Research Article

J. Algal Biomass Utln. 2013, 4 (1): 42–49

ISSN: 2229- 6905

Journal of Algal Biomass Utilization

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A Laboratory Scale Cultivation of *Spirulina platensis*using Cooling Tower Water (CTW) Supplemented with Standard Medium (CFTRI)

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ABSTRACT:

In this study, *Spirulinaplatensis* was cultivated by using cooling tower water at concentrations of 20, 30, 40, 50, 60, 70, 80, 90 and 100%. Results showed that the most suitable concentration for maximum growth of *S.platensis* (0.160 gl^{-1} dry biomass) was 100% of cooling tower water (100% CTW). The supplementation of CTW with CFTRI medium increased the growth rate at a greater extent. The maximum growth of *S.platensis*with 30% supplemented CTW was measured in terms of dry biomass (0.448 gl^{-1}) and chlorophyll a content (4.03 mgg^{-1}). The maximum removal efficiency of 30% supplemented CTW at the end of experiment was measured at 96.8%, 63.8%, 47.1% and 63.8% for TSS, TDS, COD and conductivity respectively. The nutritional status of *S.platensis* cultured in 30% supplemented CTW suggested that the biomass of *S.platensis* could be applied for use as a food nutrient in the animal farms and in fisheries. Also the use of CTW as a nutrient medium could decrease the medium cost and the production cost as well.

Key words: - *Spirulinaplatensis*, Biomass, Chlorophyll, Cooling tower water, removal efficiency

INTRODUCTION:

Spirulina is a blue green multicellular (Ciferri 1983), filamentous cyanobacterium (Richmond 1984). Its name is derived from the Latin word for helix or spiral denoting the physical configuration of the organism, when it forms swirling microscopic strands. Even though it is single celled, *Spirulina*is relatively large attaining size of 0.5 mm in length. The diameter of the cells range from 1 to 3 µm in the smaller species and from 3 to 12 in the larger. The prolific reproduction capacity of the cells and their proclivity to adhere in colonies makes *Spirulina* species a large and easily gathered plant mass (Richmond 1984).

Proteins, vitamins and iron rich microscopic *Spirulina* is commercially produced in some tropical and subtropical climatic regions of the world (Venkataraman and Becker 1985;Henrikson 1989). The large scale cultivation of microalgae and the use of its biomass as a source of certain food constituents were considered as early as the 1950. This is being developed as the food for the future because of its remarkable ability to synthesise high quality concentrated food more efficiently than any other algae. Most notably *Spirulina* is 65-71% complete protein with all amino acids in perfect balance (Becker 1994).

A basic issue in the production of *Spirulina* is to maintain a continuous culture with an optimal population density. The basic demand in this respect is to provide growth conditions that will not be too much different from the optimal for the *Spirulina* species. The primary aim in developing a culture medium is insuring that the required nutrients are present in an appropriate form and at non-inhibitory concentrations (Greasham 1997).

Cooling towers are used to cool down cooling water by using air. They remove heat from the water that is discharged from a condenser. It involves a process of admitting water into the cooling tower at the top. It then flows downwards in the cooling tower, where it is collected and fed back into the cooling tower to cool down the system again or discharged into the environment. Water evaporates during this process. This evaporation then ensures additional cooling based on the principal of evaporation chill.

Cooling towers are generally used to provide cooled water to electric power generations and mechanical processes. Power plants that are located near lakes and rivers use cooling towers to cool down the circulating water, which has been heated in the condenser.

The cooling tower water contains optimal levels of dissolved salts and residual hydrocarbons. These compounds are important nutrient source for aquatic plants and specially algae (Finlayson *et al*. 1987; Tiow-Suan and

ISSN: 2229- 6905

Anthony 1988;Servin-Reyssac1998). Algae use solar energy, while absorbing nutrients from wastewater to fix carbon substances to produce plant biomass that would constitute a valuable source of compounds for animal feed supplement and that can be at the same time, as a decaying organic pollutant that decreases water pollution.

In recent years many algae have been applied to cultivate in many kinds of wastewater to improve water quality such as *Phormidium, Spirulina, Chlorella* and *Scendesmus* (Gantar*et al.,* 1991;Ang and Abu 1996;Blier*et al.* 1996;Olguin*et al.* 1997;Phang*et al.* 2000;Traviesо*et al*.2006;Jongkon*et al*. 2008;Ungsethaphand*et al.* 2009). In the present investigation *Spirulinaplatensis* was selected to cultivate in cooling tower water. *S.platensis* is a planktonic cyanobacterium that grows fast as a valuable product (Ciferri andTiboni 1985; Cohen *et al.* 1987;Henrikson 1987).

