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LOW COST PRODUCTION OF SPIRULINA (*Spirulina platensis*) THROUGH TREATMENT OF WASTEWATER

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Spirulina is multi cellular, autotrophic and filamentous blue-green microalgae possessed rich protein, vitamins, minerals and pigments. It showed various pharmaceutical activities including anticancer and anti HIV activities. It also used as animal feed for rich protein content. Black water refers to toilet water which turned and one of the most significant sources of human pathogens and source of ground water pollution. In the present study focused to treat the wastewater through the cultivation of *S. platensis*. Culturemedium was divided into three groups (Group I (100% black water), Group II (50% black water), Group III (25% black water) and Group IV (Chemical medium). Water quality parameters were measure before and after cultivation of spirulina. A healthy and high amount of *S. platensis* was cultured in group III (25% black water) more or less similar to the chemical medium. The cost effect ratio for the synthetic medium showed the high expenses. On the other hand, in wastewater culture spirulina showed a maximum gain from zero cost management or without loss of cost. With regard to waste water treatment, the culturing of spirulina favorably altered the physical and chemical parameters of the wastewater except chlorides, total hardness and total alkalinity.The present study indicates that spirulina canbe successfully cultured in black wastewater with expected yield. Furthermore the cultured

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water can also be used for irrigation purpose with 25% addition of freshwater or as such. Since there is no bad odour and other toxic material it can also be used for toilet flush.

Keywords: Wastewater; spirulina; physicochemical parameters.

1. INTRODUCTION

Spirulina plantensis is a blue-green mesophile filamentous cyanobacteria, with high protein content, being largely used as a source of single cell protein for humans and animals. These microalgae have been used as part of the diet of people that lived in villages close to Chad Lake in Africa [1,2]. Spirulina presents high protein content and it has been used as alimentary complement in diets for weight loss and malnutrition. Since it has high protein value (60-70% in dry weight), vitamin value, mainly vitamin B12, minerals and lipids content (rich in fatty polyunsaturated acids), mainly g-linolenic acid (GLA), an antioxidant highly used in medicine [3]. Its extracts are shown biological properties, such as prevent cancers, decrease blood cholesterol levels, stimulate the immunological system, reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation [4] and it is used as anti-tumor drugs [5,6] and anti-HIV-I drugs [7]. It is also used as animal feed. For these reasons, spirulina is widely used in commercial cultivation which estimates about 3000 metric tons/year [8]. However, the current production of spirulina, is not meet out the present requirements. The culture medium is an important factor in the production cost of the algal product, massive cultivation of spirulina in wastewater media could improve the prospects for industrial production. A typical medium suitable for the growth of spirulina contains higher amounts of bicarbonate (13.6 g/l), nitrate (2.5 g/l) [9], potassium (1.0 g/l) and sodium chloride (1,0 g/l) [10].There are several culture media for the artificial cultivation of *S.platensis*. Most of them are modifications of the methods of preparation of culture medium by Zarrouk [11] based on considerations of its natural habitat and ecological parameters. Since these medium are high cost, there is a need to produce spirulina by low cost medium. Spirulina was cultivated in wastewater at many places. *S.plantensis* is a species of cyanobacteria that has been studied for wastewater treatment by a number of researchers [12,13,14]. The treatment of dairy and piggery wastewater using microalgae has been investigated by several researchers [15,16,17]. *S. platensis* was shown as the most efficient microalgae to reduce the nitrate in municipal wastewater and remove the phosphorus in most effectively [18]. The application of Spirulina cultivation in blackwater able to resolve the environmental pollution issues, produce value added product and even generate green electricity. This would benefit the society, business, and environment in achieving a sustainable circular bioeconomy. Thus, the present study was carried out with the hypothesis that it would be helpful for the production of spirulina on a large-scale with zero cost and for treating the wastewater by culturing spirulina in waste water.

2. MATERIALS AND METHODS

2.1 Spirulina Culture

2.1.1 Strain procurement, culture development and maintenance

Mother culture of *spirulina* sp. was bought from the OFERR Nallayan Research Centre for Sustainable Development, Egmore Chennai. It was inoculated (50 ml/10 litres of medium) into the Zarrouks agar medium already prepared and maintained at $4^{o}C$. Then the culture was used to the further culture. Sodium carbonate was added after autoclaving and pH was adjusted to 8.8-9.0. Growth and maintenance of the culture was done in an open natural environment under 12/12 hour sunlight –dark cycles. Manual shaking of culture was done 3 times daily. The chemical composition of synthetic medium is presented in Table 1.

2.1.2 Wastewater (Black water) collection

Black water (toilet wastewater) samples were collected with the aid of 25 liters capacity of plastic container from the point of septic tank of CVR Women hostel, Nehru Memorial College (Autonomous), Puthanampatti.

2.2 Experimental Set Up

To culture spirulina in black wastewater medium in 3 different concentrations at low cost and to treat wastewaters for irrigation purpose, the following experimental set up was designed.

