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Low cost medium formulation using cow dung ash for the cultivation of Cyanobacterium: *Spirulina* (*Arthrospira*) *platensis*

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Abstract

Spirulina (*Arthroapira*) characterized by helical/spiral shape in open left hand helix is a multicellular, free floating cyanobacterium or blue green alga. Its trichomes are cylindrical arranged in unbranched form and the width ranges from 6 to 12 µm. The basic helical feature of its filaments is unique and can be found only in liquid culture medium. *Spirulina* has been so popular in the world due to its high nutritional contents. It has been used as protein and vitamin supplement to the health food industry, in aquaculture diets and fisheries. Cyanobacterium *Spirulina* is capable to grow in various kinds of culture media. Furthermore, decomposed organic and inorganic nutrients media have been proven to be good source of culture medium for *Spirulina* cultivation by many researchers. Among many strains, *Spirulina platensis* has been cultured in various waste organic matters to evaluate its growth and biochemical components under similar controlled culture conditions. The present investigation was carried out to formulate a low cost medium using cow dung ash for the biomass production of *S. platensis*. Different concentration gradients of cow dung ash medium i.e. 10-60% with standard medium were analyzed for *Spirulina* growth at pH 9.5 ± 2, temperature 28°C ± 2 and light intensity 3 Klux. Success of the experiment was found in 20% concentration level (1.212 g/l dry biomass) having 20% cow dung ash supplemented with 80% prescribed medium i.e. CFTRI medium. The maximum removal efficiency of 20% CDAM at the end of experiment was measured at 98.06%, 51%, 83.64% and 51.01% for TSS, TDS, COD and conductivity respectively. These results indicate the potentiality of cow dung ash to provide the nutrients to the culture medium that reduces its valuable cost and make it a cheaper and economic culture for *S. platensis*. The nutritional status of *S. platensis* cultured in 20% CDAM suggested that the biomass of *Spirulina* is nutritionally rich as per standard.

Key words: Cow dung ash, Cyanobacterium, *Spirulina*, Health food industry

Introduction

Spirulina is most commonly found in natural lakes having high pH value i.e. 8 to 10 all over the earth. *Spirulina* has been consumed from a very long past time in many parts of the world as a food supplement for human as well as animals in various forms like health drink, tablets and powder etc. because of its alimentary value (Ruiz et al., 2003). Now a days, *Spirulina platensis* is gaining great interest for its cellular contents such as vitamins, minerals, polyunsaturated fatty acids, carotenoids and other pigments that have antioxidant activity (Cohen and Vonshak, 1991; Mahajan and Kamat, 1995; Bhat and Madyastha, 2000; Madhava et al., 2000; Lin et al., 2007).

Many factors are important for the production of *Spirulina* at large scale, of which most important factors are nutrient availability, temperature and light. The filamentous cyanobacteria such as *Spirulina* are found to be most compatible microorganisms for the utilization of waste and wastewaters as they are able to produce large quantity of biomass and their harvesting is also relatively easy because of their bigger size and structure. Also these wastes reduce the cost of nutrient medium and act as a source of cheap nutrient medium for cultivation of *Spirulina*. The commercial production of *Spirulina* can be made cost effective by reducing the input cost with cheap and readily available materials without sacrificing the production efficiency. Cow dung is a very common waste material that has been used by many researchers. Cow dung is commonly used as manure or agricultural fertilizer after combining with soiled bedding and urine.

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The aim of the present investigation was to formulate a low cost medium using cow dung ash for *Spirulina* production and to evaluate the nutritional status of *Spirulina* biomass.

Materials and Methods

Procurement of *Spirulina*

S. platensis strain used in the present study was procured from the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute (IARI), New Delhi, India. The culture was maintained in CFTRI medium by repeated transfer to fresh medium.

Sterilization of culture vessels

The glass wares used in the experiments were first rinsed with water properly and finally rinsed with distilled water. After draining out the water, the glass wares were sterilized in a hot air oven at 160^{o}C for 2-3 hours.

Preparation of culture medium

Cow dung ash was prepared from cow dung cakes. Cow dung cakes were collected from villagers and were dried properly in sunlight for a day or two then burned completely to form ash. After cooling, the ash was collected and filtered 4-5 times with fine mesh to remove the unburned solids and other impurities. Physico-chemical properties of cow dung ash is given in Table 1.

