Asian Journal of Chemistry Vol. 16, No. 2 (2004), 1081–1088

Efficacy of *Dunaliella salina* (Volvocales, Chlorophyta) in Salt Refinery Effluent Treatment

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The halotolerant green alga, *Dunaliella salina* (Dunal) Teod. isolated from the salt evaporation ponds (Kelambakkam near Chennai) was evaluated for its potential in the treatment of salt refinery effluent. The alga was grown in 100% effluent and its mixtures, *viz.*, effluent + seawater and effluent + basal medium in the ratio of 1 : 1. Experiments were conducted for a period of 18 d. At every six days interval, concentrations of the pigments Chl-a, Chl-b and β-carotene of the organism were recorded. UV absorption spectrum was taken to confirm the presence of β-carotene isomers at 443 nm and 475 nm. Initial and final physico-chemical features of the effluent and its mixtures namely appearance, odour, TDS, turbidity, pH, hardness, BOD, COD, NO_4—N, NO_2—N, NH_4—N, PO_4—P, K, Ca, Mg, Na, SO_4, Fe, Cl, F, Tidy's test and SiO_2 were recorded. The amount of heavy metals Ba, Al, Ag and St present in 100% effluent were also recorded. The amount of β-carotene was found high when the alga was grown in 100% effluent compared to its mixtures. Among the three different experiments, the alga grown in 100% effluent was able to reduce a maximum 77.5% of K followed by 72.5% of Na. The levels of BOD and COD were also reduced up to 54.5% and 50.5%, respectively. The removal percentages of heavy metals, *viz.*, Ba, Al, Ag and St by the alga at the above condition were 56.5, 46.0, 32.4 and 4.8%, respectively.

Key Words: *Dunaliella*, Salt effluent, BOD, COD, Nutrients, Heavy metals.

INTRODUCTION

Environmental protection has become a subject of great concern and top priority domain on a global basis. Conventional treatment technologies have not been adopted by many industries as they are not technically feasible and economically viable to achieve the required stringent standards set for the disposal of effluents into water bodies. Hence it is worthwhile to explore the possibility of using non-conventional materials in their natural and chemically activated forms. In the present investigation, the halotolerant alga, *Dunaliella salina* was used to treat the salt refinery effluent and its mixtures for the reduction of different physico-chemical parameters. This study could be the first attempt to evaluate the efficacy of *D. salina* in salt effluent treatment.

EXPERIMENTAL

The Gujarat Chemicals Ltd. is located at Tiruporur, 40 km south of Chennai, Tamil Nadu and is capable of producing one lakh tonnes of salt per year. About

280 cubic metres per day of the effluent is discharged into the receiving water body.

Ten litres of the effluent was collected in plastic containers, brought to the laboratory and the following parameters: appearance, odour, pH, TDS, hardness, BOD, COD, NO_3—N, NO_2—N, NH_4—N, PO_4—P, K, Ca, Mg, Mn, SO_4, Fe, Cl, F, Tidy's test and SiO_2 were recorded according to the standard methods[1]. The heavy metals Ba, Al, Ag and St were also recorded by inductively coupled plasma atomic emission spectroscopy (ICPAES). The test organism *Dunaliella salina*, was isolated from the salt evaporation ponds Kelambakkam near Chennai and maintained in De Walne's medium (basal medium) under 24 ± 1°C and 30 μ $Em^{-2} s^{-1}$ in 12/12 light/dark cycle. 50 mL of optimally grown algal sample was inoculated in 500 mL of (a) 100% effluent, (b) 1:1 ratio of 100% effluent and seawater and (c) 1:1 ratio of 100% effluent and basal medium, and kept for a period of 18 d under laboratory conditions. The alga grown in the basal medium was served as control. At the end of experimental period different physico-chemical characteristics of the samples were recorded. The efficacy of alga on the percentage reduction of BOD, COD and other nutrients was calculated as follows:

$$\text{Percentage reduction} = \frac{\text{Initial} - \text{final}}{\text{Initial}} \times 100$$

$$\text{Efficacy of the alga in \% reduction} = \left(\begin{array}{c}\text{\% reduction in algal}\\ \text{grown medium}\end{array}\right) - \left(\begin{array}{c}\text{\% reduction in}\\ \text{control}\end{array}\right)$$