Group I (100% Black Water)

Through –B contain 10 litres of black water collected from the Sir C.V. Raman Hostel, Nehru Memorial College, Puthanampatti.

Group II (50% Black Water + 50% Tap Water)

Through –C contains 1:1 ratio (5 litres of black water and 5 litres of tap water) collected from the Sir C.V. Raman Hostel, Nehru Memorial College, Puthanampatti.

Table 1. Chemical composition of synthetic medium

Chemicals present in synthetic medium		Concentration (g/l)
Sodium Hydrogen carbonate	$NaHCO_3$	8
Sea salt	NaCl	5
Potassium Nitrate	KNO_3	2
Magnesium Sulphate	$MgSO_4$. 7 H_2O	0.16
Ammonium Phosphate (monobasic)	NH_4PO_4. 12 H_2O	0.08
Urea	$CO(NH_2)_2$	0.015
Iron Sulphate	$FeSO_4$. 7H_2O	0.005

Group III (25% Black Water + 75% Tap Water)

Through –D contain 1:3 ratio i.e., 2.5 litres of black water and 7.5 litres of tap water collected from the Sir C.V. Raman Hostel, Nehru Memorial College, Puthanampatti.

Group IV (Chemical medium)

2.3 Pretreatment of Wastewater to alter pH

In wastewater, the pH was in acidity condition (<5pH). However, spirulina culture need alkaline condition (i,e>8 pH to 9 pH). To increase pH 1 in wastewater (to standardize multiple experiments were conducted), 20 grams of dry wood ash wasadded in 10 litres of wastewater.

2.4 Water Quality Analysis

2.4.1 Physical parameters

Temperature and Light Intensity were measured using Thermometer and Lux meter at different intervals (10 am, 1 pm, and evening 4 pm).

2.4.2 Chemical parameters

pH of the water sample was determined by using Digital pH meter. Every day the pH of the samples was measured two times one at 10.00am and at 5.00 pm.The following water quality parameters such as TSS, TDS, CO_3, Na , Mg, Fe, Ca, Po_4, K, S and total alkalinity were measure before culturing spirulina and after harvesting of spirulina, were tested. The above water quality parameters except temperature, light intensity and pH, the other parameters were tested at Department of Chemistry, Tamilnadu Agricultural Department Laboratory, Mannarpuram, Trichy.

2.5 Harvesting

Spirulina takes 3 to 4 days to mature. The matured spirulina was identified by the colour of medium (when the pale medium turns into dark green).The matured spirulina was harvested by filtration technique using cloth filter. Filtered spirulina was thoroughly washed in fresh water to remove the adhering chemicals. After washing, the spirulina were weighed under digital balance (1g accuracy model) to know their wet weight. It must be dried immediately, to preserve its quality and value. After washing and weighing, they were dried under shade for 4 days or up to complete dry.

2.6 Determination of Dry Weight of Spirulina

After drying of spirulina under shade, they weighed under digital balance with an accuracy of 1g.

2.7 Cost Effect of Spirulina Culture

The actual cost of each experiment was noted in Indian rupees, and the production of spirulina was weighed in kilogram. To find the production cost, the weight of dry spirulina produced was multiplied by the cost of 1 kg (current market value) . Then the cost effect ratio was calculated by the following formula:
Cost effect ratio = Cost of 1kg of dry spirulina/ production cost for 1kg of dry spriulina.

2.8 Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation value of respective parameter. Correlation test was made to know the relationship between the physiochemical parameters and spirulina productivity. All statistical analyses were made by using windows based statistical package (SPSS).

3. RESULTS AND DISCUSSION

Spirulina plantensis is a blue-green mesophile filamentous cyanobacteria, with high protein content, being largely used as a source of single cell protein for humans and animals. Spirulinais not only focused mainly for its rich content of protein, essential amino acids, minerals and essential fatty acids but also for its vitamins, especially vitamin B12 and provitamin A (β-carotene) and minerals, especially iron. The algae were cultivated in synthetic media made up mainly of aqueous mineral salt solutions and a carbon source from combustion gases containing carbon dioxide. The cost of chemicals was estimated to be about 15%of the total production cost [19]. Therefore a more economical way to culture *S. maxima* would be desirable. Wastewater is usually treated to get rid of undesirable substances by subjecting the organic matter to biodegradation by microorganisms such as bacteria. The biodegradation involves the degradation of organic matter to smaller molecules (CO_2, NH_3, PO_4 etc.), and requires constant supply of oxygen. The process of supplying oxygen is expensive, tedious, and requires a lot of expertise and manpower. These problems are overcome by growing microalgae in the ponds and tanks where wastewater treatment is carried out. The algae release the O_2 while carrying out the photosynthesis which ensures a continuous supply of oxygen for biodegradation.

