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ORIGINAL ARTICLE

FORMULATION OF COST EFFECTIVE RICE MILL EFFLUENT MEDIUM FOR THE MASS PRODUCTION OF SINGLE CELL PROTEIN (SCP)

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Abstract:

Spirulina is a photosynthetic, filamentous, non differentiated, multicellular blue green microalga that grows naturally in warm climate. The microalgae Spirulina is a source of protein, which is used as a protein supplement for humans, chicks and also in aquaculture. Spirulina platensis culture was isolated from two different locations namely Puducherry and Thiruvannamalai and designated as S3 and S4. The growth of S3 and S4 strains was estimated in Zarrouk's medium under laboratory condition. Various parameters like optical density, cell population and biomass was estimated. The effect of temperature and pH was determined for these strains S3 and S4 and highest biomass was observed at 30°C of pH 9.5. A new rice mill effluent (RME) medium was formulated for mass production of Spirulina by supplemented with various nutrients ($NaNO_3$, KNO_3, K_2HPO_4 and KH_2PO_4) and other cost-effective chemicals. This newly formulated RME medium generates valuable growth of Spirulina platensis. It is locally available, eco-friendly and cost effective medium.

KEYWORD:

RME medium, nitrogen, phosphorus, cost effective, Spirulina, biomass.

INTRODUCTION

Spirulina platensis is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical water bodies which have high alkaline pH value up to 11.0. The cyanobacterium Spirulina contains 74% dry weight of proteins, along with high concentrations of minerals, pigments, unsaturated fatty-acids and vitamins (Cohen, 1997) because of which it is used as a dietary supplement, nutrient source in food, feed and pharmaceutical industries especially in developing countries. It can grow in a wide range of environments like soil, sand, marshes, brackish water, sea water and fresh water (Ciferri et al., 1983).

Rice milling is a process of removing husk and part of the bran from paddy in order to produce edible rice. Parboiled rice production generally requires a large amount of water for soaking of the paddy. After soaking, the water is drained out. If this water is not properly treated could result in water pollution due to high levels of organic material present in rice mill effluent waste water. This effluent has high BOD, COD and organic contents mainly in the forms of carbon, thus having the potential to damage and deteriorate the environment (Pradhan and Sahu, 2004). Therefore, it needs to be treated before disposal, Literature reports indicate that biodegradation involving microorganisms is a suitable process for industrial waste water treatments (Noorjahan et al., 2005).

Olguin et al., 2001 reported that Spirulina has potential to reduce BOD of high carbon containing waste water due to its mixotropic nature. Rice mill effluent (RME) a rich source of starch and other nutrients can support profuse growth and aid in the mass multiplication of Spirulina. In the present study, the potential of using RME safe for disposal into the environment by reducing its BOD and supplemented with various concentration of N, P and Carbon nutrient sources and formulate low cost RME medium such a process would also generate valuable Spirulina biomass and create eco-friendly environment.

METERIALS AND METHODS

Isolation of Spirulina platensis strains and characterized

The microalga Spirulina was isolated from Pondycherry and Thiruvannamalai and named as S3 and S4. These strains was characterized based on parameters like average no. of spirals, direction of helix, distance between spirals, diameter of spirals, width, length and shape of the spirals, pH tolerance, temperature tolerance and habitat. Morphological characters were observed under microscope by using micrometric method. These strains were grown in Zarrouk's medium under laboratory condition at 30ºC light chamber for 30 days. The effect of temperature of two strains was determined by 250 ml Erlenmeyer conical flasks were used, containing 100 ml of the growth medium. Each flask was inoculated with 10 ml (0.05 mg) of the pure culture of the organisms. Each temperature regime was in triplicates. The culture were incubated in water bath at the appropriate temperature (25°C, 30°C, 35°C and 40°C) and the periodically replenished with the growth medium to prevent drying up of the cultures. The effect of pH was determined by 250 ml Erlenmeyer conical flasks were used each containing 100 ml of the growth medium. Each flask was inoculated with 10 ml (0.05 mg) of the pure culture of the organisms. Each pH (9.0, 9.5 and 10.0) was triplicate. The growth of Spirulina platensis was measured the parameters optical density, cell population and dry weight. The exhausted broth was collected for analyses. One portion was used for determination of dry cell mass concentration by optical density (OD) measurement at 560 nm using a calibration curve. Optical density was measured by using a spectrophotometer. The dry weight was measured by 100 ml of culture was sampled and filtered through what man No.1 filter paper and dried for 1 hr at 105ºC and weighed prior to filtration. The filtered wet biomass was then washed with two volumes of distilled water, dried as above and weighed. Cell population was estimated by direct microscopic count method.

