



## RESEARCH ARTICLE

# BIOCHEMICAL ANALYSIS OF *SPIRULINA PLATENSIS* (SINGLE CELL PROTEIN, SCP) UNDER IN DIFFERENT CONCENTRATION OF RICE MILL EFFLUENT

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### ABSTRACT

The present research work was carried out for assessing the optimum culture conditions for the biochemical constitution of spirulina platensis. Spirulina was cultured in conical flasks under three different concentration of rice mill effluent i.e (10 ml, 20 ml and 30 ml) under in vitro conditions. After 30 days of inoculation of cultures were on the peak of growth and ready to harvest. Dried spirulina powder was subjected to biochemical analysis. The metabolites considered for biochemical analysis were protein, carbohydrate and chlorophyll. An excellent growth performance and maximum percentage of metabolites was observed in cultures those were kept under 20 ml of rice mill effluent containing culture medium. Optical density was considered growth parameter and so deliberated after the intervals of 5 days up till 30th days. The higher percentage of protein, carbohydrate and chlorophyll were recorded in 20 ml of rice mill effluent containing medium compare to other concentrations.

**Key words:** spirulina, cyanobacteria, single cell protein and metabolites.

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### INTRODUCTION

*Spirulina* are multicellular and filamentous blue-green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. It grows in water, can be harvested and processed easily and has very high macro- and micro-nutrient contents. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is naturally found – for instance those living adjacent to Lake Chad in the Kanem region have very low levels of malnutrition, despite living on a spartan millet-based diet. This traditional food, known as *dihé*, was rediscovered in Chad by a European scientific mission, and is now widely cultured throughout the world. In many countries of Africa, it is still used as human food as a major source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as health food. Spirulina has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry, and increasingly as a protein and vitamin supplement to aquafeeds. China is using this micro-alga as a partial substitute of imported forage to promote the growth, immunity and viability of shrimp.

The primary objective of this study is therefore the growth performance and biochemical assessment of *Spirulina platensis* cultivation in different concentration of rice mill effluent under in vitro condition.

### MATERIALS AND METHODS

#### Collection of Rice Mill Effluent (RME)

The Rice Mill Effluent (RME) was collected from the Parvathi Rice Mill at Meetuppattai in Pudukkottai District, Tamil Nadu. The collected Rice Mill Effluent sample was stored in the plastic container and stored at room temperature. *Spirulina platensis* cultivated in three different concentration of rice mill effluent (10 ml, 20ml and 30ml) on growth performance and cellular constituents. *Spirulina platensis* was cultivated in conical flasks and these flasks filled with freshly prepared Zarrouk's medium and Zarrouk's medium with different concentration of rice mill effluent (10 ml, 20 ml and 30 ml) with 100 ml of mother culture. These cultures were subjected to grow under in vitro conditions. Growth of *spirulina* was measured uv spectrophotometer at 560 nm. Algal slurry was sun dried for 8-14 hours depending on climatic conditions. Sun dried algal flasks were grinded by pastel mortar and then biochemically analyzed. Protein was estimated by the method as described by lowry *et al.*, (1951). The estimation of carbohydrate was carried out by simple Anthrone method (Roe

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et al., 1955). Chlorophyll was estimated by the procedure and equation, suggested by of Parson and Strickland (1965).

### Direct Microscopic Count

A loopful of culture was placed on a slide and a cover slip was placed over the culture. The wet mount was observed and counted under 10X objective.

## RESULTS

*Spirulina platensis* was cultured under in Zarrouk's medium and Zarrouk's medium with three different concentration of Rice Mill Effluent under *in vitro* conditions.

**Table 1. optical density (1st to 30th day) of spirulina platensis cultures kept under different concentration of rice mill effluent compared to zarrouk's medium**

s.no	Treatments	1 <sup>st</sup> day	10 <sup>th</sup> day	20 <sup>th</sup> day	30 <sup>th</sup> day
1.	T1- zarrouk's medium + 100 ml mother culture	1.298	2.294	4.325	19.870
2.	T2- zarrouk's medium + 100 ml mother culture + 10 ml rice mill effluent	1.098	1.971	3.124	14.205
3.	T3- zarrouk's medium + 100 ml mother culture + 20 ml rice mill effluent	1.325	2.638	5.764	28.785
4.	T4- zarrouk's medium + 100 ml mother culture + 30 ml rice mill effluent	1.309	2.354	4.457	20.098

**Table 2. Biochemical analysis of spirulina platensis cultured under different concentration conditions**

Treatments	Protein	Carbohydrate	Chlorophyll
T1- zarrouk's medium + 100 ml mother culture	56.6%	14.50%	1.24%
T2- zarrouk's medium + 100 ml mother culture + 10 ml rice mill effluent	55.3%	14.20%	1.21%
T3- zarrouk's medium + 100 ml mother culture + 20 ml rice mill effluent	59.6%	15.98%	1.34%
T4- zarrouk's medium + 100 ml mother culture + 30 ml rice mill effluent	57.35%	14.98%	1.20%

The optical density of each measured by regular intervals of 5 days to know about growth performance of cultures in different conditions. Due to the variable growth parameters, the growth rate of all three sets was also variable. Growth performance of *spirulina platensis* under different culture conditions was measured in terms of optical density. The maximum growth was reported from Zarrouk's medium with 20 ml of rice mill effluent containing medium. The od value were reported in 20 ml rice mill effluent containing medium (1.325, 2.638, 5.76, and 28.78) from 1<sup>st</sup> 30<sup>th</sup> days. While the minimum growth rate represented in 10 ml rice mill effluent containing medium, the od value were reported (1.309, 1.97, 3.12 and 14.20) from 1<sup>st</sup> to 30<sup>th</sup> days. (Table.1). All the flasks were harvested after 30 days of inoculation by nylon cloth of 65 mesh. The algal slurry was washed with fresh water for 3-4 times to remove the adhered salts and dewatered by pressing gently for few minutes and finally dried under the sun or keeping in the hot air oven and the dried powder was subjected to biochemical analysis. Blue green microalga *spirulina platensis* is an important source of nutrients, proteins, amino acids, carbohydrate, chlorophyll and minerals. The highest chlorophyll content was recorded in 20 ml of rice mill effluent inoculated culture medium *spirulina platensis* possess 1.34%, while it was 1.21% chlorophyll was recorded in 10 ml rice mill effluent inoculated culture medium and 1.24% chlorophyll was recorded in Zarrouk's medium. The highest protein and carbohydrate content were recorded in t3- (59.6% and 15.98%) it was containing 20 ml of rice mill effluent cultured medium, and the lowest protein and carbohydrate content were recorded in t2- (55.3% and 14.20%) it was containing 10 ml of rice mill effluent inoculated cultured medium. In t1 containing zarrouk's medium without rice mill effluent (56.6% and 14.50%) of protein and carbohydrate content recorded. (Table.2).

## DISCUSSION

*Spirulina platensis* was cultured under three different conditions for *spirulina* cultivation. The dried powder which came from three different culture conditions also analysed to predict the best culture conditions. the metabolites which considered analysis were proteins, carbohydrate and chlorophyll. Due to variable growth parameters, the growth rate of all three sets was also variable. Growth performance of *spirulina platensis* under different culture conditions was measured in terms of optical density. Among four different conditions the optimum performance of *spirulina platensis* was observed under 20 ml of rice mill effluent containing medium.

The minimum growth performance and biochemical analysis indicated that the observed in 10 ml rice mill inoculated medium.

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