3.1 Spirulina Culture in Synthetic Medium

The chemical composition of the synthetic medium is given in the Table 1. Healthy growth of spirulina was observed in synthetic medium and it was fully grown at fifth day. Appearance of also shifted to light green to dark green (Fig. 1). In proportion to increasing cell mass. After harvesting, they were dried under shade and dry weight of spirulina was measured as 10.6 g/ 10 litres culture medium. The temperature ranging between 25°C and 30°C during morning time and 26°C and 33°C during evening time. The intensity of lux varied from 170 lux to 749 lux during morning, 250 to 643 lux at noon period and it was found to be low 35 to 52 lux during evening time. The pH for five days found in the range of 8.0 to 8.6. The gross productivity of spirulina was obtained about 10.6 g/10 litres.

3.2 Cost effect of Spirulina Culture

Actual cost of expenses of spirulina culture and the productive value (Market value) are given in Table 4. The cost effective ratio for the synthetic medium showed the high expenses with loss. On the other hand, black water showed maximum gain without initial cost.

3.3 Effect of Spirulina in the Treatment of Wastewater

3.3.1 Characters of black water at time of collection

Black water has a high content of solids bad odour and black colour. At the time of collection, the black water exhibited highly acidic condition. The pH ranged from 4.0 to 4.5 and chemical characteristic features of black water showed in high concentration.

3.4 Yield of Spirulina in Black Water System

Spirulina was cultivated at different concentrations of black water (0, 50 and 75%) diluted with water. Spirulina is grow in alkaline medium, thus, the pH of the test water was increased up to pH 8 by adding ash of cow dung cake at require amount. Spirulina was started their growth on the first day of inoculation. It was grown and ready to harvest at 5th day of the experimental period. Maximum amount (10.6 ± 0.10 g/l) of spirulina was cultured in group III and minimum amount were achieved in group I (6.7 ± 0.10 g/l). After cultivation of spirulina the almost all water quality parameters were remarkably altered.

3.5 Physical Characters and Chemical Characters of Black Water after Spirulina Culture

3.5.1 Colour of the water sample

Black water in all groups turned green colourfrom black colour on the 2nd day of treatment and remained dark green colour up to the end of the experiment day (Fig. 1).

3.5.2 Odour

Black water showed foul odour even in the first day of experiment and it was decreased day by day and 3rd day onwards there were no bad odour.

3.5.3 Temperature

During the experimental period temperature was observed at morning, noon and evening. The temperature ranged between 25°C and 30°C during morning and 26°C to 33°C during the evening time.

3.5.4 Light

The light intensity varied from 170 lux unit to 749 lux unit during morning. It varied from 250 lux unit to 643 lux unit during noon while it was found to be low (35 lux unit to 52 lux unit) during evening.

3.6 Chemical Parameters

The levels of all chemical parameters in black water, before and after the cultivation of spirulina are given in Table 2 and differences between the values of chemical parameters before cultivating spirulina and after cultivating spirulina (Student 't' Test values) are illustrated in the Table 3 and Fig. 2.

Table 2. Observed Chemical Parameters during, before and after spirulina Culture in Black Water (Bathroom Waste water).Mean Values within the Parantheses are Ranges of Respective Mean

Parameter	Period	Group I	Group II	Group III
Total suspended solid (mg/l)	Before culture	342.0 ± 1.00 (341.0 - 343.0)	171.0 ±0.50 (170.5 - 171.5)	85.5 ± 0.25 (85.3 - 85.8)
	After culture	50.0 ± 5.00 (45.0 - 55.0)	25.0 ± 2.50 (22.5 - 27.5)	12.5 ± 1.25 (11.3 - 13.4)
Total dissolved solids (mg/l)	Before culture	3264.0 ± 1.00 (3263.0 - 3265.0)	1632.0 ± 0.50 (1631.5 - 1632.5)	816.0 ± 0.25 (815.8 - 816.3)
	After culture	1005.0 ± 5.00 (1000.0 - 1010.0)	502.5 ± 2.50 (500.0 - 505.0)	251.3 ± 1.25 (250.0 - 252.0)
Chlorides(mg/l)	Before culture	675.0 ± 1.00 (674.0 - 676.0)	337.5 ± 0.50 (337.0 - 338.0)	168.8 ± 0.25 (168.5 - 169.0)
	After culture	263.3 ± 15.28 (250.0 - 280.0)	131.7 ± 7.64 (125.0 - 140.0)	65.8 ± 3.81 (62.5 - 70.0)
Sulphates (mg/l)	Before culture	26.0 ± 1.00 (25.0 - 27.0)	13.0 ± 0.50 (12.5 - 13.5)	6.5 ± 0.25 (6.3 - 6.8)
	After culture	51.7 ± 2.89 (50.0 - 55.0)	25.8 ± 1.44 (25.0 - 27.5)	12.9 ± 0.72 (12.5 - 13.8)
Sulphides(mg/l)	Before culture	15.2 ± 0.15 (15.0 - 15.3)	7.6 ± 0.08 (7.5 - 7.7)	3.8 ± 0.04 (3.7 - 3.8)
	After culture	3.7 ± 0.58 (3.0 - 4.0)	1.8 ± 0.29 (1.5 - 2.0)	0.9 ± 0.14 (0.8 - 1.0)
Total phosphates (mg/l)	Before culture	11.5 ± 0.50 (11.0-12.0)	5.8 ± 0.25 (5.5 - 6.0)	2.9 ± 0.13 (2.8 - 3.0)
	After culture	6.0 ± 1.00 (5.0 - 7.0)	3.0 ± 0.50 (2.5 - 3.5)	1.5 ± 0.25 (1.3 - 1.8)
Total iron (mg/l)	Before culture	0.1 ± 0.01 (0.04 - 0.1)	0.03 ± 0.01 (0.02 - 0.03)	0.01 ± 0.00 (0.01 - 0.01)
	After culture	1.5 ± 0.50 (1.0 - 2.0)	0.8 ± 0.25 (0.5 - 1.0)	0.4 ± 0.13 (0.25 - 0.50)
Sodium (mg/l)	Before culture	26.2 ± 1.04 (25.0 - 27.0)	13.1 ± 0.52 (12.5 - 13.5)	6.5 ± 0.26 (6.3 - 6.8)
	After culture	229.0 ± 3.61 (225.0 - 232.0)	114.5 ± 1.80 (112.5 - 116.0)	57.3 ± 0.90 (56.3 - 58.0)