No .of spirulina per ml of sample = average no.of spirulina cell x no.of microscopic field per sq.cm x dilution factor.
= x x n x 100

The dry weight was measured by 100 ml of culture was sampled and filtered through what man No.1 filter paper and dried for 1 hr at 105ºC and weighed prior to filtration. The filtered wet biomass was then washed with two volumes of distilled water, dried as above and weighed. The biomass concentrations in the cultures were determined through the cell weight measured by the method of Vonshak et al., 1982.

COLLECTION OF RICE MILL EFFLUENT

For laboratory experiment the rice mill effluent was collected from Parvathi rice mill from Sethiyathopu, Cuddalore district, Tamilnadu. The physico-chemical characters of the effluent were analysed and were presented in Table 2.

RME MEDIA FORMULATION

The collected effluent was filtered through what mann No.1 filter paper to remove the dust particles. Four 250 ml conical flask were taken and 100 ml of rice mill effluent was added, in each conical flask various nitrogen ($NaNO_3$ and KNO_3 1gL-1) and phosphorus (K_2HPO_4 and KH_2PO_4 0.5 gL^{-1}) sources were added separately, and add trace amount of $CaCl_2$, $FeSO_4$ and EDTA in each flask, pH was adjusted with 10 g-1 $NaHCO_3$ and Rice Mill Effluent (RME) broth was formulated. It was sterilized in an autoclave. The S_3 and S_4 strains were inoculated to the medium and incubated for 30 days in light chamber, after 30 days optical density value and dry weight was estimated. The highest growth was observed in both $NaNO_3$ and K_2HPO_4 added media.

Mass production of Spirulina SCP in RME media compare with routine ZM media

The newly formulated Rice Mill Medium (RME) was prepared and transfer in 1 liter Erlenmeyer conical flask, standard inoculums (50 ml) of two strains were separately inoculated and maintained at room temperature in light chamber for 30 days. After 30 days of growth the parameters such as optical density, cell population and dry weight were estimated by various methods are discribed earlier.

RESULT AND DISCUSSION

The isolated algal samples S3 and S4 srains general morphological characteristic like average no.of spirals, direction of helix, distance between spirals, diameter, width, length and shape of spirals were observed under microscope. And pH tolerance, temperature tolerance and habitat were also observed. The two strains S3 and S4 were identified as Spirulina platensis. It was presented in Table 1. The strain S3 and S4 was grown in Zarrouk's medium for further analysis for 30 days in light chamber. The growth parameters optical density cell population and dry weight of S3 and S4 strains were estimated. All the growth parameters were highest in S3 followed by S4. The effect of temperature and pH determined for the strains S3 and S4 highest biomass was observed at 30℃ of pH 9.5. In this optimum condition the biomass observed S3 and S4 were found to be 4.6 and 4.2 mg/100 ml. It has been shown by previous workers (Danesi et al., 2001; Vonshak, 1997) that the optimal growth temperature for S. platensis is between 30℃ and 35℃ with 40℃ definitely being deleterious to this cyanobacterium. In respect to increase in biomass, the best responses were obtained at 30℃, which agrees with the studies by Danesi et al. (2001).

