

## Cultivation of *Spirulina* sp. Using Organic Substrates

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The large scale production of *Spirulina* biomass depends on many factors, the most important are nutrient availability, temperature and light. The nutritional requirements of algae are replaced with organic substrates such as neem cake, groundnut cake and press mud. Organic cultivation of *Spirulina* sp. using neem cake and groundnut cake rich in nitrogen and pressmud rich in potassium and phosphorous had been tried. Various concentrations of neem cake & pressmud and groundnut cake & pressmud were tried. The concentrations enhancing the growth of *Spirulina* were selected and organic media were constituted. The organic media (OM1) with neem cake and pressmud has 15g of each constituent and 16.8g of baking soda and 1g of sea salt. The other organic media (OM2) has 10g of groundnut cake and 15g of pressmud along with 16.8g of baking soda and 1g of sea salt. The growth, chlorophyll content and protein content of *Spirulina* sp. grown in the organic media were comparable with that of the cultures grown in Zarrouk medium.

**Key words:** *Spirulina*, Zarrouk medium, Neem cake, Groundnut cake, Pressmud, Organic medium, Growth OD, Chlorophyll, Protein.

*Spirulina* is one of the most promising microalgae. It is rich in protein, GLA and pigments such as phycocyanin. Commercial production of the magic blue green microalgae *Spirulina* is steadily increasing around the world. *Spirulina* can be easily manipulated and grown in nutrient medium with high alkaline pH (Alava *et al.*, 1997).

*Spirulina* is an outstanding candidate for outdoor cultivation being both thermophilic and alkalophilic, with optimum growth conditions

being 35-37°C and pH of about 10. The high optimum growth temperature is also responsible for increased productivity. The unique chemical composition of *Spirulina* offers an additional benefit for the large scale cultivation of this algae (Cohen, 1997). The outdoor cultivation of *Spirulina* in India started in the eighties.

The cultivation of microalgae is used for the production of valuable chemical compounds including natural pigments, biofuels and dietary supplements. Progress has been made in cultivation techniques and in designing high efficiency photobioreactors (Andrade & Costa, 2007).

*Spirulina* is largely grown as monoculture in outdoor cultivation systems (Venkataraman *et al.*, 1995). The cost of growth medium accounts for major share in the production of *Spirulina*.

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Raoof *et al* (2006) reported the incorporation of selected nutrients within the standard Zarrouk medium with other cost effective alternate chemicals, to facilitate low cost production of *Spirulina*.

To reduce the cost of medium agricultural byproducts can be used. Andrade and Costa (2007) reported about the use of molasses as an organic substrate for mixotrophic cultivation of *Spirulina platensis*.

In the present study an attempt has been made to use organic manures like neem cake, groundnut cake and pressmud to substitute the nitrogen, phosphorous and potassium requirements of Zarrouk medium and to reduce the cost of media.

## MATERIAL AND METHODS

The *Spirulina platensis* inocula were obtained from three sources namely

- *Spirulina* Production, Research and Training Centre, Antenna trust, Madurai (C<sub>1</sub>)
- Thulasi Saroja health Care Service Centre, Mfrs Spiros, *Spirulina* culture, Madurai (C<sub>2</sub>)
- CFTRI, Mysore (C<sub>3</sub>)

The culture were grown in traditional Zarrouk medium at 28-36°C in open sunlight in 2 litre beakers.

### Media

Zarrouk medium (ZM) (Zarrouk, 1966) was used as the standard medium, (ZM). The organic substitutes used in the low cost media included neem cake (NC), groundnut cake (GnC) and pressmud (PM)

In the traditional Zarrouk medium the N, P and K constituents were supplemented with various concentrations of i) NC viz 15g, 30g and 45g per litre. ii) GnC viz 10g, 30g and 50g per litre and (iii) PM viz 15g, 30g and 45g per litre individually. Along with the various concentrations of NC, GnC and PM, baking soda at 16.8g/l, sea salt @1g/l were added as carbon

**Table 1.** Response of the *Spirulina* cultures to neem cake

S. No.	Treatment	OD			Chlorophyll (µg/ml)			Protein (%)		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
1	ZM	1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
2	ZM*+NC 15g	0.8	0.9	0.8	1.06	1.24	0.97	48	42	48
3	ZM*+NC 30g	0.7	0.7	0.6	0.69	0.69	0.67	32	32	21
4	ZM*+NC 45g	0.1	0.2	0.2	0.2	0.2	0.2	6	8	9
	SED	0.0530			0.0587			1.6320		
	CD 5%	0.1080			0.1196			3.3245		

Zm - Zarrouk medium, NC - Neem Cake, ZM\* - ZM without NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub> & K<sub>2</sub>SO<sub>4</sub>

**Table 2.** Response of the *Spirulina* cultures to groundnut cake

S. No.	Treatment	OD			Chlorophyll (µg/ml)			Protein (%)		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
1	ZM	1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
2	ZM*+GnC 10g	0.7	0.8	0.9	0.83	0.97	0.97	42	48	48
3	ZM*+ GnC 30g	0.4	0.5	0.6	0.56	0.67	0.69	16	21	21
4	ZM*+ GnC 50g	0.2	0.1	0.3	0.2	0.3	0.3	6	7	9
	SED	0.0562			0.0682			1.0801		
	CD 5%	0.1146			0.1389			2.2002		

Zm – Zarrouk medium, GnC – Groundnut Cake, ZM\* - ZM without NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub> & K<sub>2</sub>SO<sub>4</sub>

source and to maintain the alkaline pH of 10-11.