Table 1. Physico-chemical properties of cow dung ash.

Properties	Value
pH	9.21
EC (dS/m) (1:2.5)	0.22
Organic Carbon %	0.17
Available P_2O_5 “Kg/Ha”	60.00
Available K_2O “Kg/Ha”	1613
Zn (ppm)	3.76
Cu (ppm)	1.02
Fe (ppm)	4.88
Mn (ppm)	3.10
Total N%	0.08

Cow Dung Ash Medium (CDAM) was used as growth medium to grow *Spirulina platensis* in pure form (100% CDAM) without any supplementation and in formulated form supplemented with CFTRI medium (prescribed medium) in different concentrations of cow dung ash medium. To prepare cow dung ash medium, 80 gm of cow dung ash was dissolved in 1000 ml of sterilized distilled water (8% concentrated solution; wt/v) and left for 2 hours. After 2 hours, the solution was filtered first with fine mesh to remove the undissolved solid particles and then with filter paper to get a clear solution. This form of solution was called as cow dung ash medium (CDAM). Different concentration levels of CDAM i.e. 10%-60% were prepared using standard CFTRI medium. CFTRI medium was used as control in the experiments.

Standardization of inoculum density and day of harvest

Exponentially growing 14 days old culture with OD 0.8-1.0 at 560 nm was used as inoculum and 10% were inoculated in each flask. The cultures were grown for a period of 24 days and after harvesting growth were measured in the form of dry biomass of all concentration levels of cow dung ash medium.

Experimentation

Experiment I. Cultivation of *S. platensis* in 100% cow dung ash medium (100% CDAM)

An experiment was conducted to evaluate the potentiality of cow dung ash medium in pure form without any supplementation for *Spirulina platensis* cultivation. The medium was taken in duplicate with a control in 500 ml conical glass flasks containing 250 ml medium. For cultivation of *S. platensis*, the pH of CDAM was adjusted to 9.5 ± 2 with the help of 1 N NaOH and temperature 28°C ± 2. The photoperiod was given 12/12 alternative light and dark period with light intensity 3 Klux by white fluorescent tubes. All three flasks were incubated with 10% (v/v) (OD at 0.8-1.0) of inoculum. Medium agitation was carried out by orbital shaker three times a day for two minutes. Experiment was run for 24 days. The results were compared with control.

Experiment II. Cultivation of *S. platensis* in Formulated Cow Dung Ash Medium (Formulated CDAM)

In second experiment, *S. platensis* was cultivated in cow dung ash medium formulated/supplemented with CFTRI medium. Constituents of the basal medium were eliminated in step wise manner and substituted with increasing percentage volume amount of cow dung ash medium (CDAM) in the decreasing percentages volume amount of CFTRI medium i.e. CFTRI%+CDAM% in 100%+0%, 90%+10%, 80%+20%, 70%+30%, 60%+40%, 50%+50%, 40%+60% and named as control, 10%, 20%, 30%, 40%, 50% and 60% respectively. Their effects on growth were studied in order to find out the optimum concentration of CDAM with CFTRI medium. All the culture conditions were kept same as in experiment I. The experiment was conducted in duplicate in a completely randomized design. The results were compared with control.

Estimation of growth performance

Spirulina platensis as an experimental organism was grown in CFTRI medium, 100% CDAM and CFTRI+CDAM in different combinations. Initial growth performance was observed in terms of dry biomass of *S. platensis* and compared with control. After finding the best medium combination of CFTRI+CDAM for *S. platensis* cultivation on the basis of highest amount of dry biomass, the physico-chemical analysis of medium was carried out before inoculation and after harvesting before mass cultivation of *S. platensis*. The nutritional analysis of *S. platensis* was also done.

Physico-chemical analysis

Before the mass culture of *S. platensis* in best medium combination of CFTRI+CDAM; medium was first analyzed before inoculation 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 analyzed again in the filtrate after harvesting the biomass of *S. platensis* at the end of the experiment to assess the efficiency of formulated cow dung ash medium. The results of physico-chemical analysis were statistically analyzed through analysis of variance (ANOVA) at 95% confidence level.

