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# Chlorophyll production from Spirulina platensis (single cell protein, SCP); cultivation with sodium chloride in Rice mill waste water

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**Abstract:** Paddy soaked rice mill effluent (RME) was collected and characterized. The potential of using RME safe for disposal into the environment by reducing its BOD and supplemented with and N, P, K and Carbon nutrient sources and formulate low cost RME medium, increased salinity various concentration of NaCl (1.2, 1.4, 1.6, 1.8 and 1.0 $gL^{-1}$) such a process would also generate valuable *Spirulina* biomass and chlorophyll content and most over create eco-friendly environment. The higher cellular growth was focused in 1.6$gL^{-1}$ sodium chloride contained media, the optical density, biomass productivity and chlorophyll content were 1.980, 0.562 $mgml^{-1}$ and 0.035 $mgml^{-1}$ respectively. The conclusion of this study focused RME medium was locally available, cost effective and eco friendly medium, make a pollution free environment and scale up production of valuable protein rich food " green gold" *Spirulina.*
**Key words:** Chlorophyll production, *Spirulina platensis*,single cell protein, SCP, cultivation with sodium chloride ,Rice mill waste water.

## Introduction

Chlorophyll is a naturally occurring pigment present in photosynthetic plants, including algae, and in some photosynthetic bacteria, known as cyanobacteria. The greater part of industrially prepared chlorophyll-derivatives is destined for the increasing demand for natural colorants for food and beverages. Some of the industrial production is also destined for the cosmetic and toiletry market, and to the pharmaceutical market[1].

*Spirulina platensis* is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids[2,3]. More recently, special attention has been given to *S. platensis* as a potential source of pharmaceuticals, and other high value products such as chlorophyll[4,5]. The utilization of chlorophyll from *S. platensis* is an attractive alternative that should be considered due to its high content of this pigment, and ease of cultivation. The cyanobacterium *S. platensis* possesses a high tolerance to alkaline pH, for ease of cultivation; a large size for its cell aggregates for ease of harvest; and an easily digestible cell wall[6]. 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. Rice mill effluent does not contain toxic compounds or pathogenic bacteria; but it can contain the traces of pesticide overdose, more so in the third world countries. Discharge into soil or water bodies on a continuous basis causes major environmental problems. The stagnant water emits off-odor. Off-odor during soaking can be generated due to fermentative changes. The growth of natural flora is effected due to discharge of effluent into the soil. Since it is rich in nutrients it triggers the growth of algae in water bodies. 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[7].

Olguin *et al*.[8] 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 and N, P, K and Carbon nutrient sources and formulate low cost RME medium, increased salinity various concentration of NaCl such a process would also generate valuable *Spirulina* biomass and chlorophyll content and most over create eco-friendly environment.

## Materials and methods

### *Spirulina* strain and growth medium

The *Spirulina platensis* was obtained from Department of Microbiology, Annamalai University and named as S5. The strain was maintained in a modified Zarrouk's medium[9] in 500mL flasks containing 200mL of culture at room temperature. The composition of the medium was per liter of distilled water: 10 g $NaHCO_3$, 0.5 g $K_2HPO_4$, 2.5 g $NaNO_3$, 1.0g $K_2SO_4$, 1.0 g NaCl, 0.20 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.08 g EDTA and 0.04 g $CaCl_2$ (Ravelonandro *et al*., 2008). The medium was autoclaved for 20 min at 121˚C.

### Collection of rice mill waste water and characterized

The rice mill waste water was collected from Ambiga rice mill, Annamalai nagar, Chidambaram, Cuddalore district. The paddy soaked waste water was collected and characterized.

### Measurement of Other Parameters

Electrical conductivity, salinity, total dissolved solids, and temperature and other micro nutrient elements (sodium, calcium, potassium and magnesium) were analyzed by Atomic absorbance spectrophotometer (AAS) Department of Soil Science, Annamalai University.

The collected effluent was filtered through Whatman No.1 filter paper to remove the dust particles. Erlenmeyer conical flask were taken and 100 ml of rice mill effluent was added, in each conical flask, $NaNO_3$1g$L^{-1}$ as a nitrogen source and $K_2HPO_4$ 0.5g$L^{-1}$ as a phosphorus sources were added separately, and add trace amount of $CaCl_2$, $FeSO_4$ and EDTA in each flask, pH was adjusted with 10 g$L^{-1}$ $NaHCO_3$ and Rice mill waste water converted as liquid medium for growth of *Spirulina platensis*. It was sterilized in an autoclave. The S5 strain was inoculated into the medium and incubated for 30 days in light chamber, after 30 days optical density value and biomass and chlorophyll content were estimated and maintained as control.