The rice mill effluent was collected from Parvathy rice mill from Sethiyathopu. The waste water observed that the colour was pale yellow, the odour was unpleasant and the effluent were turbidity with an acidic pH (6.09) with low concentration of DO (0.2 – 1.0), BOD (350), COD (550), nitrate (2.5mg), sodium (100.09 ppm), calcium (50.36 ppm), pottasium (8 ppm), magnesium (43.78 ppm). Moreover the waste water was rich major minerals like sodium, calcium and magnesium (Table 3). It was stored in cold room temperature at 4ºC.

Spirulina strains S_3 and S_4 were cultivated in RME broth. Four 250 ml conical flask were taking and add 100 ml of rice mill effluent, in each conical flask various Nitrogen ($NaNO_3$ and KNO_3 $1gL^{-1}$) and Phosphorus (K_2HPO_4 and KH_2PO_4 $1gL^{-1}$) were added separately. In both nitrogen sources high algal biomass was observed in S_3, $NaNO_3$ (dry weight 3.80 mg / 100 ml) followed by KNO_3 (dry weight 2.80 mg / 100 ml). In S_4, $NaNO_3$ (dry weight 3.73 mg / 100 ml) followed by KNO_3 (dry weight 2.73 mg / 100 ml) was observed (Table 4). Carvalho et al, 2010 reported that the presence of nitrogen source was necessary to ensure higher cell mass concentration. Thus justifying the traditional use of nitrate in Spirulina platensis culture media. Nitrogen deficiency, as visually confirmed by the yellowish colouring of cells observed up to the 10th day of cultivation. Afterward, the use exponentially increasing flow rates of nitrogen sources were likely responsible for higher amount of nitrogen provided to the system, for increased pigment formation and then for restoring of cell dark green colour.

Phosphorus is a major nutrient required for the growth of alga and determines its primary productivity. Mostert and Grobbelaar, 1981 have indicated the essential role of phosphorus in maintaining high production rates of microalgae mass cultures. In phosphorus sources high algal biomass was observed in S_3, K_2HPO_4 (dry weight 4.20 mg / 100 ml) followed by KH_2PO_4 (dry weight 2.73 mg / 100 ml). In S_4, K_2HPO_4 (dry weight 4.00 mg / 100 ml) followed by KH_2PO_4 (dry weight 2.50 mg / 100 ml) was observed.

The low cost RME media was standardized and compared with routine ZM media composition shown in Table (5). This investigation was taken up with the basic aim of providing a simple, locally available, eco-friendly and low cost medium.

Spirulina has a high bicarbonate requirement, which acts not only as a carbon source but helps to maintain alkaline conditions, and increase the growth of Spirulina. Since laboratory grade sodium bicarbonate is costly in the Indian context, in RME medium 10 gL-1 compare with ZM media 16.8 gL-1 Therefore, the significant of the RME medium are clearly emphasized, not only as a low-cost alternative but also as a highly productive input, which can be used profitably by the rural population for large-scale biomass production of protein-rich Spirulina. In tropical countries, especially developing countries such as India, emphasis is placed more on the production costs. Therefore the present investigation was aimed towards the formulation of a new cheaper, low cost RME medium for the growth of cyanobacterium Spirulina (a rich source of proteins), using locally available rice mill waste water and create eco-friendly environment. The present study, the above discussed results are related to the formulation of low cost RME media, and production of Spirulina SCP and reduce the cost of production.

Table.1. General Characteristics of *Spirulina platensis* strain

S. No	Characters	S3	S4
1.	Average number of spirals	5-14	5-15
2.	Direction of helix	Right	Right
3.	Distance between spirals (µm)	0.5	0.2
4.	Diameter of spirals (µm)	2-10	12-15
5.	Width of spirals (µm)	0.2	2.0
6.	Length of spirals (µm)	0.5	0.1
7.	Shape of spirals	Tight	Loose
8.	pH tolerance	Alkaline	Alkaline
9.	Temperature tolerance	Mesophile (30°C)	Mesophile (30°C)
10.	Habitat	Marine, fresh and brackish water	Marine, fresh and brackish water