Nutritional analysis

For the study of cellular contents of *S. platensis* in best formulated cow dung ash medium (on the basis of highest amount of dry biomass of *Spirulina*), *Spirulina* was mass cultured. The biochemical contents like protein, carbohydrate, pigments (chlorophyll a, carotenoids, phycocyanin, phycoerythrin and allophycocyanin), minerals and free amino acids of *Spirulina* were estimated by following standard methods. Among pigments, chlorophyll a and carotenoids content of the cells were estimated by the method of Mackinney (1941) using absolute methanol while the phycobiliproteins (phycocyanin, phycoerythrin and allophycocyanin) content was estimated by Benette and Bogourd (1973) method. Total cellular protein content was estimated by the method of Lowry et al. (1951). The method of Duboi's et al. (1956) was employed for determination of total carbohydrate of the cell. This is also known as phenol-sulphuric acid method. Free and bound amino acids were separated by standard methods given by Awapara (1969) and Krishnamurthy and Swaminathan (1955) respectively. Then quantitative estimation of free amino acids was carried out by standard methods of Lee and Takahashi (1966). Among minerals, DDPA extractable exchangeable cations (Zn, Cu, Mn and Fe) were estimated by Atomic absorption spectrophotometer following standard method (Lindsey and Norvell, 1978) while Na% was calculated by standard flame photometer following standard method (Lindsey and Norvell, 1978).

Statistical analysis

Results of the growth evaluation were subjected to analysis of variance (ANOVA) and Tukey's Honest Significance Difference (HSD) test with confidence level of 95 % ($p<0.05$) in order to verify significant difference in different concentration levels and biomass. Also the results of physico–chemical analysis and nutritional analysis were analyzed statistically.

Results

Growth performance of *Spirulina platensis* in cow dung ash medium (CDAM)

When *Spirulina platensis* was grown in 100% CDAM without any supplementation of CFTRI medium, the growth was better, although some small clumps were formed in this medium. The amount of dry biomass of *S. platensis* was found 0.524 g/l. The growth of *S. platensis* was nearly half of the control (CFTRI medium) i.e. 1.112 g/l (Table 2). In order to improve the growth performance of *S. platensis*, CDAM was formulated by supplementation of CFTRI medium at concentration levels from 10% to 60%.

When *S. platensis* was grown in formulated CDAM from concentration level 10% to 60%, growth could found in every medium. Some amount of small clumps also formed in every formulated medium, their quantity was more in higher concentrations. The maximum dry biomass of *S. platensis* found in 20% formulated CDAM was 1.212 g/l that was higher than dry biomass obtained in CFTRI medium (1.112 g/l). It was followed by 10% and 30% formulated CDAM (dry biomass 0.812 g/l and 0.620 g/l respectively). While the minimum amount of dry biomass obtained in 60% formulated CDAM (0.240 g/l) followed by 50% and 40% formulated CDAM (dry biomass 0.300 g/l and 0.380 g/l) (Table 2; Figure 1).

Table 2. Growth Performance of *Spirulina platensis* in 100% Cow Dung Ash Medium (100% CDAM) and in different concentrations of formulated CDAM compared with control (CFTRI Medium).

Medium	Dry Biomass (g/l)
Control	1.112±0.14
100% CDAM	0.524±0.21
10% Formulated CDAM	0.812±0.01
20% Formulated CDAM	1.212±0.40
30% Formulated CDAM	0.620±0.47
40% Formulated CDAM	0.380±1.02
50% Formulated CDAM	0.300±1.31
60% Formulated CDAM	0.240±0.52

*Mean ± Standard Deviation

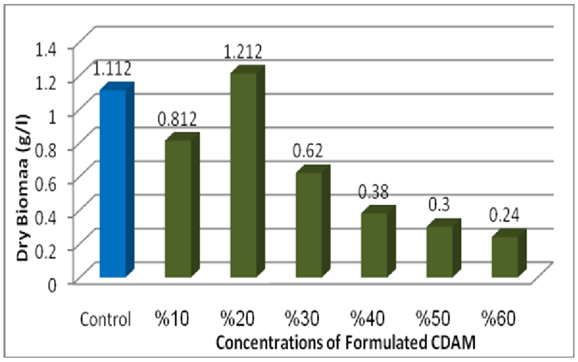

Figure 1. Comparative growth performance of *S. platensis* in formulated Cow Dung Ash Medium (CDAM) at different concentration levels compared with control (CFTRI medium).