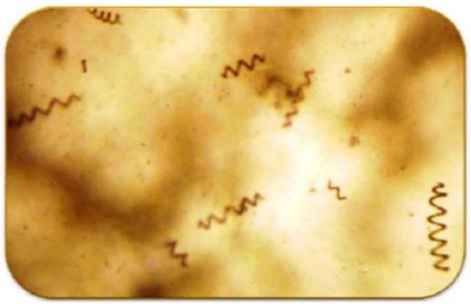
Parameter	Period	Group I	Group II	Group III
Potassium (mg/l)	Before culture	48.9 ± 0.79 (48.0 - 49.5)	24.5 ± 0.39 (24.0 - 24.8)	12.2 ± 0.19 (12.0 - 12.4)
	After culture	417.7 ± 15.37 (400.0 - 428.0)	208.8 ± 7.69 (200.0 - 214.0)	104.4 ± 3.84 (100.0 - 107.0)
Total hardness (mg/l)	Before culture	1065.0 ± 5.00 (1060.0 - 1070.0)	532.5 ± 2.50 (530.0 - 535.0)	266.3 ± 1.25 (265.0 - 267.5)
	After culture	317.0 ± 28.58 (300.0 - 350.0)	158.5 ± 14.29 (150.0 - 175.0)	79.3 ± 7.15 (75.0 - 87.5)
Total alkalinity (mg/l)	Before culture	1280.0 ± 5.00 (1275.0 - 1285.0)	640.0 ± 2.50 (637.5 - 642.5)	320.0 ± 1.25 (318.8 - 321.3)
	After culture	171.7 ± 10.41 (160.0 - 180.0)	85.8 ± 5.20 (80.0 - 90.0)	42.9 ± 2.60 (40.0 - 45.0)
Calcium (mg/l)	Before culture	241.7 ± 1.53 (240.0 - 243.0)	120.8 ± 0.76 (120.0 - 121.5)	60.4 ± 0.38 (60.0 - 60.7)
	After culture	13.7 ± 3.79 (11.0 - 18.0)	6.8 ± 1.89 (5.5 - 9.0)	3.4 ± 0.95 (2.8 - 4.5)
Yield(g)	After culture	6.7 ± 0.10 (6.6 - 6.8)	7.1 ± 0.10 (7.0 - 7.2)	10.6 ± 0.10 (10.5 - 10.7)

Table 3. Comparison ('t' Test) of chemical parameters in all groups between before and after the cultivation of spirulina in Black Water

Parameter	Group I			Group II			Group III		
	df	't' value	P value	df	' t'value	P value	df	' t'value	P value
Total suspended solid (mg/l)	2	126.440	0.000	2	126.440	0.000	2	126.440	0.000
Total dissolved solid (mg/l)	2	702.742	0.000	2	702.742	0.000	2	702.742	0.000
Chlorides (mg/l)	2	45.616	0.000	2	45.616	0.000	2	45.616	0.000
Sulphates (mg/l)	2	21.356	0.002	2	21.356	0.002	2	21.356	0.002
Sulphides (mg/l)	2	45.696	0.000	2	45.696	0.000	2	45.696	0.000
Total phosphates (mg/l)	2	11.000	0.008	2	11.002	0.006	2	11.004	0.003
Total iron (mg/l)	2	4.972	0.038	2	4.974	0.035	2	4.975	0.032
Sodium (mg/l)	2	127.576	0.000	2	127.576	0.000	2	127.576	0.000
Potassium (mg/l)	2	43.707	0.001	2	43.707	0.001	2	43.707	0.001
Total hardness(mg/l)	2	53.248	0.000	2	53.248	0.000	2	53.248	0.000
Total alkalinity (mg/l)	2	251.346	0.000	2	251.346	0.000	2	251.346	0.000
Calcium (mg/l)	2	109.528	0.000	2	109.528	0.000	2	109.528	0.000

