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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (9): 1554-1558, 2004
ISSN 1028-8880

The Potential for Using Wastewater from Household Scale Fermented Thai Rice Noodle Factories for Cultivating *Spirulina platensis*

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Abstract: In this study, the influence of various concentrations of wastewater medium, nitrogen, phosphorus, potassium and soda ash (Na_2CO_3) using batch cultivation for *S. platensis* growth were examined. It was found that modified wastewater medium, which contains a 1:11 dilution ratio of wastewater, supplemented with 0.09 g L^{-1} nitrate, 0.59 g L^{-1} phosphate, 0.18 g L^{-1} potassium and 3 g L^{-1} Na_2CO_3, shows great potential for cultivating *S. platensis*. The growth of the algae was smooth in the modified culture. Apart from chlorophyll (2.36 mg g^{-1}), approximately 1.0 g L^{-1} biomass, 59% protein and 14% phycocyanin were detected which was almost identical to that of Zarrouk medium.

Key words: *Spirulina platensis*, Zarrouk medium, modified wastewater medium

INTRODUCTION

Spirulina platensis is a photosynthetic and blue-green microalgae. Its chemical composition includes proteins (55-70%), lipids (4-7%), carbohydrates (13.6%), RNA (2.2-3.5%), DNA (0.6-1%), essential fatty acids (18%), carotenoids (0.47%), xanthophylls (0.22%), chlorophyll (1.0%) and phycocyanin (14%)[1,2]. Moreover, Spirulina contains essential amino acids; the highest values are leucine (10.9% of total amino acids), valine (7.5%) and isoleucine (6.8%)[3]. Spirulina has essential fatty acids: linoleic acid ($C_{18:2}$) and γ-linolenic acid ($C_{18:3}$)[4]. Spirulina is an excellent human food since it contains a high quality and quantity of nutritional components. Apart from a high protein concentration, its chemical compositions have corrective properties against viral attacks, anemia and tumor growth. For example, γ-linolenic acid can reduces serum cholesterol levels[1]; β-carotene can counteract free radicals that alter cells causing cancer[5,6], B_{12} is of great value in the treatment of pernicious anemia[7-9]. Calcium-Spirulan (Ca-SP) from Spirulina showed activity against HIV[1,10] and phycocyanin which has antioxidative properties which may be used as a therapeutic agent and in the cosmetic industry[11,12].

Spirulina grows quickly, continually and provides 20 times more protein than soya beans[1,13] and is easily manipulated and grown in Zarrouk (high bicarbonate) medium. Therefore, Zarrouk medium is frequently used for Spirulina isolation and production processes[14,15]. In Thailand, household scale fermented rice noodle factories are scattered around many regions and many tons of their wastewater are drained away to the surrounding environment. Wastewater composition from a fermented Thai rice noodle factory consists of carbonate, bicarbonate, nitrate, phosphate, sodium chloride, potassium, magnesium, calcium and iron. Since Spirulina can grow in a wide range of water compositions, this study aimed to verify the possibility of using wastewater from household scale fermented Thai rice noodle factories, using chemical fertilizer supplementation, such as nitrogen, phosphorus and potassium sources for *S. platensis* cultivation. *S. platensis* yields (protein, biomass, chlorophyll and phycocyanin) obtained from wastewater cultivation were evaluated and compared with a Zarrouk medium (control).

MATERIALS AND METHODS

Microorganism and inoculum: The microorganism used was *S. platensis*. The inoculum was obtained by liquid cultivation using 500 mL of Zarrouk[14] medium in 1000 mL Erlenmeyer flasks. The cultivation conditions were maintained in a light-dark photocycle (8/16 h); illumosity (lumunance) 3500 lux from fluorescent lamps; aeration to maintain dissolved oxygen at a level of 8-9 mg L^{-1} and temperature 32±21°C. The inoculum concentration (OD) was determined by spectrophotometry at λ = 560 and its growth rate at OD = 0.6 was used as an initial optimal inoculum concentration. Growth culture obtained from Zarrouk medium was used as the control.