Removal efficiency of *Spirulina platensis* in 20% formulated cow dung ash medium (20% formulated CDAM)

20% formulated CDAM was found to be best when *S. platensis* cultivated in different concentrations of formulated CDAM. The results of physico-chemical analysis of 20% formulated CDAM and CFTRI medium are summarized in Table 4.

Before inoculation, the amount of TSS, TDS, COD and conductivity obtained for the 20% formulated CDAM were 13937 mg/l, 8940 mg/l, 1760 mg/l and 14900 mS/m respectively. After the cultivation of microalga *S. platensis*, the solids like TSS, TDS, COD and conductivity were found significantly ($p<0.05$) lower. The TSS, TDS, COD and conductivity values were 270 mg/l, 4380 mg/l, 288 mg/l and 7300 mS/m respectively. In CFTRI medium, before inoculation of *Spirulina* culture; the amount of TSS, TDS, COD and conductivity were 193 mg/l, 4200 mg/l, 592 mg/l and 7000 mS/m respectively. It can be clearly observed from the Table 4, that the amount of TSS was nearly 72 folds higher and COD was 3 folds higher in 20% formulated CDAM as compared to CFTRI medium, while TDS and conductivity values were two times higher in 20% CDAM than control before *Spirulina* cultivation.

The final COD concentration was smaller than before culturing of *S. platensis* evidencing the COD removal during the time of this research study indicating good biomass production of *Spirulina* in 20% formulated CDAM. The removal efficiency of COD in 20% formulated CDAM was very high i.e. 83.64%. The % removal of TSS and TDS was 98.06% and 51% respectively.

After cultivation of *Spirulina,* the decrease in TSS, TDS, COD and conductivity values was found 150 mg/l, 1740 mg/l, 240 mg/l and 2900 mS/m in CFTRI medium. It can be seen that the utilization of these solids was high in 20% formulated cow dung ash medium than CFTRI medium. The % removal of TSS, TDS and COD in CFTRI medium was 22.3%, 58.6% and 59.5% as compared to 98.06%, 51% and 83.64% respectively in 20% formulated CDAM. The pH value increased from 9.5 to 9.78 in control while increment in pH value was high in 20% formulated CDAM i.e. 9.5 to 9.99 (Table 4; Figure 2).

Nutritional Status of Spirulina platensis in 20% formulated cow dung ash medium (20% formulated CDAM)

At constant culture conditions; temperature 28°C-30°C, pH 9.5 and light intensity 3 Klux, the highest mean dry biomass i.e. 1.212 g/l of *Spirulina* was observed in 20% formulated CDAM. In 20% formulated CDAM, the amount of protein, carbohydrate and free amino acids (FAN) was observed 509 µg/ml, 0.196 mg/ml and 0.598 ppm respectively. Minerals such as Zn, Cu, Fe, Mn and Na were present 0.12 ppm, 0.18 ppm, 0.31 ppm, 0.11 ppm and 0.039% respectively in 20% formulated CDAM (Table 3).

The protein content of *S. platensis* in control was found 31% higher as compared to 20% formulated CDAM. The amount of protein in control was 611.6 µg/ml while in 20% formulated CDAM was 509 µg/ml. The carbohydrate content of *S. platensis* was only 16.9% higher while FAN (free amino acids) content was 34.6% higher in control than in 20% formulated CDAM. The amount of carbohydrate and FAN content of *S. platensis* in 20% formulated CDAM was found 0.196 mg/ml and 0.598 ppm respectively while in control was found 0.21 mg/ml and 0.74 ppm respectively.

Table 3. Comparative study of nutritional status of *Spirulina platensis* in 20% formulated cow dung ash medium (20% formulated CDAM) and in CFTRI Medium.

Item	Unit	CFTRI Medium	20% Formulated CDAM
Protein	µgml^{-1}	611.6±0.35	509.0±0.12
Carbohydrate	mgml^{-1}	0.21±0.21	0.196±0.01
Free Amino Acids	ppm	0.74±1.02	0.598±0.03
Pigments			
Chlorophyll a	mgml^{-1}	0.019±1.03	0.023±0.01
Carotenoids	mgml^{-1}	0.0037±0.23	0.0033±0.21
Phycocyanin	mgml^{-1}	0.074±0.12	0.052±0.40
Phycoerythrin	mgml^{-1}	0.023±1.025	0.022±0.60
Allophycocyanin	mgml^{-1}	0.028±0.25	0.034±0.20
Minerals			
Zn	ppm	0.07±0.56	0.12±0.12
Cu	ppm	0.16±0.48	0.18±0.15
Fe	ppm	0.28±1.36	0.31±0.12
Mn	ppm	0.16±1.24	0.11±0.10
Na	%	0.03±1.02	0.039±0.10
Cost of medium	` hl^{-1}	432.0	345.6