MATERIALS AND METHODS:

Cooling tower water (CTW) used in the study was collected from Skol Breweries Ltd, Unit- Tripti, Banmore, Distt. Morena (MP).*Spirulinaplatensis* strain used in this work was procured from the Centre for Conservation and Utilization of Blue Green Algae, IARI, New Delhi. The culture was maintained by repeated transfer to CFTRI medium.

***Experiment I. Cultivation of S. platensis In Cooling Tower Water without any supplementation*:** For the cultivation of *S.platensis*, CTW was first filtered and sterilized. It was diluted with distilled water to produce dilution percentages of 20, 30, 40, 50, 60, 70, 80, 90 and 100. The experiment was set in 500ml Erlenmeyer conical flasks and replicated three times in a completely randomized design. The stock solution of *S.platensis* was maintained at $30^oC\pm1$ with photoperiod of 12hrs light/dark period provided by fluorescent lamps at the light intensity of 3 Klux. pH was adjusted at 9.0±1. Experiment was initiated with 10% (v/v) (OD at 1.0) of stock solution of *S.platensis*. The experiment run for 24 days at temperature $25^oC\pm2$, pH 10±1 and photoperiod of 12/12 light /dark with light intensity 3 Klux by fluorescent tubes, aerated 3 times for 2 minutes in a day by orbital shaker.

Experiment II. Cultivation of S. platensis In Supplemented Cooling Tower Water: In second experiment, *S.platensis* was cultivated in supplemented CTW. For this, CTW was diluted with CFTRI medium (it was used as control in experiment) to produce dilution percentage of 20, 30, 40, 50, 60, 70, 80 and 90. All the culture conditions were kept same as in experiment I.

***Growth Evaluation*:** Growth was estimated in terms of dry biomass and chlorophyll a content. At the end of experiment after 24 days, biomass was filtered with 0.45 µm cellulose acetate filter paper, oven dried for 4 to 6 hrs at 75°C and the difference between the initial and final weight of filter paper was taken as the dry weight of *S.platensis*. Spectrophotometricestimation of the chlorophyll a content was carried outfollowing the method of Mackinney (Mackinney 1941).

***Statistical Analysis*:** Results were submitted to analysis of variance (ANOVA) and Tukey honest significance difference (HSD) test with confidence level of 95 % ($p<0.05$) in order to verify significant difference in dilutions, dry biomass and chlorophyll a content.

***Cost Analysis and Nutritional Value*:**The cost of formation of new medium i.e. 30% supplemented CTW and the standard cost of CFTRI medium was calculated. The profit was calculated in terms of percentage. Samples of *S.platensis* were analyzed for their nutritional contents (protein, pigments, minerals and free amino acids (FAN)) using standard methods.

***Physico-Chemical Analysis*:**Before the mass culture of *S.platensis* in 30% supplemented CTW, the medium was first analyzed for its physico-chemical properties viz. pH, Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS) and Conductivity following standard methods (Clesceri*et al.* 1989). Same physico-chemical properties were determined again in the filtrate after harvesting the biomass of *S.platensis* at the end of the experiment to assess the efficiency of 30% supplemented CTW medium. The results of analysis were statistically analyzed through analysis of variance (ANOVA) at 95% confidence interval.

RESULTS AND DISCUSSION:

***Experiment I**:* Growth performance of *S.platensis* cultured in cooling tower water with different dilutions in distilled water in terms of dry biomass and chlorophyll a content is illustrated in table I. It was found that the cyanobacterium*S.platensis* was able to grow well in cooling tower water only in higher dilutions, because it was not able to adapt itself in low dilutions due to scarcity of dissolved nutrients in the medium. At higher dilutions i.e. from 70% to 100%, the cyanobacterium was able to survive all throughout the experimental period. On the other hand, at lower dilutions i.e. from 20% to 60% it could not survive and died almost completely at the end of experiment because the feed was not sufficient for growth. The maximum dry biomass i.e. 0.60 gl^{-1} was found in pure

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CTW i.e. in 100%CTW. The chlorophyll a content in 100% CTW was 2.89 mgg^{-1}. This was followed by 90% CTW which produced 0.152gl^{-1} dry biomass and 2.5mgg^{-1} chlorophyll a content and 80% CTW produced 0.136 gl^{-1} dry biomass and 2.5mgg^{-1} chlorophyll a. Choonawala andSwalaha (2004) reported 0.010 gl^{-1} dry biomass of *Spirulina*, when grown in Brine Effluent Medium (BEM) obtained from cooling tower water, while in Synthetic Spirulina Medium (SSM) found 0.081gl^{-1} dry biomass. In order to improve the growth performance of *S.platensis*, cooling tower water was modified by supplementation of CFTRI medium at dilution level from 20% to 90%. CFTRI medium was used as control during the experimental study.