Liquid culture medium preparations: Wastewater from the household scale fermented Thai rice noodle factory

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was collected, left to stand for at least 6 h to allow insoluble particle sedimentation. Insoluble solids were removed, then the filtrated wastewater was used for *S. platensis* cultivation. Five hundred mL of cultivation medium (adjusted initial pH to 9) was done using wastewater in 1000 mL Erlenmeyer flask. The growth of *S. platensis* was studied in relation to wastewater medium, nitrate, phosphate, potassium and Na_2CO_3 (soda ash) concentration. In this experiment, chemical fertilizers such as 21:0:0, 0:46:0 and 0:0:60 were used as nitrate, phosphate and potassium sources. Once the optimal initial wastewater medium concentration had been determined, a batch culture was carried out with various additions of nitrate, phosphate, potassium and Na_2CO_3.

Wastewater medium concentration: wastewater from a household fermented Thai rice noodle factory was diluted with tap water at different ratios of 1:8, 1:9, 1:10, 1:11 and 1:12. 0.09 g L^{-1} of nitrate, phosphate and potassium was supplemented into each wastewater medium preparation.

Nitrate variation: Four concentrations (0.09, 0.15, 0.21 and 0.27 g L^{-1}) of nitrate were added into the optimal wastewater medium concentration.

Phosphate variation: Ten concentrations (0.09, 0.19, 0.29, 0.39, 0.49, 0.59, 0.69, 0.79, 0.89 and 0.99 g L^{-1}) of phosphate were added into the optimal wastewater medium concentration.

Potassium variation: Ten concentrations (0.09, 0.15, 0.18, 0.21, 0.24, 0.27, 0.51, 0.61, 0.72 and 0.81 g L^{-1}) of potassium were added into the optimal wastewater medium concentration.

Na_2CO_3 variation: Four concentrations (2.0 , 3.0 , 4.0 and 5.0 g L^{-1}) of Na_2CO_3 were added into the optimal wastewater medium concentration.

Culture conditions: Ten percent of inoculum was added to each liquid medium culture, cultivation period was 20 days and cultivation conditions were similar to the inoculum culture.

Analytical methods

1. Protein content was examined by the Kjeldahl method[16].
2. Chlorophyll content was examined according to Mackinney[17].
3. Phycocycanin content was examined according to Boussiba and Richmond[18].

Statistical analysis: The data were analyzed using SPSS for Windows 7.5.2. Treatment means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Wastewater composition was evaluated by Khon Kaen Provincial (Zone 6, Thailand) Water Works and Department of Soil Science, Faculty of Agriculture (Khon Kaen University). Wastewater (pH 3.56) contained 181.50 mg L^{-1} Na, 84.00 mg L^{-1} Ca, 66.19 mg L^{-1} Mg, 7.15 mg L^{-1} Fe, 60.19 mg L^{-1} NH_4^+-N, 3.49 mg L^{-1} NO_3^+-N, 22.60 mg L^{-1} P, 12.05 mg L^{-1} K, 2,550 mg L^{-1} BOD, 4,400 mg L^{-1} COD and 5.5 mg L^{-1} total solids (TS). Major mineral ingredients found in this wastewater are similar to those found in Zarrouk medium, which are necessary to support *S. platensis* growth.

The concentration of diluted wastewater at a ratio 1:11 gave the highest biomass (0.59 g L^{-1}) when compared with other wastewater mediums, but its biomass was identical to those found in ratio 1:9 (0.52 g L^{-1}), 1:10 (0.54 g L^{-1}) and 1:12 (0.56 g L^{-1}) (Table 1). However, *S. platensis* protein, biomass and pigments acquired in Zarrouk medium were greater than in wastewater medium. This may be a consequence of the Zarrouk medium containing all necessary macro and micronutrients to support *S. platensis* growth better than wastewater. Apart from carbon, *S. platensis* requires the usual major biological nutrients: N, P, K, S, Mg, Ca, Fe, plus a number of micronutrients[19,20]. Nitrate, phosphate and potassium (0.09 g L^{-1}) supplemented into 1:11 diluted wastewater medium was sufficient for *S. platensis* growth. Even Spirulina biomass (0.59 g L^{-1}) and chlorophyll contents (2.26 mg g^{-1}) were lower and significantly different to Zarrouk medium but protein (50.76%) and phycocyanin (12.98%) contents were insignificantly different.