### Effect of salinity

The salinity of the medium was varied by addition of sodium chloride NaCl concentration. Five medium salinities were tested (1.2, 1.4, 1.6, 1.8 and 1.0 g$L^{-1}$) and the chlorophyll content was determined by[10] method. Algal growth can be measured most accurately in terms of pigment content. The algal growth was harvested by centrifugation 10 ml broth at 6000 rpm in a centrifuge for 10 mins. The algal pellet was washed twice with distilled water and supernatant in 10 ml of 95% methanol. The tubes containing algal suspension were kept in a water bath at 60˚C for 30 mins. Intermittent shaking of the tubes ensured complete extraction of pigment. The tubes were removed from water bath, allowed to cool to room temperature and centrifuged again to remove the cell debris. Clear supernatant containing the pigment was transferred to a volumetric flask and volume was made up to 10 ml by adding methanol. Optical density was measured by using a spectrophotometer at 560nm. The biomass concentrations in the cultures were determined through the cell weight measured by the method of Vonshak *et al*[11].

**Table.1. Physico-chemical nature of rice mill wastewater**

| Parameter | Ranges |
|---|---|
| Colour | Pale yellow, Brown yellow |
| Odour | Unpleasant |
| Electrical conductivity (EC) | 1.75 |
| pH | 6.09 |
| Total solids (mg $l^{-1}$) | 582 |
| Suspended solids (mg $l^{-1}$) | 150 |
| Dissolved solids (mg $l^{-1}$) | 432 |
| BOD (mg/L) | 550 |
| COD (mg/L) | 2100 |
| DO (mg/L) | 0.2 – 0.8 |
| Na (ppm) | 100.09 |
| Ca (ppm) | 50.36 |
| K (ppm) | 8 |
| Mg (ppm) | 43.78 |

**Table.2. Composition of RME medium**

| Rice mill effluent media (RME) | Composition ($gL^{-1}$) |
|---|---|
| $NaHCO_3$ | 10 |
| $K_2HPO_4$ | 0.5 |
| $NaNO_3$ | 1.0 |
| $K_2SO_4$ | - |
| NaCl | - |
| $MgSO_4.7H_2O$ | - |
| $CaCl_2.2H_2O$ | Trace |
| $FeSO_4.7H_2O$ | Trace |
| EDTA | Trace |
| pH | 9.5 |
| Rice mill effluent | 1000ml |

**Table.3. Comparison of biomass concentration and chlorophyll content of *Spirulina platensis* cultivation for different medium salinity in rice mill waste water**

| NaCl concentration (g/l) | Rice mill waste water (1000ml) | | | |
|---|---|---|---|---|
| | S5 | | | |
| | Optical density (560nm) | Biomass ($mgml^{-1}$) | Chlorophyll content($mgml^{-1}$) | Chlorophyll content(%) |
| Control | 1.147 | 0.223 | 0.009 | 4.0 |
| 1.2 | 1.652 | 0.401 | 0.023 | 5.7 |
| 1.4 | 1.700 | 0.492 | 0.029 | 5.8 |
| 1.6 | 1.980 | 0.562 | 0.035 | 6.2 |
| 1.8 | 1.543 | 0.398 | 0.020 | 5.0 |
| 2.0 | 1.235 | 0.225 | 0.010 | 4.4 |
| $SE_D$ | 0.126 | 0.056 | 0.004 | 0.354 |
| CD (P=0.5) | 0.252 | 0.112 | 0.008 | 0.708 |

## Result and discussion

The *Spirulina platensis* S3 strain was obtained from Department of Microbiology, Annamalai University. It was maintained in modified Zarrouk's medium.

It was observed that the colour of the effluents was yellow brown, the odour was unpleasant and the effluents were turbid. The waste water showed an acidic pH ( 6.25) with low concentration of DO (0.2 – 0.8), BOD (550), COD (2100), nitrate ( 2.5mg), sodium (100.09 ppm), calcium (50.36 ppm), potassium (8 ppm), magnesium (43.78 ppm). Table.1. In a similar kind of study, the detailed process and effluent characteristics of a rice mill were studied by Pradhan and Sahu (2004)[16]. Thus, literature reports on physico-chemical analysis of industrial effluents reveal that rice mill effluents possess low BOD, COD and organic matter in comparison to effluents generated by other industries, however, in quantitative terms it compels for treatment before disposal. Thus the waste water was used and formulated new cost effective medium Rice mill effluent (RME) liquid medium[17]. The medium salinity was varied from 1.2 to 2.0g $L^{-1}$. The higher cellular growth was focused in 1.6g $L^{-1}$ sodium chloride contained media, the optical density, biomass productivity and chlorophyll content were 1.980, 0.562 $mgml^{-1}$ and 0.035 $mgml^{-1}$ respectively. The growths kinetic are presented in terms of optical density, biomass and chlorophyll content in Table.3.