Table I. Growth performance of *S.platensis* cultivated in cooling tower water with different dilutions in distilled water.

Dilutions	**Dry biomass (gl^{-1})**	**Chlorophyll a content ($\times 10^{-3} mgml^{-1}$)**
20% CTW	0.008±0.03	nd
30% CTW	0.008±0.01	nd
40% CTW	0.096±0.10	nd
50% CTW	0.104±0.23	nd
60% CTW	0.120±0.61	nd
70% CTW	0.120±0.03	1.65±0.13
80% CTW	0.136±0.05	2.50±0.56
90% CTW	0.152±1.06	2.65±0.21
100% CTW	**0.160±0.02**	2.89±0.59
Control	0.600±0.21	7.31±1.03

*Mean ± SD; nd= not determined

Experiment II: From this study, the results of dry biomass and chlorophyll a content areshown in table II. The cyanobacterium*S.platensis* had a great increment in growth after supplementation with CFTRI medium. The increase in growth performance was observed more in lower dilutions in comparison to higher dilutions after supplementation. The best amount of dry biomass (0.448 gl^{-1}) and chlorophylla content (4.03 mgg^{-1}) were found in 30% supplemented CTW, while in control dry biomass of *S.platensis* was 0.60 gl^{-1} and 7.31 mgg^{-1} chlorophyll a content. Manikandavelu and Murugan (2009), in their study, found 0.34 gl^{-1} and 0.520 gl^{-1} dry biomass of *S.platensis* in swine waste filtrate and in control (Zm) respectively. This finding is similar to the present results. Promya*et al*. (2008) found 0.80 gl^{-1} dry biomass of *S.platensis* in 100% kitchen waste water (kw) and 0.0728gl^{-1} in 90% kw. The difference in findings is due to the different source of nutrient medium for culturing *Spirulina*. After supplementation, the amount of dry biomass of *S.platensis* found in 30% supplemented CTW was comparable to the amount of dry biomass obtained in control (0.448 gl^{-1} and 0.60 gl^{-1}). This was followed by 20%supplimented CTW, where dry biomass and chlorophyll a content were found 0.40 gl^{-1} and 4.03 mgg^{-1} respectively. The increase in growth of *S.platensis* due to supplementation of CFTRI medium at lower dilutions was due to the presence of adequate amount of chemical load/ nutrients in the CTW medium as supported by the results of chemical analysis of 30% supplemented CTW. When the dilution of CTW increased with CFTRI medium, the chemical load of medium also increased gradually and thus excess load affected the rate of photosynthesis of *S.platensis* and caused the reduction of biomass at higher dilutions with CFTRI medium. The lowest amount of dry biomass i.e. 0.03 gl^{-1} was observed in 90% supplemented CTW followed by 0.04 gl^{-1} in 80% supplemented CTW.

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Table II. Growth performance of *S.platensis*cultivated in cooling tower water with different dilutions in CFTRI medium.

Dilutions	Dry biomass (gl^{-1})	Chlorophyll a content ($\times 10^{-3} mgml^{-1}$)
20% CTW	0.400±0.05	3.85 ±0.01
30% CTW	**0.448±0.03**	4.03 ±0.05
40% CTW	0.320±0.13	3.62 ±0.13
50% CTW	0.160±0.20	1.89 ±0.09
60% CTW	0.080±0.15	0.75 ±1.03
70% CTW	0.080±1.30	0.73 ±2.01
80% CTW	0.040±0.02	0.362±2.03
90% CTW	0.030±0.06	0.396±0.03
Control	0.600±0.21	7.31 ±1.03

*Mean ± SD

ANOVA test indicated that in Experiment I, there was statistically significance difference ($p<0.05$) in the mean value of chlorophyll a content among all dilutions. The difference between the mean value of biomass among the dilutions 30%, 40% and 60% were non-significant, while among other dilutions, it was significant ($p=0.024$). In Experiment II, ANOVA test indicated statistically significance difference among the mean value of chlorophyll a content and biomass in dilutions 30%, 50%, 60%, 80% and 90% while in dilutions 20%, 40% and 70%, there was no significant difference among the mean values of chlorophyll a content. The values of dry biomass were statistically significant ($p=0.024$).