S. platensis growth carried out in the 1:11 diluted wastewater, with 0.09 g L^{-1} of phosphate and potassium added and supplemented with nitrate fertilizer (21:0:0). The effect on growth was studied for different initial nitrate concentrations (0.09 B 0.27 g L^{-1}). For the wastewater medium, the result showed that additions of nitrate concentration between 0.09 - 0.21 g L^{-1} had no significant effect on Spirulina growth. Amounts of protein (52 -54 %) and phycocyanin (12%) which were obtained from wastewater were not significantly different from Zarrouk medium, but its biomass (0.21-0.24 g L^{-1}) was significantly different and two times less than those found in Zarrouk medium (Table 2). Even though Spirulina can grow well at a nitrate concentration of 0.61 g L^{-1}[21], the result obtained

Table 1: The influence of wastewater medium concentration on *S. platensis* protein, biomass and pigments; 1:8, 1:9, 1:10, 1:11 and 1:12 being the dilution ratio between wastewater and tap water

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	57.22±6.47^{a}	0.97±0.09^{a}	13.35±0.64^{a}	7.53±2.07^{a}
Ratio 1:8	29.99±0.52^{cd}	0.38±0.00^{c}	6.06±0.75^{c}	2.10±0.03^{b}
Ratio 1:9	26.01±1.12^{d}	0.52±0.04^{bc}	7.29±0.80^{bc}	1.69±0.01^{b}
Ratio 1:10	49.53±1.60^{b}	0.54±0.04^{bc}	8.58±0.81^{b}	1.47±0.23^{b}
Ratio 1:11	50.76±0.54^{ab}	0.59±0.06^{b}	12.98±1.28^{a}	2.26±0.04^{b}
Ratio 1:12	35.02±1.20^{c}	0.56±0.08^{b}	12.84±0.40^{a}	1.88±0.63^{b}

Means±SD in each column with different superscripts indicate significant differences (P<0.05)

Table 2: The influence of nitrate concentration (g L^{-1}) supplemented in wastewater (1:11 ratio) medium for *S. platensis* protein, biomass and pigments

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	52.42±3.63^{NS}	0.53±0.00^{a}	13.11±0.12^{NS}	14.18±0.19^{a}
N = 0.09	54.09±1.65	0.24±0.04^{b}	12.76±1.27	10.49±1.95^{ab}
N = 0.15	53.38±0.11	0.22±0.03^{b}	12.52±0.56	8.95±1.69^{b}
N = 0.21	52.74±1.58	0.21±0.01^{b}	12.82±0.68	11.30±0.28^{ab}
N = 0.27	-		-	--

Means±SD in each column with different superscripts indicate significant differences (P<0.05) and NS = non significant (P>0.05)
P.S. data at nitrate concentration 0.27 g L^{-1} was excluded from statistical analysis

Table 3: The influence of phosphate concentration (g L^{-1}) supplemented in wastewater (1:11 ratio) culture medium for *S. platensis* protein, biomass and pigments

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	53.45±0.28^{abc}	0.63±0.01^{a}	14.21±0.14^{ab}	11.76±0.21^{a}
P = 0.09	52.99±2.61^{abc}	0.23±0.00^{c}	12.99±1.43^{ab}	9.31±0.56^{abc}
P = 0.19	55.68±2.10^{ab}	0.35±0.03^{bc}	14.48±1.29^{ab}	7.45±0.70^{bcd}
P = 0.29	56.05±0.11^{ab}	0.29±0.13^{bc}	16.12±0.57^{a}	9.45±3.74^{abc}
P = 0.39	59.54±3.86^{a}	0.33±0.07^{bc}	14.28±0.90^{ab}	8.47±1.21^{abc}
P = 0.49	52.52±2.64^{abc}	0.29±0.03^{bc}	12.48±1.32^{bc}	8.44±1.77^{abc}
P = 0.59	47.27±1.95^{c}	0.46±0.10b	13.08±0.62^{ab}	4.02±0.87d
P = 0.69	46.40±1.41^{c}	0.34±0.10^{bc}	15.59±0.02^{ab}	7.74±2.62^{abcd}
P = 0.79	47.12±7.16^{c}	0.40±0.09^{bc}	13.39±0.56^{ab}	5.86±1.25^{cd}
P = 0.89	51.61±4.62^{abc}	0.39±0.11^{bc}	9.96±3.07^{c}	6.18±1.12^{cd}
P = 0.99	49.90±0.28^{bc}	0.23±0.07^{c}	13.33±0.96^{ab}	11.02±0.23^{ab}