In the second step, various concentrations of (i) NC and PM and (ii) GnC and PM were tried.

Combinations yielding better results were selected and new organic media were formulated with various concentrations of NC, GnC and PM along with baking soda and sea salt. About 50 ml of the culture was added to 200 ml of the media as initial inoculum. The performance was measured in terms of growth (OD at 540 nm), chlorophyll content (Talling & Driver, 1961) and protein content (Vonshak 1997), on the 9<sup>th</sup> day of growth.

The low cost organic media formulated OM1 has NC and PM and OM2 has GnC and PM.

The cultures were grown in open sunlight at 28-36°C under 12/12 hr light dark cycle. Sparger was used for mixing the cultures.

#### Statistical analysis

The data recorded were subjected to analysis of variance (ANOVA) using Agres statistical package. SED and CD at 5% significance level are given in the tables.

### RESULTS AND DISCUSSION

For the successful growth of algae in a culture, the environment must be conditioned to meet as many of the intrinsic requirements of that organism as possible. The environmental factors may be either physical such as temperature and light or chemical which provides all the raw

**Table 3.** Response of the *Spirulina* cultures to pressmud

S. No.	Treatment	OD			Chlorophyll (µg/ml)			Protein (%)		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
1	ZM	1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
2	ZM*+PM 15g	0.7	0.6	0.7	0.93	0.96	0.93	37	29	37
3	ZM*+ PM 30g	0.5	0.4	0.4	0.39	0.28	0.39	15.9	15.9	15.9
4	ZM*+ PM 45g	0.5	0.3	0.4	0.39	0.14	0.14	15.9	12.8	15.9
	SED	0.0577			0.0527			1.2019		
	CD 5%	0.0117			0.1073			2.1482		

Zm – Zarrouk medium,

PM - Pressmud, ZM\* - ZM without NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub> & K<sub>2</sub>SO<sub>4</sub>

**Table 4.** Response of *Spirulina* cultures to combination of Neem cake and press mud

S. No.	Treatment		OD			Chlorophyll (µg/ml)			Protein (%)		
	NC g/l	PM g/l	C1	C2	C3	C1	C2	C3	C1	C2	C3
1	5	5	0.5	0.6	0.7	0.56	0.56	0.69	21	21	32
2	5	10	0.6	0.6	0.7	0.69	0.69	0.69	21	32	32
3	5	15	0.5	0.6	0.6	0.56	0.69	0.69	21	32	32
4	10	5	0.7	0.7	0.8	0.56	0.69	0.83	21	37	32
5	10	10	0.6	0.6	0.7	0.69	0.69	0.83	32	37	37
6	10	15	0.6	0.7	0.8	0.56	0.69	0.69	37	37	37
7	15	5	0.9	0.8	0.8	0.69	0.69	0.83	42	37	42
8	15	10	0.9	0.8	0.9	0.83	0.83	0.97	42	42	42
9	15	15	0.9	1.1	0.9	0.83	0.97	0.97	48	42	48
10	ZM		1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
	SED		0.0658			0.0641			3.8371		
	CD 5%		0.1310			0.1275			7.6363		

ZM – Zarrouk medium Treatments 1-9 have Baking soda 16.8g and sea salt 1g along with neem cake and Pressmud

materials used for the structural and protoplasmic synthesis of the algal cells.

Studies on the nutritional requirements of algae, using replacement techniques as employed for higher plants, have revealed that the requirements of algae are similar to those for the phanerogams. In 1896, Molisch had observed that the mineral nutrition of the algae was not different from that of higher plants. The major absolute requirements include carbon, phosphorous, nitrogen sulfur, potassium and magnesium (Becker, 1994).

Nitrogen is the second important element in algal nutrition. Generally algae are able to utilize nitrate, ammonia or other organic sources of nitrogen.

In order to reduce the cost of production of *Spirulina* compounds like Single Super

Phosphate (SSP), Murate of Potash (MOP) (Raouf *et al.*, 2006), urea (Costa *et al.*, 2004; Luna *et al.*, 2004), Ammonium chloride (Sassano *et al* 2007) and molasses (Andrade and Costa, 2007) were supplemented.

Phosphorous is a major nutrient required for normal growth of all algae it is essential for almost all cellular processes. Potassium is a requirement for all algae. Under potassium deficient conditions growth and photosynthesis are reduced and respiration is high (Becker, 1994). The present study of organic cultivation of *Spirulina* reveals that neem cake @ 15g/l (Table 1) enhance the growth of *Spirulina*.