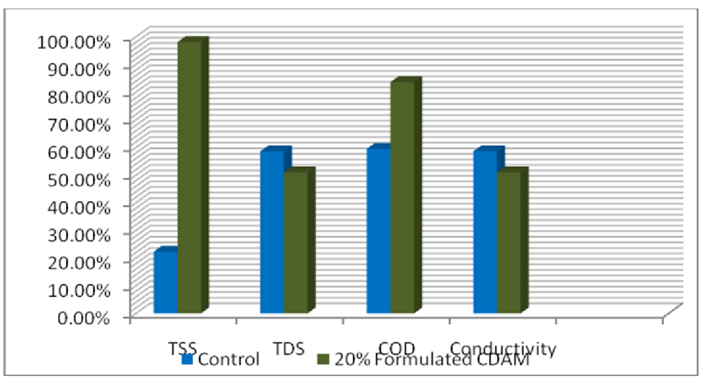

Figure 2. Comparative removal efficiency of *S. platensis* (in terms of % removal) in CFTRI medium and in 20% Formulated Cow Dung Ash Medium (CDAM).

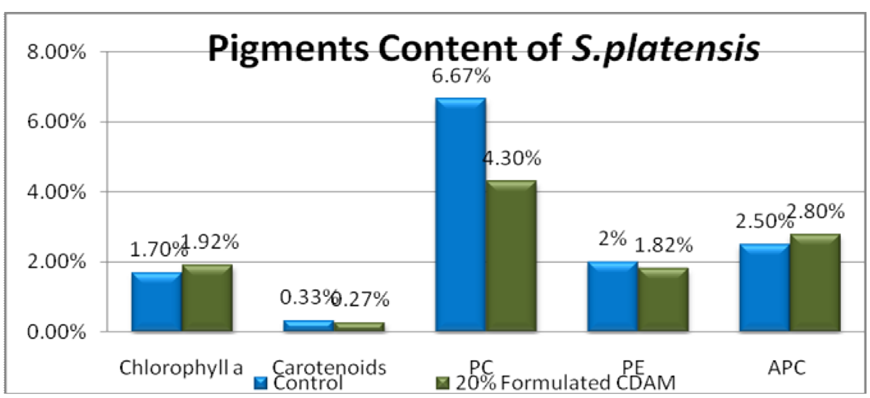

Figure 3. Comparative study of pigments content of *S. platensis* in control and 20% formulated cow dung ash medium (CDAM).

Table 4. Results of physico- chemical analysis and percentage removal in 20% formulated cow dung ash medium compared with control (CFTRI medium).

Physico-chemical Parameter	Unit	20% Formulated CDA Medium			CFTRI Medium		
		Before Culture	After Culture	%Removal	Before Culture	After Culture	%Removal
pH		9.5±2	9.99±2	-	9.5±2	9.78	-
TSS	mg/l	13937±0.01	270±1.04	98.06%±0.02	193±1.60	150±0.38	22.3%±0.05
TDS	mg/l	8940±1.02	4380±1.05	51.0%±0.01	4200±0.06	1740±0.31	58.6%±0.70
COD	mg/l	1760±1.05	288±0.12	83.64%±1.05	592±0.01	240±1.30	59.5%±0.10
Conductivity	mS/m	14900±1.00	7300±0.02	51.01%±1.04	7000±1.02	2900±0.08	58.6%±0.02
Hardness	mg/l	22	Nd	-	nd	nd	-