Means±SD in each column with different superscripts indicate significant differences (P<0.05)

Table 4: The influence of potassium concentration (g L^{-1}) supplemented in wastewater (1:11 ratio) culture medium for *S. platensis* protein, biomass and pigments

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	54.22±0.86^{a}	0.62±0.02^{ab}	14.82±0.49^{a}	10.41±0.25^{a}
K = 0.09	50.19±2.23^{ab}	0.30±0.04^{cd}	11.06±0.07^{cde}	5.45±0.72^{c}
K = 0.15	50.62±0.27^{ab}	0.63±0.05^{a}	13.33±1.80^{abc}	2.93±0.20^{d}
K = 0.18	47.11±0.86^{ab}	0.65±0.01^{a}	12.94±0.33^{abc}	2.39±0.16^{d}
K = 0.21	38.34±9.25^{c}	0.62±0.04^{ab}	11.98±0.11^{bcd}	2.62±0.01^{d}
K = 0.24	50.15±4.03^{ab}	0.56±0.09^{ab}	14.79±0.95a	2.84±0.26^{d}
K = 0.27	44.89±0.84^{bc}	0.51±0.06^{b}	14.57±2.06^{ab}	4.10±2.06^{cd}
K = 0.51	53.25±1.05^{ab}	0.35±0.07^{c}	11.50±1.77^{bc}	4.94±1.08^{c}
K = 0.61	48.08±2.58^{ab}	0.39±0.04^{c}	9.79±0.38^{de}	4.07±0.34^{cd}
K = 0.72	46.15±2.14^{abc}	0.30±0.03^{cd}	8.61±0.69^{e}	5.12±0.53^{c}
K = 0.81	46.12±4.37^{abc}	0.22±0.03^{d}	9.44±1.00^{de}	7.55±0.07^{b}

Means±SD in each column with different superscripts indicate significant differences (P<0.05)

Table 5: The influence of Na2CO3 concentration (g L^{-1}) supplemented in wastewater (1:11 ratio) culture medium for *S. platensis* protein, biomass and pigments

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	53.62±2.84^{ab}	0.51±0.01^{ab}	14.23±1.29^{a}	11.12±1.08^{a}
Na_2CO_3 = 2	41.19±2.84^{c}	0.45±0.10^{ab}	9.53±0.81^{c}	3.45±0.74^{c}
Na_2CO_3 = 3	51.96±2.91^{b}	0.56±0.04a	11.21±0.39^{bc}	3.53±0.51^{c}
Na_2CO_3 = 4	51.40±0.60^{b}	0.49±0.11^{ab}	12.41±1.58^{ab}	4.34±1.08^{c}
Na_2CO_3 = 5	59.23±0.06^{a}	0.34±0.04^{b}	12.33±0.51^{ab}	6.81±0.19^{b}

Means±SD in each column with different superscripts indicate significant differences (P<0.05)

Table 6: Comparison of *S. platensis* protein, biomass and pigment obtained from Zarrouk medium and modified wastewater medium (diluted ratio 1:11, 0.09 g L^{-1} nitrate, 0.59 g L^{-1} phosphate, 0.18 g L^{-1} potassium and 3 g L^{-1} Na_2CO_3)

Liquid medium	Protein (%)	Dry weight (g L^{-1})	Phycocyanin (%)	Chlorophyll (mg g^{-1})
Zarrouk	59.38±5.23^{NS}	1.29±0.13^{NS}	14.06±1.85^{NS}	4.98±0.17^{a}
Modified	58.77±1.86	0.99±0.06	14.46±1.75	2.36±0.21^{b}

Means±SD in each column with different superscripts indicate significant differences (P>0.05), NS = non significant (P±0.05)

from this work showed that *S. platensis* growth was inhibited when nitrate concentrations were increased to 0.27 g L^{-1}. Jourdan[21] suggesting that when sugar or other easily oxidizable organic materials are used as a source of carbon in the presence of nitrates, they may reduce nitrates to ammonia that is toxic above 0.30 g L^{-1}. Moreover, urea made up of ammonia and CO_2, is an excellent nutrient for Spirulina, but its concentration in the medium must be kept low (below about 0.60 g L^{-1})[21].