When neem cake was used @15g/l, the maximum growth of the 3 cultures were in the range of  $0.8 \pm 0.1$ , the chlorophyll content and protein content were in the maximum of 1.27  $\mu\text{g/l}$

**Table 5.** Response of *Spirulina* cultures to combination of groundnut cake and press mud

S. No.	Treatment		OD			Chlorophyll ( $\mu\text{g/ml}$ )			Protein (%)		
	GnC g/l	PM g/l	C1	C2	C3	C1	C2	C3	C1	C2	C3
1	5	5	0.6	0.5	0.5	0.56	0.56	0.69	21	32	21
2	5	10	0.5	0.5	0.6	0.56	0.69	0.69	32	32	32
3	5	15	0.6	0.7	0.6	0.69	0.69	0.56	21	21	32
4	10	5	0.8	0.8	0.7	0.59	0.83	0.83	32	32	32
5	10	10	0.9	0.9	0.9	0.83	0.83	0.83	37	37	42
6	10	15	0.9	0.9	1.0	0.83	0.97	0.97	42	48	48
7	15	5	0.8	0.8	0.8	0.69	0.69	0.83	42	42	42
8	15	10	0.7	0.8	0.7	0.69	0.56	0.69	37	42	37
9	15	15	0.8	0.9	0.9	0.69	0.69	0.69	37	37	37
10	ZM		1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
	SED		0.0691			0.0620			1.5107		
	CD 5%		0.1376			0.1233			3.9891		

ZM – Zarrouk medium Treatments 1-9 have Baking soda 16.8g and sea salt 1g along with neem cake and Press mud.

**Table 6.** Growth of *Spirulina* in organic media constituted with neem cake, groundnut cake and pressmud combination

S. No.	Treatment	OD			Chlorophyll ( $\mu\text{g/ml}$ )			Protein (%)		
		C1	C2	C3	C1	C2	C3	C1	C2	C3
1	OM1	0.9	1.0	0.9	0.83	0.97	0.97	48	42	48
2	OM2	0.9	0.9	1.0	0.83	0.97	0.97	42	48	48
3	ZM	1.2	1.3	1.2	1.6	1.8	1.6	64	63	64
	SED	0.0649			0.0873			1.2981		
	CD 5%	0.1340			0.1801			2.6793		

OM1 – NC 15g; PM 15g; BS 16.8g, seasalt 1g per litre, OM2 – GNC 10g; PM 15g; BS 16.8g, seasalt 1g per litre, ZM – Zarrouk medium

ml and 48% respectively. When 45g of NC was used there was no significant growth.

Groundnut cake @ 10g/l (Table 2) showed better performance. The results obtained showed growth of  $OD\ 0.8\pm0.1$ ,  $0.90\pm0.07$  ig/ml chlorophyll and  $45\pm3\%$  protein at the concentration of 10g of groundnut cake. When groundnut cake was used at 50g/l bleaching of the culture occurred. It may be due to excessive nitrogen supply which the cultures may not tolerate.

When *Spirullia* was grown in pressmud substituted media (Table 3) shows growth OD of  $0.7\pm0.1$ , chlorophyll of  $0.93\pm0.03$  ig/ml and protein of  $34\pm3\%$ . Comparatively the cultures growing in pressmud alone showed lesser growth, chlorophyll and protein than along with neem cake and groundnut cake grown cultures.

NC and GnC are rich in nitrogen than in P & K whereas PM is rich in P and K than N. So combination trial was done with NC and PM and GnC and PM.

Among the combinations employed neem cake 15g pressmud 15g showed better performance (Table 4). Maximum growth among treatments recorded were  $1.0\pm0.1$  growth OD,  $0.93\pm0.04$  ig/ml chlorophyll and  $45\pm3\%$  protein. Similarly when groundnut cake (10g) and press mud 15g were employed (Table 5), similar results were obtained, the growth of  $OD\ 1.0\pm0.1$  chlorophyll  $0.09\pm0.07$  ig/ml and protein  $45\pm3\%$ . Media with neem cake (15g) and PM (15g) was designated OM1 and media with GnC (10g) and PM (15g) was designated OM2. The constituents of OM1 are Baking soda 16.8g, neem cake 15g, Pressmud 15g, Sea salt 1g for one litre of medium and that of OM2 are Baking soda 16.8g, groundnut cake 10g, Pressmud 15g, sea salt 1g for one litre of the medium. Culture growing in OM1 and OM2 measured a maximum growth OD of  $0.9\pm0.1$  and chlorophyll content of  $0.9\pm0.07$  ig/ml. The protein content of the cultures were  $45\pm3\%$ .

The organic media OM1 and OM2 were compared with Zarrouk medium. Both the media performed on par with each other (Table 6). The approximate production cost of one litre of Zarrouk medium is Rs.5.50 where as that of the organic media in Rs.0.50 to 0.75.

## CONCLUSION

Present study emphasis is the possibility to cultivate *Spirulina* organically. By using organic supplements low cost media could be formulated for production of *Spirulina*.

The growth was at the maximum during 7, 8, 9 days after inoculation. The results of this study highlighted the potential of neem cake, groundnut cake and pressmud as organic substrate for the cultivation of *Spirulina*.

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