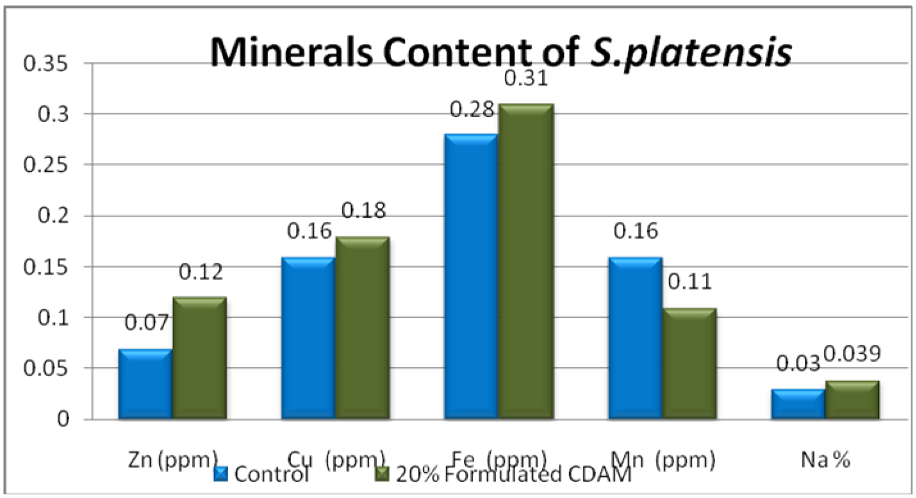

Figure 4. Comparative study of minerals content of *S. platensis* in control and 20% formulated cow dung ash medium (CDAM).

The amount of pigments like chlorophyll a, carotenoids, phycocyanin, phycoerythrin and allophycocyanin of *S. platensis* in 20% formulated CDAM was found 0.023 mg/ml, 0.0033 mg/ml, 0.052 mg/ml, 0.022 mg/ml and 0.034 mg/ml respectively while in control was found 0.019 mg/ml, 0.0037 mg/ml 0.074 mg/ml, 0.023 mg/ml and 0.028 mg/ml respectively. Although the amount of pigments of *Spirulina* like, carotenoids, phycoerythrin and phycocyanin was less in 20% formulated CDAM than the values of control; the pigments like, allophycocyanin and chlorophyll a were higher in *S. platensis* cultured in 20% formulated CDAM (Figure 3). It is clearly indicated from the Figure 4 that the amount of minerals of *S. platensis* grown in 20% formulated CDAM was almost similar to that of *S. platensis* grown in CFTRI medium, even Zn, Cu, Fe and Na content was higher in 20% formulated CDAM that in control.

Assessment of cost effect

The cost of preparation of CFTRI medium and 20% formulated CDAM was calculated. The 20% formulated CDAM, which possessed the minimum cost of Rs. 345.6 hl^{-1}, yielded considerable cost saving (20 %) as compared to the CFTRI medium (Rs. 432 hl^{-1}). 20% formulated CDAM was not only less expensive than CFTRI medium; even it was more effective than CFTRI medium in the biomass production too i.e. 1.212 g/l and 1.112 g/l respectively (Table 3).

Discussion

When cow dung ash medium used as a cheap nutrient growth medium for *S. platensis* biomass production in lab conditions, yield was found better in 100% CDAM (8% wt/v) (used without any supplementation of CFTRI medium). The biomass content of *S. platensis* observed 0.524 g/l (dry basis) in 100% CDAM as compared to 1.112 g/l dry biomass obtained in standard medium. After formulating CDAM with CFTRI medium at concentration levels 10% to 60%, best biomass production of *S. platensis* obtained in 20% formulated CDAM. Although, blooming of *S. platensis* was successfully carried out in every concentration of formulated CDAM. Biomass in 20% formulated CDAM (1.212 g/l) was significantly higher than that obtained in standard medium (1.112 g/l) (Table 2; Figure 1).

The *S. platensis* biomass obtained in 20% formulated CDAM was nutritionally rich as contained 42% protein, 16.17% carbohydrate and 0.023 mg/ml chlorophyll a content (Table 3). Although, the protein and carbohydrate content of *S. platensis* in 20% formulated CDAM was significantly lower than that obtained in *S. platensis* grown in CFTRI medium (55% protein, 18.9% carbohydrate), was within the range of the value prescribed for *S. platensis* (Vonshak, 1997). Carbohydrate to protein ration (C/P) of *S. platensis* grown in 20% formulated CDAM was significantly higher (0.385) than the ratio observed in standard medium (0.344). The lower value of C/P ratio in standard medium is due to absence of organic source of C in it. Higher and lower C/P ratios are observed in nutrient limited and nutrient sufficient medium respectively (Kaushik et al., 2006).