The photosynthetic pigments namely Chl-*a*, Chl-*b* and β-carotene of *D. salina* were extracted[2] and β-carotene globules were isolated[3] from *Dunaliella salina* as per reported method. The sample was then read at UV absorption spectrum in Beckman spectrophotometer at 443 nm and 475 nm.

RESULTS AND DISCUSSION

Effect of *D. salina* on 100% salt refinery effluent treatment

Dunaliella salina was able to survive in 100% effluent during the study period. The initial turbid effluent became a clear solution at the end of the study period due to the algal growth, whereas, the effluent remained turbid in control. A maximum reduction of 50.5% was encountered in hardness (Table-1). The percentage reduction of different nutrients, *viz.*, NO_3—N, NO_2—N, NH_4—N, PO_4—P, K, Ca, Mg, Na, Fe, SO_4, Cl, F and SiO_2 was ranging from 38.5 to 77.5%. Among the nutrients a maximum percentage reduction was achieved in K (77.5%) followed by Na (72.5%). The efficacy of *D. salina* for the reduction of BOD and COD levels was found high when compared to control. The BOD reduction in control was only 2.1%, whereas 54.5% was achieved by growing *D. salina*. Similarly the reduction of COD was found up to 50.5% in *D. salina* grown effluent (Table-1).

TABLE-1
EFFICACY OF *D. SALINA* ON PERCENTAGE REDUCTION OF PHYSICO-CHEMICAL PARAMETERS IN 100% SALT REFINERY EFFLUENT

Parameters	Percentage reduction in control	Efficacy of *D. salina* in percentage reduction
Appearance	Turbid	Colourless
Odour	H_2S	Odourless
Turbidity NTU	2.06	49.40
TDS	0.17	16.87
pH*	7.75	7.63
Hardness $CaCO_3$	0.10	50.50
BOD	2.10	52.37
COD	0.96	50.50
NO_3	0	48.40
NO_2	0	31.30
NH_4	0.39	43.90
PO_4	0.71	41.87
K	0.60	77.50
Ca	0.26	51.76
Mg	0.71	41.87
Na	0.03	72.50
SO_4	0.02	53.40
Fe	0	56.90
Cl	7.12	59.29
F	0	50.10
Tidy's test	1.28	66.70
SiO_2	0.03	38.50

*Indicates only the values

The heavy metals, *viz.*, Ba, Al, Ag and St present in *D. salina* grown 100% effluent were found reduced when compared to control (Table-2). The effluent did not contain the following heavy metals: Cu, Cd, Co, Pb, Ni, Hg and Cr.

TABLE-2
EFFICACY OF *D. SALINA* ON THE PERCENTAGE REDUCTION OF HEAVY METALS IN 100% SALT REFINERY EFFLUENT

Heavy Metals	Control	Percentage reduction by *D. salina*
Barium	65.19	56.5
Aluminium	20.58	46.0
Silver	7.674	32.4
Strontium	4.686	4.8

Effect of *D. salina* on 1:1 ratio of 100% salt refinery effluent and seawater treatment

The test alga, *D. salina,* was able to grow in 1:1 ratio of 100% effluent and seawater mixture. The initial turbid colour and a distinct H_2S odour of the mixture became colourless and odourless, respectively, towards the end of study period. *Dunaliella salina* was able to reduce the hardness, 15.5%. Among the macro nutrients, *viz.*, NO_3—N, NO_2—N, NH_4—N, PO_4—P and K the alga was able to reduce a maximum reduction 96.0% in NH_4—N followed by 91.0% in PO_4—P, 70.2% in NO_3—N, 25.0% in K and 22.2% in NO_2—N. The percentage reduction of Ca, Mg, Na, Fe, Cl, F, SO_4 and SiO_2 was found to vary between 25.2% and 91.0%. Further, the alga was able to reduce 38.0% of BOD and 52.0% of COD (Table-3).