Physico-Chemical Analysis:The results of Physico-chemical analysis of 30% supplemented CTW and CFTRI medium are summarized in table 3. After completing the 24 days experimental period, it was found that the solids like TSS, TDS and COD were significantly ($p<0.05$) lower. Here, both organic and inorganic loads were mixed up which were used by the *Spirulina*as the source of nutrients. Similar results were found in the study of Habib*et al.* (1997) and Toyub*et al.* (2007).

Table III. Physico-chemical analysis and percentage removal in 30% supplemented cooling tower water and CFTRI medium.

Physico-chemical Parameter	Unit	CFTRI Medium			30% Supplemented CTW		
		Before Culture	After Culture	%Removal	Before Culture	After Culture	%Removal
pH		9.0	9.78	-	9.0	9.82	-
TSS	mgl^{-1}	193±1.6	150±0.38	22.3%±0.05	625±0.01	20±0.08	96.8%±0.02
TDS	mgl^{-1}	4200±0.06	1740±0.31	58.6%±0.7	6960±0.03	2520±0.6	63.8%±0.5
COD	mgl^{-1}	592±0.1	240±1.30	59.5%±0.1	544±0.02	288±0.1	47.1%±1.2
Conductivity	mSm^{-1}	7000±1.02	2900±0.08	58.6%±0.02	11600±0.5	4200±.0.2	63.8%±1.3

*Mean ± SD

ISSN: 2229- 6905

Both the media showed an increase in pH at the end of the experiment. During commercial growth of *S.platensis*, the medium has high pH (9.5 to 12) and high salinity and they are particularly selective for this organism, an important factor in preventing contamination of the culture by bacteria, algae and protozoa (Walach*et al.*1987). The amount of TSS found 193 mgl^{-1} and 625 mgl^{-1} before culturing*S.platensis* in CFTRI medium and 30% supplemented CTW respectively. As the CFTRI medium is a purely high grade chemical medium, the TSS content is naturally less in it while the other one contains good amount of TSS because of its contact with the other processes in cooling tower and with the surrounding. After culture of *S.platensis*, TSS content was 150 mgl^{-1} and 20 mgl^{-1} in CFTRI medium and 30% supplemented CTW respectively. The removal efficiency was measured 22.3% and 96.8% respectively. The TDS content before culture was 4200 mgl^{-1} in CFTRI medium while 6960 mgl^{-1} in 30% supplemented CTW. The removal efficiency was recorded 58.6% and 63.8% respectively. The % removal of TSS and TDS of 30% supplemented CTW was found higher than CFTRI medium after culturing *Spirulina.*

The COD values were 592 mgl^{-1} and 544 mgl^{-1} in CFTRI medium and 30% supplemented CTW respectively. The value of COD of 30% supplemented CTW was within the range as the highest COD value 500 to 980 mgl^{-1} with either aerobic or anaerobic digestion is not harmful for aquaculture (Phang 1990). With 30% supplemented CTW and CFTRI medium , the COD value decreased 47.1% and 59.5% respectively, but somewhat less than in the cultivation of *S.platensis* in 100% kitchen waste water and 90% kitchen waste water as reported by Promya*et al.* (2008), where COD decreased by 72.6% and 73% respectively. The present findings were also less than in the cultivation of *S.platensis* in waste water from the production of sago starch as reported by Canizares (Canizares 1993), where COD decreased by 98.0% and in the cultivation of*S.platensis in* 30% effluent from pig manure biogas digester reported by Promya and Traichaiyaporn (Promya and Traichaiyaporn 2003), where COD decreased by about 97% using different waste sources.

The physico-chemical analysis of 30% supplemented CTW under the present study indicated that the medium is nutritionally rich. Wing and Chang (2002) analyzed the chemical composition of palm kernel meal, which is less agreed with the present findings. Toyub*et al*. (2007), analyzed chemical parameters of fertilizer factory effluent and sweetmeat factory waste which are more or less similar to the present findings. Habib*et al.* (1998) determined the chemical contents of raw latex conc. rubber effluent, standard Malaysian rubber effluent and palm oil mill effluent which are not exactly similar to the present study. It may be due to the different source of effluent. The % removal of conductivity was 58.6% and 63.8% in CFTRI medium and 30% supplemented CTW respectively.