Among pigments, chlorophyll a (0.023 mg/ml) and allophycocyanin (2.8 mg/ml) contents of *S. platensis* were high in 20% formulated CDAM than that in control (0.019 mg/ml chlorophyll a and 0.028 mg/ml allophycocyanin) (Figure 3). While among minerals content, all minerals (except Mg) were found high in *S. platensis* cultivated in 20% formulated CDAM. All the values were found within the range of the recommended range for *S. platensis* (Vonshak, 1997). However, there was no statistically significance difference among Cu and Fe content of *S. platensis.*

The removal efficiency of COD (83.64%) and TSS (98.06%) was found very high after cultivating *S. platensis* in 20% formulated CDAM as compared to found in CFTRI medium. The % removal of TDS and conductivity was comparable to the values of standard medium (Table 4; Figure 2).

Similar results have been demonstrated by various researchers worked on cattle wastes, swine wastes and poultry wastes (Ungsethaphand et al., 2009; Canizares and Dominguez, 1993; Canizares-Villanueva et al., 1995; Silva et al., 2003; Ayala and Vargas, 1987: Mezzomo et al., 2010; Mitchell and Richmond, 1988 etc.) Mitchell and Richmond (1988) used cattle waste leachate to enhance the power of Zarrouk's medium for the production of *Spirulina*. The results showed that the maximum cell density of *Spirulina* obtained in medium containing 16.3% waste leachate and 83.7% Zarrouk's medium. The effluent of anaerobically digested cow manure was used to supplement the medium by Ayala and Vargas (1987) in experiment for the cultivation of *S. maxima*. The results showed growth rates of 45.3 mg/L /d. These studies supported the present findings.

The present findings were also supported by Oron et al. (1979). They used raw cow manure for the cultivation of *S. maxima* under field conditions. The results showed 3158 mg/L biomass production of *Spirulina*. Lincoln et al. (1996) used anaerobically digested cattle manure in 1:1 ratio for the cultivation of *Spirulina.* The yield of *Spirulina* biomass was found 70 mg/L/ d for laboratory experiments and 24 mg/L/d for outdoor cultivations. N-NH_3 removal was recorded 24 mg/L/ d. At concentration level <75 mg N-NH_3/L, the *Spirulina* growth was smooth while at concentration levels >100 mg N-NH_3/L, *Spirulina* did not grow well.

Churnbarn and Peerapornpisol (2010) cultivated *Spirulina* in a medium containing effluent from anaerobically treated swine wastewater at concentration gradients from 0 to 100%. The results demonstrated best concentration of swine wastewater for *Spirulina* growth was 10%. They declared that 8.0 g/l $NaHCO_3$ and 1.5 g/l $NaNO_3$ are also required along with 10% diluted swine wastewater to obtain highest growth rates of *S. platensis* that support the present findings.

The present results were also confirmed by the work done by Canizares et al. (1993) who found 25% and 50% swine waste best for nutrient removal and biomass production of *S. maxima*. To find out the treatment efficiency of suspension and immobilized systems through the growth response of *S. maxima* towards the waste, different concentration gradients of aeration stabilized swine waste were compared in the experiment. In suspension system, 50% swine waste was found to be best in terms of nitrogen removal and biomass concentration while in immobilized system, 25% and 50% of swine waste were best for nitrogen removal (>90%) and *S. maxima* cultivation.

Canizares and Dominquez (1993) used supernatant of aerated swine wastewater in their study for the biomass production of *S. maxima*. The growth of *Spirulina* was found in every concentration of swine wastewater and also in its pure form. However, 50% dilution of swine wastewater was best for highest growth rate. At this concentration, maximum 75% $N\text{-}NH_4^+$ removal and 53% total phosphate removal was recorded.

In another study of Canizares-Villanueva et al. (1994) effluent of anaerobically digested pig waste was used to cultivate another microorganism *Phormidium*. The results showed that the dilution levels from 10 to 50% of effluent were suitable for the growth of *Phormidium* sp. Among these dilutions, 25% was found best for maximum removal efficiency of nutrients. The removal of 48%, 100%, 87% and 68% was recorded for Ammonium, $P\text{-}PO_3^{4-}$, $N\text{-}NO_3^-$ and total phosphorus respectively. The removal of COD was 91%.