Table 4. Cost effect of spirulina in different culture medium

Sl. No	Medium	Expenses (Rs) For the production of 1kg of spirulina	Productivity Dry weight (kg)	Market valueof 1 kg of spirulina (Rs)	Cost effect ratio (Production cost: market value)
1	Chemical Medium	270.00	10.6	600	1:2.2
2	Black water Medium	0	8.1	600	0:600



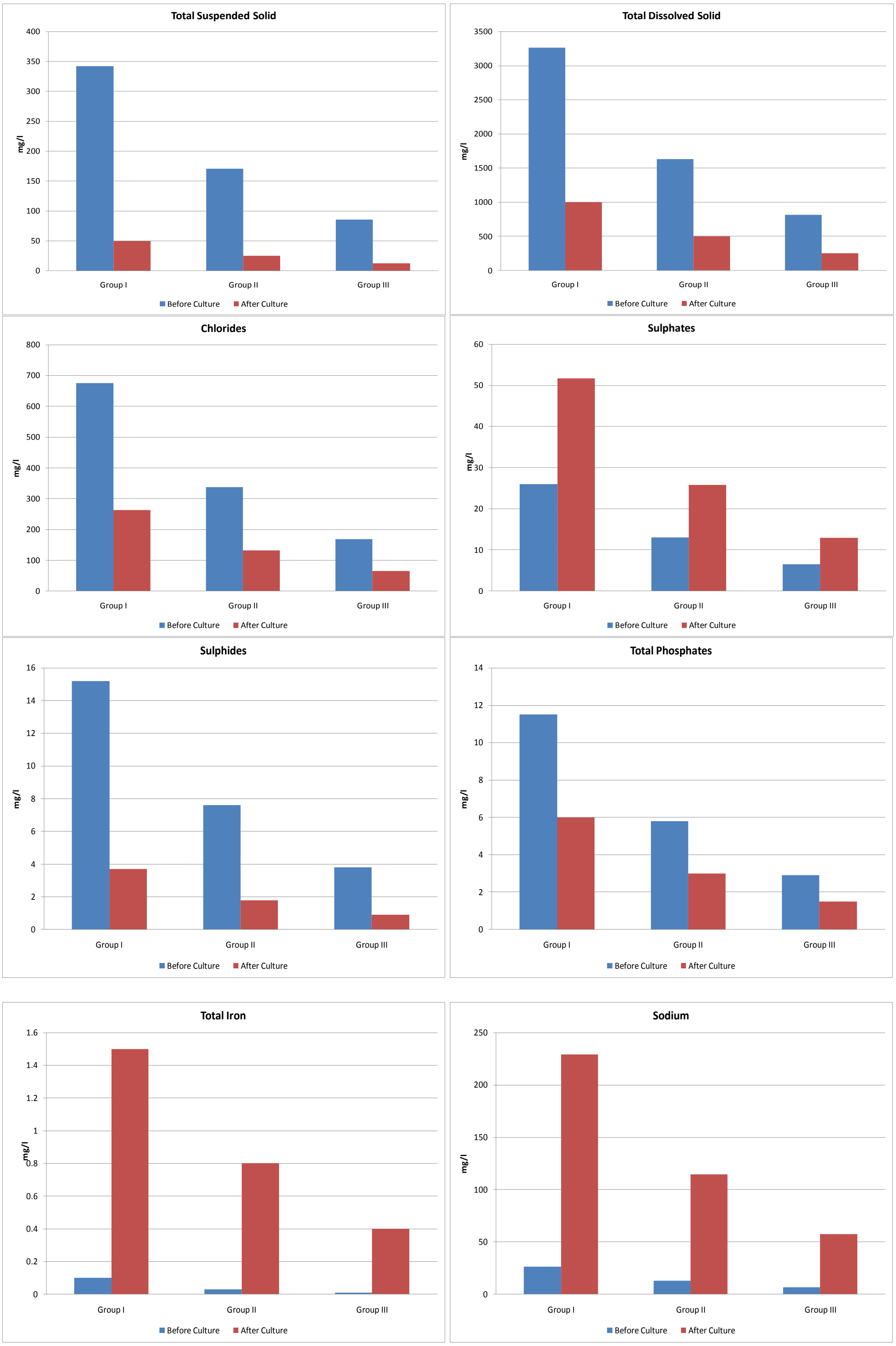
A. Microscopical view of *S. platensis*

B. Harvesting of spirulina

C. Black water before treatment

D. Black water after culturing spirulina

Fig. 1. Microscopical view of *S. platensis*, harvesting of spirulina from black waterand nature of blackwater before and after treatment


