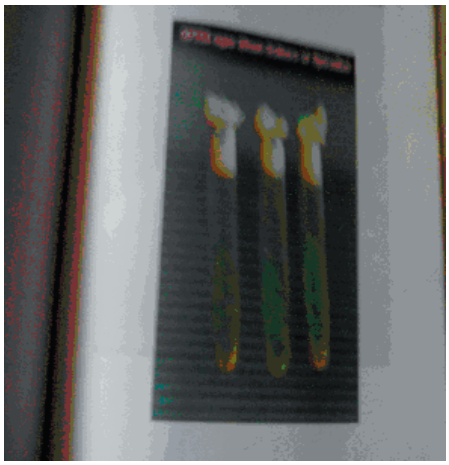

Figure 3. *Spirulina* growth in CFTRI broth

Table 2: Biomass, protein, carbohydrate, chlorophyll and carotinoid contents of *Spirulina* cultured in different media and sources after different days of incubation (values are mean values of three replicates)

S. No	Name of the Medium	Time of incubation (in Days)	Biomass (g/l)	Protein (g/l)	Carbohydrate (g/l)	Chlorophyll (g/l)	Carotenoid (g/l)
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 3. Biomass, protein and carbohydrate contents of yeast cultured in different media and sources (Values are means of three replicates)

S.No	Name of the Medium	Time of incubation (In Days)	Biomass (g/l)	Protein (g/l)	Carbohydrate (g/l)
1	YEMA (Yeast Extract Mannitol Agar)	0	0.01	0.0053	0.0033
		3	6.23	3.3019	2.0559
		5	8.20	4.346	2.706
		7	12.90	5.247	3.267
		10	12.90	6.837	4.257
2	Mollases	0	0.06	0.0318	0.0198
		3	7.22	3.8266	2.3826
		5	9.10	4.823	3.003
		7	13.93	6.3229	3.9369
		10	13.93	7.4094	4.6134
3	Yeast Sludge	0	0.04	0.0212	0.0132
		3	5.98	3.1694	1.9734
		5	8.15	4.3195	2.6894
		7	11.82	5.2046	3.2406
		10	11.82	6.3176	3.9336

Biomass production

The biomass of *Spirulina* from various media and sources were estimated and the results are given in Table 2. The biomass was highest in CFTRI medium, when compared to other media, which was obtained after 10 days of incubation (Table 2). In the agro based wastes also the productivity was highest after 10 days of incubation.

Biochemical constituents of *Spirulina* Biomass

Among the two different media tested the highest amount of protein was observed after 10 days of incubation in CFTRI medium (5.554 g/l) when the protein content was only 5.22 g/l/ in the molasses source (Table 2). The protein content was higher in molasses when compared to yeast sludge (Table 2). The higher biomass of *Spirulina* in enriched media, when compared to their growth on agro based wastes, might be due to the differences in the substrates of the growth medium which governs the protein content of microorganisms (Litchfiled, 1979).

After 10 days of incubation the amounts of carbohydrate, chlorophyll and carotenoids were highest in CFTRI medium when compared to others; however the values obtained from media containing agro-based wastes were almost equal (Table 2).

The biomass, protein and carbohydrate levels of yeast produced when cultured in molasses medium was higher when compared to other media (Table 3).

Amount of protein produced was higher in *Spirulina* when compared to the yeast. This might be due to differences in the components of substrates especially high carbon and low nitrogen level (Litchfield, 1979). In conclusion, results of the present study indicated that agro based wastes could be successfully used for the production of SCP from *Spirulina* and yeast.

REFERENCES

Arnon, D.L. 1949. A copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaries* plant physiol., 24: 1-15.

Bull, A., Holt, T., and Lilly, M.D. 1983. *Biotechnology: International Trends and Perspectives*, Oxford and IBH Publ. Co. Pvt. Ltd., New Delhi.

Clement, G. 1968. *In: Single Cell Protein*. Mateles, R.I. and Tannebaum, S.A. (Eds.), MIT Press, Cambridge, Massachutets P. 306-308.

Ferry, C.N. 1930. Nutrient preparation and low-cost basis construction for village production of Spirulina. In algal biotechnology. *Ing. Eng. Chem.*, 22: 1154-1162.

Gantar, M., Obrebt, Z. and Dalmaejia. 1991. Nutrient removal and algal succession during the growth of *Spirulina platensis* and *Scenedesmus quadricauda* on Swine waste water. *Bio Resource Technol.*, 36: 167-171.

Hedge, J.E. and Hofreiter, B.T. 1962. Determination of reducing sugars and carbohydrate. *In: Methods in Carbohydrate chemistry*, Whistler R.L. and Miller, J.N. (Eds.). Academic Press, New York, P. 380.

Litchfiled, J.H. 1979. Production of Single Cell Protein for use in food or feed. *In: Microbial Technology*. H.J. Peppler and D. Perlman (Eds.). P. 93-155.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Schwartz, J., Shklar, G.I. and Sudha, D. 1986. Carcinogens. 7: 711-715.

Scrimshaw, N.S. 1968. *In single cell protein*. *In:* Mateles, R. I. and Tannenbavim. S.R. (Eds.), MIT press, Cambridge, Mass. P. 3-7.

Subba Rao, N.S. 1982. *Biofertilizers in Agriculture*. Oxford and IBH Publ. Co., New Delhi.

Tannenbaum, S.R. and Wagt, E.I.C. 1975. *Single Cell Protein*. MIT press, Cambridge, Mass. P. 176-178.

Venkataraman, H. 1983. *Single Cell Protein*, MIT press, Cam-