A decrease in salinity was observed during growth because of the assimilation of irons by the microalgae but the variation was not very large and did not influence the growth of *Spirulina platensis*. The optimum microalgae growth was obtained with salinity of 13g $L^{-1}$ even though *A.platensis* from Toliara was isolated from a salt lake. As reported in the literature, cyanobacteria are able to adapt to the alkaline habitat, but high salinity could became limiting[12,13]**.** Morphological changes were also observed as the number of spirals decreased with the increased salinity. Some studies have already observed that addition of NaCl inhibits growth of the helicoidal morphine[14,15]. In this study, the salinity was increased up to 2.0g $L^{-1}$, the optimal level was concluded at 1.6g $L^{-1}$ of NaCl concentration. In RME medium the alkaline condition was maintained by using $NaHCO_3$ 10g $L^{-1}$. In this salinity level the biomass and chlorophyll content were also increased. When the NaCl concentration was decreased and increased in the level of 1.6g $L^{-1}$, the biomass and chlorophyll content was also affected. In 1.2, 1.4, 1.8 and 2.0g$L^{-1}$ of NaCl concentration the biomass and chlorophyll content was 1.235, 1.652, 1.700 and 1.543 $mgml^{-1}$ and 5.7, 5.8. 5.0 and 4.4% respectively. When increased salinity it indicates the growth of *Spirulina* was inhibited and also the chlorophyll content also decreased.

**Fig.1. Effect of salinity on biomass productivity**

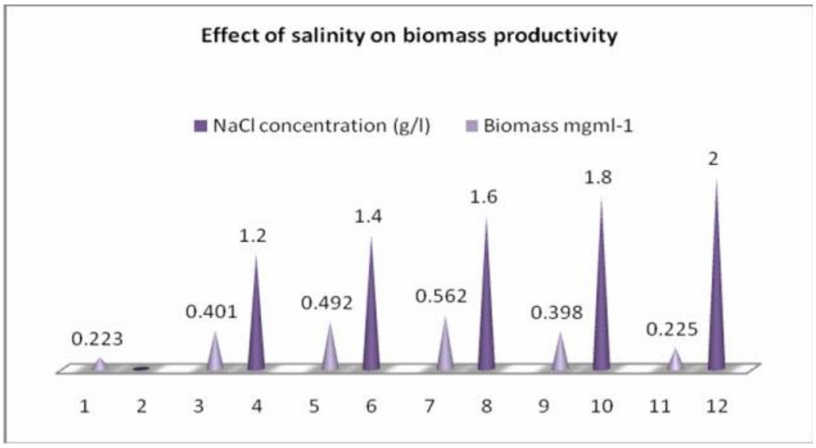
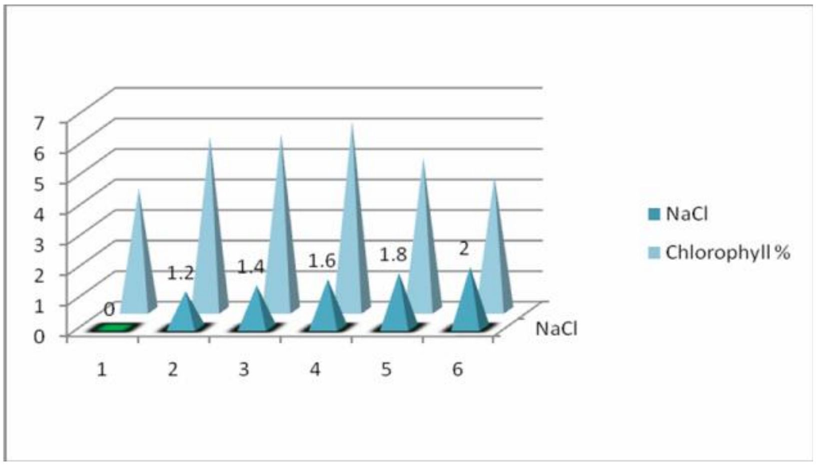



**Fig.2. Effect of salinity on chlorophyll content**



## Conclusion

Many literatures reported that the *Spirulina* SCP was cultivated in various waste water from noodles factory, digested pig waste, olive oil mill and sea water. In this study the paddy soaked rice mill waste water was collected and converted newly cost effective medium for cultivation of *Spirulina platensis* with increased amount of chlorophyll content. And also RME medium was locally available, cost effective and eco friendly medium. The conclusion of this study focused make a pollution free environment and scale up production of valuable protein rich food "green gold" *Spirulina.*

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