Total Suspended Solid
mg/l
400
350
300
250
200
150
100
50
0
Group I
Group II
Group III
Before Culture
After Culture
Total Dissolved Solid
mg/l
3500
3000
2500
2000
1500
1000
500
0
Group I
Group II
Group III
Before Culture
After Culture
Chlorides
mg/l
800
700
600
500
400
300
200
100
0
Group I
Group II
Group III
Before Culture
After Culture
Sulphates
mg/l
60
50
40
30
20
10
0
Group I
Group II
Group III
Before Culture
After Culture
Sulphides
mg/l
16
14
12
10
8
6
4
2
0
Group I
Group II
Group III
Before Culture
After Culture
Total Phosphates
mg/l
14
12
10
8
6
4
2
0
Group I
Group II
Group III
Before Culture
After Culture
Total Iron
mg/l
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Group I
Group II
Group III
Before Culture
After Culture
Sodium
mg/l
250
200
150
100
50
0
Group I
Group II
Group III
Before Culture
After Culture

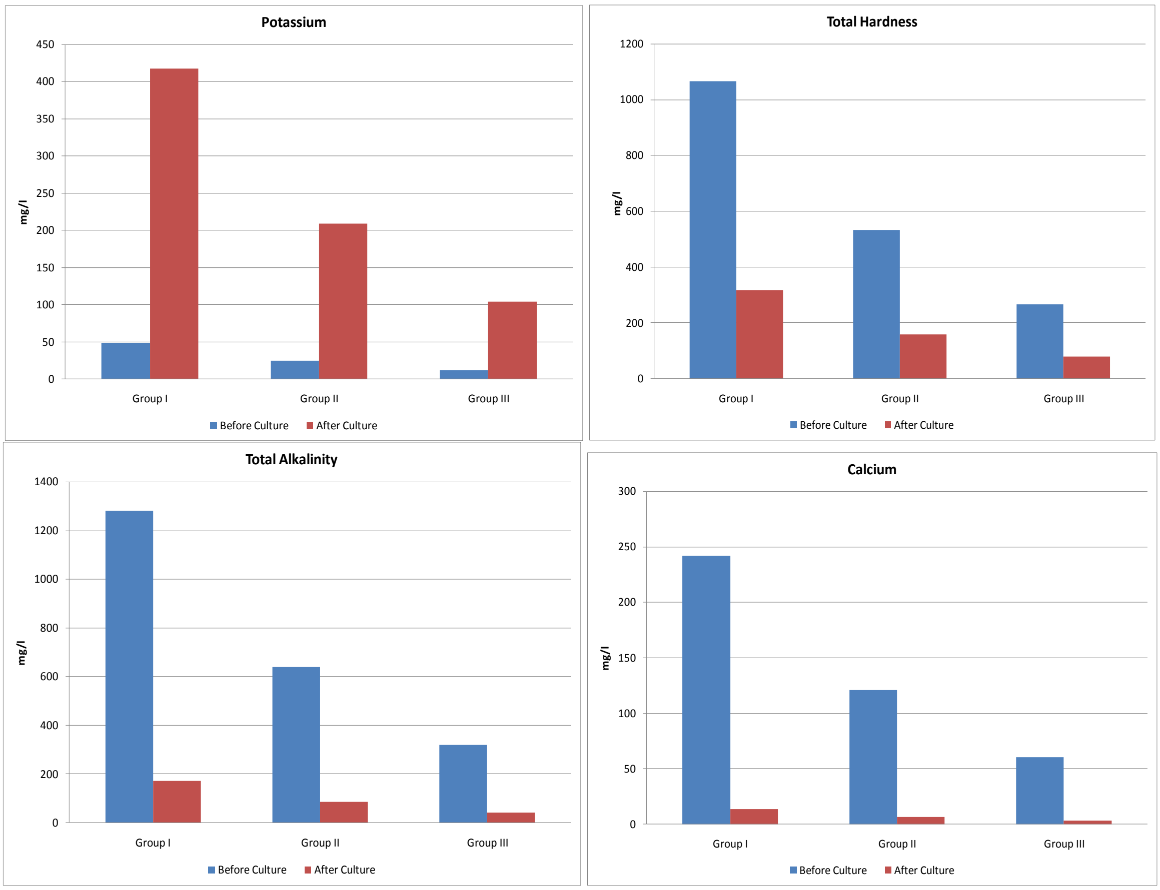


Fig. 2. Mean values of chemical parameters during before and after the culture of Spirulina in Black water

3.6.1 pH

Black water has low pH at the time of collection however it was altered manually by adding required amount of cow dung cake ash up to it turn in alkaline condition. The normal pH range for irrigation water is from 6.5 to 8.4. Irrigation water with a pH outside the normal range may cause a nutritional imbalance or may contain a toxic ion [20,21]. The most important negative effect on the environmental caused by agricultural wastewater is the increases in soil salinity, which if not controlled, can decrease productivity in long term [22]. In the present study, after cultivation of spirulina, the wastewater showed slight alkaline pH. High level of pH may cause nutrient imbalance however, the pH of the treated water under study was observed to close to normal range.