Cost Analysis and Nutritional Value:The 30% supplemented CTW medium, which possessed the minimum cost of ` 303 hl^{-1}, yielded considerable cost saving (29.9%) as compared to the CFTRI medium. The protein content of *S.platensis* cultured in CFTRI medium and 30% supplemented CTW was 46% and 25.3% resp. (Table IV). In general, *S.platensis* contains a range of 50-70% protein (Hill 1980; Nakumura 1982; Venkataraman 1983;Colla*et al.* 2005), depending mainly on the quality of nutrients present in the medium. The study of pigment analysis of *S. platensis* cultured in CFTRI medium and 30% supplemented CTW, concluded that the amounts of phycobilliproteins, chlorophyll a and carotenoids were almost comparable to that of CFTRI medium. Also, the mineral contents were almost equal in both the media (Figure I); however the protein content was approximately two times less in 30% supplemented CTW than in CFTRI medium. The nutritional status of *S.platensis*in 30% supplemented CTW indicated the healthy biomass which can be feed as an animal diet supplement that may lead to improve animal health (Belay *et al*. 1996). The biomass of *S.platensis* was evaluated as a good nutritional food source for fish, especially, *Oreochromis* sp. (Tuptim tilapia). Young tilapias are easily weaned and grow fast to market size when fed the formulated diet (Mahajan and Kamat 1995). Fast growth rates are common when fish are fed foodstuffs containing levels of 20-30% protein for fish.

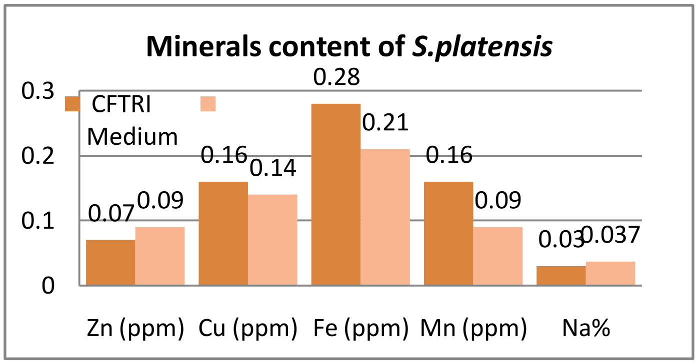

Figure I. Comparative minerals study of *S.platensis* in CFTRI medium and 30% supplemented CTW

Table IV. Cost analysis and nutritional value of *S.platensis* cultured in CFTRI medium and 30% supplemented cooling tower water.

Item	**CFTRI Medium**	**30% Supplemented CTW**
Cost	`432 hl^{-1}	`303 hl^{-1}
Biomass Production (Dry weight gl^{-1})	0.60±0.21	0.448±0.03
Protein (%)	46%±0.35	25.3%±1.05
Pigments		
Chlorophyll a (mgml^{-1})	7.31±1.03	4.03 ±0.05
Carotenoids (mgml^{-1})	3.65±0.23	4.8±0.15
Phycocyanin (mgml^{-1})	6.3±0.12	7.4±0.14
Phycoerythrin (mgml^{-1})	0.43±1.025	0.418±2.14
Allophycocyanin (mgml^{-1})	1.55±0.25	0.226±1.02
Free Amino Acids (ppm)	7.38±1.02	4.33±3.03
Minerals		
Zn (ppm)	0.07±0.56	0.09±0.21
Cu (ppm)	0.16±0.48	0.14±0.14
Fe (ppm)	0.28±1.36	0.21±0.15
Mn (ppm)	0.16±1.24	0.09±0.15
Na%	0.03±1.02	0.037±0.48

*Mean ± SD

CONCLUSION:

The supplementation of CTW with CFTRI medium at 30% dilution gave the good results in terms of dry biomass and nutritional value of *S.platensis*. A higher % decrease of TSS, TDS, COD and conductivity was observed in the 30% supplemented CTW as compared to that of CFTRI medium. It shows that cooling tower water is a promising wastewater medium for cultivation of *S.platensis*. On the other hand, the use of cooling tower water as medium for Spirulina cultivation is 29.9% cost saving as compared to the standard medium i.e. CFTRI medium. This kind of biomass can be utilize to feed animals and in fisheries.

ISSN: 2229- 6905

Thus, further study is required to evaluate its application in large scale production.

ACKNOWLEDGEMENT:

The authors would like to thank Mr. Anand Jain, QAE, SKOL Brewery Ltd, Unit- Tripti, Banmor, Distt- Morena, (MP) for providing the cooling tower water and help in analytical approach of the cooling tower water for successful completion of the research work.

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