Table.2. Effect of pH and temperature on the dry weight of *Spirulina platensis*

Dry weight of *Spirulina platensis* (mg/100 ml)												
Temp	**25° C**			**30° C**			**35° C**			**40° C**		
pH	**9.0**	**9.5**	**10.0**	**9.0**	**9.5**	**10.0**	**9.0**	**9.5**	**10.0**	**9.0**	**9.5**	**10.0**
S3	3.8	3.4	3.3	4.4	4.6	4.0	3.9	3.0	2.5	2.9	2.8	2.0
S4	2.9	3.0	2.2	4.0	4.2	3.8	4.0	2.8	2.7	2.5	2.6	2.2

Table.3. Physico-chemical nature of Rice mill effluent

S. No	Parameter	RME
1.	Colour	Pale yellow
2.	Odour	Unpleasant
3.	Electrical conductivity (EC)	1.75
4.	pH	6.09
5.	Total solids (mg l^{-1})	582
6.	Suspended solids (mg l^{-1})	150
7.	Dissolved solids (mg l^{-1})	432
8.	BOD (mg/L)	850
9.	COD (mg/L)	2530
10.	DO (mg/L)	0.2 – 1.0
11.	Na (ppm)	100.09
12.	Ca (ppm)	50.36
13.	K (ppm)	8.00
14.	Mg (ppm)	43.78
15	Nitrate (ppm)	0.5-0.8

Table.4. Effect of various nitrogen and phosphorous sources in RME media

Strains	Nutrient sources	Range (gL^{-1})	After 30 days	
			Growth OD value (560 nm)	Dry weight (mg/100ml) biomass
S3 (N)	$NaNO_3$	1	1.350	3.80
	KNO_3	1	1.296	2.80
S4 (N)	$NaNO_3$	1	1.320	3.73
	KNO_3	1	1.285	2.70
S3 (P)	K_2HPO_4	0.5	1.375	4.20
	PO_4	0.5	1.283	2.73
S4 (P)	K_2HPO_4	0.5	1.310	4.00
	PO_4	0.5	1.205	2.50

* Contain 1000gl^{-1} rice mill effluent, 10 gl^{-1} $NaHCO_3$, $CaCl_2$, $FeSO_4$ and EDTA- Trace, pH 9-11.

Table.5. Comparison of standard (ZM) and newly formulated low cost (RME) medium

S.No	Zarrouk's media (ZM)	Composition (g)	Rice mill effluent media (RME)	Composition (g)
1.	$NaHCO_3$	16.8	$NaHCO_3$	10
2.	K_2HPo_4	0.5	K_2HPo_4	0.3
3.	$NaNo_3$	2.5	$NaNo_3$	1.0
4.	K_2So_4	1.0	K_2So_4	-
5.	NaCl	1.0	NaCl	-
6.	$MgSo_4.7H_2o$	0.2	$MgSo_4.7H_2o$	-
7.	$CaCl_2.2H_2o$	0.04	$CaCl_2.2H_2o$	Trace
8.	$FeSo_4.7H_2o$	0.01	$FeSo_4.7H_2o$	Trace
9.	EDTA	0.08	EDTA	Trace
10.	pH	8-9	pH	8-9
11	Distilled water	1000ml	Rice mill effluent	1000ml

Table.6. Growth parameters of *Spirulina platensis* in RME and Zorrouk's medium under laboratory condition (After 30 days).

Strains	Optimum condition		OD (560 nm)	Cell population	Dry weight (gL^{-1})
	Temp (^oC)	pH			
S3(ZM)	30	9.5	1.870	2,50,000	2.50
S4(ZM)	30	9.5	1.835	2,10,000	2.30
S3(RME)	30	9.5	1.862	2,70,000	3.00
S4(RME)	30	9.5	1.821	2,45,000	2.80

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ADDITIONAL QUALIFICATION:
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