3.6.2 Total suspended solid

Total suspended solid definedthe fraction of sediment small enough to remain suspended in the water. It can be related to microbial pollution and interfere with disinfection, clogging of irrigation systems and deposition. After spirulina cultivation, a significant reduction (t test; $p<0.05$) in TSS was observed in all groups. Before the culture of spirulina, the mean value of TSS was about 342.0 ± 1.00 mg/l in group I, 171.0 ± 0.50 mg/l in group II and in 85.5 ± 0.25 mg/l in group III. However, after the culture of spirulina, the mean level of TSS was about 50.0 ± 5.00 mg/l in group I, 25.0 ± 2.50 mg/l in group II and in group III it was about 12.5 ± 1.25 mg/l.High amount of suspended solids may cause turbidity and restrict light penetration through water resulting in a negative effect on the health of aquatic ecosystems and aquatic plant growth however the high TSS would not be affected the plant growth [23].

3.6.3 Total dissolved solid

Before cultivating spirulina the black water exhibited a high amount of TDS in all groups (3264.0 ±1.00 mg/l for group I; 1632.0 ± 0.50 mg/l for group II and 816.0 ± 0.25 mg/l for group III). However, it was remarkably reduced to 1005.0 ± 5.00 mg/l, 502.5 ± 2.50 mg/l and 251.3 ±1.25 mg/l, respectively for group I, group II and group III. After cultivation of spirulina, black water showed a significant ('t' test; $p<0.05$) reduction in the TDS.Irrigation water with total dissolved solids (TDS) less than 450 mg/l is considered good, and that with greater than 2000 mg/l is unsuitable for irrigation purpose [24]. In the present study, the quantity of TDS after the culture of spirulina was about 1005.00 mg/l in black water. In the present study, TDS level was significantly reduced and found to the range of normal irrigation water.

3.6.4 Chlorides

In black water, the chloride level was significantly ('t' test; $p< 0.05$) reduced. Before the cultivation, chlorides level was about 675.0 ± 1.00 mg/l, 337.5 ± 0.50 mg/l and 168.8 ± 0.25 mg/l for group I, group II and group III respectively. However, it was about 263.3 ± 15.28 mg/l (group I), 131.7 ± 7.64 mg/l (group II) and 65.8 ± 3.81 mg/l (group III).Chloride (Cl^-) is most common toxicity in the irrigation water. If the Cl^- concentration in the leaves exceeds the tolerance of the crop, injury symptoms develop such as leaf burn or drying of leaf tissue. These symptoms occur when leaves accumulate from 0.3 to 1.0 percent chlorides in the water [25]. The present result showed the chloride content was higher in wastewater after treatment by spirulina culture compared recommended level. Thus it may cause the contamination in plants and may affect the soil nature.

3.6.5 Sulfates

Before culture the black water has 26.0 ± 1.00 mg/l in group I, 13.0 ± 0.50 mg/l in group II and 6.5 ± 0.25 mg/l in group III. After harvesting of spirulina, the sulfate level was remarkably increased to 51.7 ± 2.89 mg/l, 25.8 ± 1.44 mg/l, 12.9 ± 0.72 mg/l, respectively for group I, group II and group III. There was a significant ('t' test; $p< 0.05$) different between the levels of Sulfate during the time of collection and after harvesting of spirulina**.** Sulfate is second to bicarbonate as the major anion in hard water reservoirs [26]. Sulfate ion is a major contributor to salinity in many irrigation waters. Sulfate in irrigation water has fertility benefits, and irrigation water often has enough sulfates for maximum production for most crops. From our result the sulfate content in treated wastewater medium were lower than the normal range.

3.6.6 Sulphide

After harvesting spirulina, the sulphide level has been reduced to 3.7 ± 0.58 mg/l, 1.8 ± 0.29 mg/l and 0.9 ± 0.14 mg/l in group I, group II and group III, respectively. Student's 't' test showed a significant different ($p<0.05$) between the levels of sulphide in the waste water at before and after harvesting of spirulina. From our results the Sulfide range is lower than the normal level.

3.6.7 Total phosphate

Phosphorus is one of the major nutrients for healthy growing of crop. Total phosphate content showed a significant reduction ('t' test; $p< 0.05$) in all groups. Before cultivation, it was about 11.5 ± 0.50 mg/l, 5.8 ± 0.25 mg/l and 2.9 ± 0.13 mg/l, in group I, group II and group III, respectively. Nevertheless, after harvesting of spirulina the phosphate level showed a reduction to 6.0 ± 1.00 mg/l in group I, 3.0 ± 0.50 mg/l in group II and 1.5 ± 0.25 mg/l in group III. Phosphate levels are expected to range from 0.01 to 0.05 mg/L for healthy freshwater systems. Elevated levels of phosphorus, however, can cause shifts in this balance and is the most common cause of undesirable growth of aquatic weeds and algae [26]. In the present study, the wastewaters showed high level of phosphate levels after spirulina culture.