The biochemical components of *S. maxima* (Canizares and Dominquez, 1993; Canizares et al., 1993) and *Phormidium* sp. were found by Caiiizares-Villanueva et al. (1994) to be differing in respect to the growth medium. In a medium with 50% diluted aerated pig wastewater the lipids were 6%, protein content was 36% and carbohydrates 44% in *S. maxima* biomass, whereas in *Phormidium* sp. the lipids were 9.4%, protein content was 53.4%, and carbohydrates 27.5% (Converti et al., 2006).

Mezzomo et al. (2010) found 5.0 and 8.5% concentrated swine wastewater best for maximum cellular concentration and maximum specific growth rate of *S. platensis* and 26.5% and 30.0% swine wastewater best for maximum COD removal. In their study, the biological treatment of swine waste water was done by the cultivation of microalgae *Spirulina platensis*. This work was done to study the best dilution of the wastewater for maximum biomass production and for removal of COD, ammonia and phosphorus to the microalgae. The cultivation of *S. platensis* strain Paracas presented maximum cellular concentrations and maximum specific growth rates in the wastewater concentration of 5.0 and 8.5%. The highest COD removal occurred with 26.5 and 30.0% of waste water in the medium. The maximum removal of total phosphorus (41.6%) was with 8.5% of wastewater, which is related to the microalgae growth. These results also support the results of CDAM in present study.

Gantar et al. (1991) studied on the swine wastewater for its nutrient removal in terms of nitrogen and phosphorus in algal suspension during the growth of *Spirulina platensis* and *Scenedesmus quadricauda*. In their study, they found that the growth rate of introduced alga varied with the concentration of waste used and the time of cultivation of alga was also an important factor for its growth. The introduced alga was found growing with autochtonous algae only at 10% to 20% concentration of waste. On the other side, when the concentration of manure was increased, the introduced alga did not compete with autochtonous algae.

Ratana et al. (2010) demonstrated that the use of 0.2 g/L urea and 4.5 g/L $NaHCO_3$ to supplement anaerobically digested pig waste at 20% dilution made the medium cheaper than the modified Zarrouk's medium. They calculated 4.4 times cheaper cost for this medium as compared to modified Zarrouk's medium. The removal rates of nitrogen and phosphorus were recorded 34 mg/L/d and 4 mg/L/d respectively. For pilot scale conditions and for laboratory conditions, the average productivities were found 12 $g/m^2/d$ and 19.9 $g/m^2/d$ respectively. Effluents from chicken and cow sheds and from piggeries were tested for growth of *Spirulina* by Yunus (1990). Half the strength of Zarrouk's medium was used as control and it was observed that effluents from piggery and cowsheds could be used as good substitutes for *Spirulina* cultivation. All these findings are in corroboration with the present results on cow dung ash medium.

20% formulated CDAM yielded considerable cost saving (20%) as compared to the CFTRI medium. 20% formulated CDAM was not only less expensive than CFTRI medium; even it was more effective than CFTRI medium in the biomass production too (1.212 g/l and 1.112 g/l respectively).

Conclusion

When cow dung ash medium used as a cheap nutrient growth medium for *S. platensis* biomass production in lab conditions, the biomass of *S. platensis* in 100% CDAM was lower as compared to control. After formulating CDAM with CFTRI medium at concentration levels 10% to 60%, best biomass production of *S. platensis* obtained in 20% formulated CDAM. The *S. platensis* biomass obtained in 20% formulated CDAM was nutritionally rich as contained 42% protein, 16.17% carbohydrate and 0.023 mg/ml chlorophyll a content. The protein content of *S. platensis* in control was found 31% higher as compared to 20% formulated CDAM. The carbohydrate content of *S. platensis* was only 16.9% higher while FAN (free amino acids) content was 34.6% higher in control than in 20% formulated CDAM. The removal efficiency of COD (83.64%) and TSS (98.06%) was found very high after cultivating *S. platensis* in 20% formulated CDAM as compared to found in CFTRI medium. The % removal of TDS and conductivity was comparable to the values of standard medium.

The nutritional analysis of *S. platensis* grown in 20% formulated CDAM demonstrated that it is a rich source of protein and carbohydrate. 20% formulated CDAM yielded considerable cost saving (20%) as compared to the CFTRI medium. 20% formulated CDAM was not only less expensive than CFTRI medium; even it was more effective than CFTRI medium in the biomass production too.

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