Phosphate additions at various initial concentrations (0.09 B 0.99 g L^{-1}) were supplemented into 1:11 diluted wastewater medium and 0.09 g L^{-1} of nitrate and potassium was added in each concentration. The *S. platensis* growth was examined and compared with Zarrouk medium. The maximum biomass (0.63 g L^{-1}) was revealed in Zarrouk medium while 0.46 g L^{-1} was obtained when 0.59 g L^{-1} of phosphate concentration was added into wastewater medium (Table 3). The biomass acquired from wastewater medium declined when phosphate concentrations was greater or less than 0.59 g L^{-1}. By contrast chlorophyll, protein and phycocyanin contents obtained from wastewater were almost similar to those found in Zarrouk medium. Microalgae have the ability to take up and utilise phosphorus as a carbon source[22]. In Zarrouk medium the inorganic phosphate is present in form of K_2HPO_4 (0.50 g L^{-1}). Phosphate, magnesium and calcium cannot be increased much without precipitating magnesium or calcium phosphate, possibly leading to imbalances in the solution[21].

Potassium enrichments at different initial concentrations (0.09 B 0.81 g L^{-1}) into 1:11 diluted wastewater medium, (0.09 g L^{-1} of nitrate and phosphate) was supplied to each concentration. The results (Table 4) showed that additions of potassium at 0.18 g L^{-1} provided the maximum biomass yield (0.65 g L^{-1}) which was quite similar to Zarrouk medium (0.62 g L^{-1}). However, Spirulina biomass was decreased to 0.22 g L^{-1} when a potassium concentration of 0.81 g L^{-1} was supplemented. Potassium concentration can be increased and it should not become more than five times the sodium concentration[21]. Inhibition of Spirulina growth may occur when the ratio of K^+: $Na^+ > 5$[23]. Since sodium concentration present in this wastewater was 0.18 g L^{-1}, therefore low biomass productivity was found when a high concentration of potassium was supplemented into wastewater medium.

The effects of Na_2CO_3 on growth of *S. platensis* was investigated and compared with normal growth from Zarrouk medium. *S. platensis* was batch cultured in 1:11 diluted wastewater medium and 0.09 g L^{-1} of nitrate, phosphate and potassium was supplemented. Different concentrations of Na_2CO_3 (2.0 B 5.0 g L^{-1}) were added to the wastewater medium. The highest biomass (0.56 g L^{-1}) was obtained when 3.0 g L^{-1} of Na_2CO_3 was added to the wastewater medium and it was slightly higher than Zarrouk medium (0.51 g L^{-1}), but there was no significant difference between both values (Table 5). However, phycocyanin and chlorophyll contents observed from all wastewater medium samples were less than that found in Zarrouk medium. And also, chlorophyll content examined from wastewater (3.45 B 6.81 mg g^{-1}) was significantly different from Zarrouk medium (11.12 mg g^{-1}). Suitable water composition for Spirulina growth should contain 5.0 g L^{-1} Na_2CO_3[21], however its biomass was decreased to 0.34 g L^{-1} when 5.0 g L^{-1} of Na_2CO_3 was added to wastewater medium. It may have been caused by high alkalinity, above the optimal Spirulina pH (8.2 B 11.0) range. High pH occurs in the medium when CO_3^{2-} is oxidised to CO_2 and OH^-. Spirulina can adapt to a gradual change in pH; however the culture may rapidly deteriorate when pH is changed abruptly, as may happen in a growth medium that is not well buffered[23].

Wastewater from household scale fermented Thai rice noodle factory constitute an excellent medium for growing algae since it contains many valuable nutrients such as nitrogen, phosphorus, potassium and trace elements to support *S. platensis* production. Hence, modified wastewater medium which consists of 1:11 diluted ratio of wastewater, supplemented with 0.09 g L^{-1} of nitrate, 0.59 g L^{-1} of phosphate, 0.18 g L^{-1} potassium and 3 g L^{-1} Na_2CO_3 showed a great possibility for cultivating *S. platensis*. The growth of the algae was smooth in the modified culture, approximately 1.0 g L^{-1} biomass, 59% protein and 14% phycocyanin were detected and they were identical to Zarrouk medium (Table 6). However, chlorophyll content obtained from modified culture (2.36 mg g^{-1}) was less than half that of Zarrouk medium (4.98 mg L^{-1}) but still, using wastewater from a fermented Thai rice noodle factory for cultivating *S. platensis* is economical, adaptable and a safe wastewater treatment system.

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