3.6.8 Total iron

In the black water the total iron content was found to be low while it was significantly increased ('t' test; $p< 0.05$) after cultivation of Spirulina. An average of 0.1 ± 0.01 mg/l in group I, 0.03 ± 0.01 mg/l in group II, 0.01 ± 0.00 mg/l in group III total iron was recorded in group I, group II and group III, respectively at initial period. However, it showed notable increase from the level of initial period as about 1.5 ± 0.50 mg/l, 0.8 ± 0.25 mg/l and 0.4 ± 0.13 mg/l, respectively for group I, group II and group III. Fe (iron) is not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum [25]. Our result showed the level of iron content inblack water and (1.5mg/l) after cultivation of spirulina. These levels were lower than the normal level however, it would not cause any unwanted changes in soil and agriculture water and it is permissible level for irrigation

3.6.9 Sodium

Interestingly, it exhibited a significant increase ('t' test; $p<0.05$) from the initial level. At the time of collection, black water showed low level of sodium and about 26.2 ± 1.04 mg/l, 13.1 ± 0.52 mg/l and 6.5 ± 0.26 mg/l in group I, group II and group III, respectively. However, it was about 229.0 ± 3.61 mg/l in group I, 114.5 ± 1.80 mg/l group II and 57.3 ± 0.90 mg/l in group III. Level of sodium was 229 mg/l in black water after the culture of spirulina. It is slightly high to the normal level. High level of sodium salts absorbed by plants can be produce harmful effect for the plants [20, 27].

3.6.10 Potassium

Black water exhibited a remarkable increase (‘t’ test; $p<0.05$) in the potassium level after spirulina cultivation. Potassium contents of group I was about 48.9 ± 0.79 mg/l, group II was about 24.5 ± 0.39 mg/l, and group III was about 12.2 ± 0.19 mg/l. However, it was about 417.7 ± 15.37 mg/l for group I, 208.8 ±7.69 mg/l for group II and 104.4 ± 3.84 mg/l for group III.The presence of potassium ions in excessive amount does not constitute any risk and may even supplement crops needs as only values exceeding 50cmol/l may be considered as posing any serious risk factor with irrigation water. The present result showed potassium level was increased in the spirulina cultured wastewater.

3.6.11 Total hardness

It was significantly decreased (‘t’ test; $p<0.05$) from the initial level. At the time of collection, total hardness was about 1065.0 ± 5.00 mg/l, 532.5 ± 2.50 mg/l and 266.3 ± 1.25 mg/l in group I, group II and group III, respectively. Further it was reduced to 317.0 ± 28.58 mg/l, 158.5 ± 14.29 mg/l and 79.3 ± 7.15 mg/l for group I, group II and group III.Hardness is an indication of the amount of calcium and magnesium in the water and is expressed as mg $CaCO_3$, or parts per million $CaCO_3$. Water with hardness in the range of 100 to 150 mg $CaCO_3$ is considered desirable for plant growth. Plants tolerate high levels of these elements, so toxicity is not normally a problem. Before the culture of spirulina the total hardness of test system was very high and it was reduced to normal range after the culture of spirulina [28].

3.6.12 Total Alkalinity

Black water showed a significant reduction (‘t’ test; $p<0.05$) after cultivation in all groups. It was recorded as 1280.0 ± 5.00 mg/l in group I, 640.0 ± 2.50 mg/l in group II and 320.0 ± 1.25 mg/l in group III at initial period. However, after cultivation of spirulina the total alkalinity level was reduced to 171.7 ± 10.41 mg/l, 85.8 ± 5.20 mg/l and 42.9 ± 2.60 mg/l respectively for group I, group II and group III.High alkalinity indicates that the water will tend to increase the pH of the soil or growing media; possibly to a point that is detrimental to plant growth. Low alkalinity could also be a problem in some situations. From our results the hardness of wastewater elevated to the normal irrigation water, it may cause unwanted changes in pH and may cause the sodium hazards in irrigation water. Furthermore, the level of bicarbonate and carbonate ions were reduced by adding water and used for the irrigation purposes. Among the components of water, alkalinity and bicarbonates are normally the most significant concern. In the present study, total alkalinity was significantly reduced in the spirulina cultured wastewater [28].

3.6.13 Calcium

Black water showed a remarkable reduction (‘t’ test; $p<0.05$) in the calcium level after the cultivation of spirulina. At the time of collection the calcium level was 241.7 ± 1.53 mg/l in group I, 120.8 ± 0.76 mg/l in group II and 60.4 ± 0.38 mg/l in group III. However iron content showed a strong reduction in all groups.Generally, calcium and magnesium maintain a state of equilibrium in most waters [29]. High concentration of Ca^{2+} and Mg^{2+} ions in irrigation water can increase soil pH, resulting in reducing of the availability of phosphorous [30]. Water containing Ca^{2+} and Mg^{2+} higher than 200 mg/l cannot be used in agriculture [31]. Based on our result the calcium content after the culture of spirulina in black wastewater was 13.0 mg/l.

4. CONCLUSION

The present study indicates that spirulina can besuccessfully cultured in black waterwith expected yield and low expensive. Further the cultured water can also be used for irrigation purpose with 25% addition of freshwater or as such. Since there is no bad odour and other toxic material it can also be used for toilet flush.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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