TABLE-3
EFFICACY OF *DUNALIELLA SALINA* ON PERCENTAGE REDUCTION OF DIFFERENT PHYSICO-CHEMICAL PARAMETERS IN 1:1 RATIO OF 100% SALT REFINERY EFFLUENT AND SEAWATER

Parameters	Percentage reduction in control	Efficacy of *D. salina* on percentage reduction
Appearance	Turbid	Colourless
Odour	—	—
Turbidity NTU	3.90	13.2
TDS	0.006	9.7
pH*	7.70	7.5
Hardness $CaCO_3$	0.03	15.5
BOD	0.74	38.0
COD	0.75	52.0
NO_3	1.50	70.2
NO_2	11.10	22.2
NH_4	1.10	96.2
PO_4	0.70	91.0
K	0.94	25.2
Ca	0.10	39.9
Mg	0.65	32.7
Na	0.04	39.2
SO_4	0.10	53.6
Fe	4.00	91.0
Cl	0.04	37.7
F	0.10	47.9
Tidy's test	0.95	86.8
SiO_2	4.30	6.9

*Indicates only the values

Effect of *D. salina* on 1:1 ratio of 100% salt refinery effluent and basal medium treatment

Dunaliella salina was capable of turning the mixture from turbid to colourless at the end of study period. The alga showed a maximum percentage reduction in hardness (56.0%). A minimum percentage reduction was encountered in total $CaCO_3$ (3.1%). Among the macronutrients a maximum percentage reduction was achieved by the alga towards NO_3—N (66.1%). The efficacy of *D. salina* on the percentage reduction of BOD and COD was 20.0 and 42.0% when compared to only 3.0 and 1.0%, respectively, in control (Table-4).

TABLE-4
EFFICACY OF *DUNALIELLA SALINA* ON PERCENTAGE REDUCTION OF DIFFERENT PHYSICO-CHEMICAL PARAMETERS IN 1:1 RATIO OF 100% SALT REFINERY EFFLUENT AND DE WALNE'S MEDIUM

Parameters	Percentage reduction in control	Efficacy of *D. salina* on percentage reduction
Appearance	Turbid	Colourless
Odour	—	—
Turbidity NTU	2.60	12.66
TDS	0.48	23.30
pH*	7.41	0.40
Hardness $CaCO_3$	0.13	55.70
BOD	3.00	20.00
COD	1.00	42.00
NO_3	1.80	66.10
NO_2	1.30	25.40
NH_4	4.90	8.83
PO_4	0.45	36.55
K	0.90	19.10
Ca	0.45	33.62
Mg	0.62	34.40
Na	0.05	16.40
SO_4	0.09	44.07
Fe	3.30	13.87
Cl	0.02	18.04
F	10.60	40.50
Tidy's test	0.44	21.80
SiO_2	0.08	28.50

*Indicates only the values

PIGMENTS

The concentration of Chl-*a*, Chl-*b* and β-carotene of the alga was found high in control than the alga grown in effluent and its mixtures. *Dunaliella salina* grown in the three different experimental conditions revealed that the concentration of pigments were found high in 100% effluent followed by 1:1 ratio of 100% effluent and seawater and 1:1 ratio of 100% effluent and medium (Fig. 1). UV absorption spectrum of the sample was taken at 443 nm and 475 nm indicating the presence of 9-*cis*-β-carotene and all-*trans* β-carotene isomers (Fig. 2).

Occurrence of different algal communities in polluted environments depends

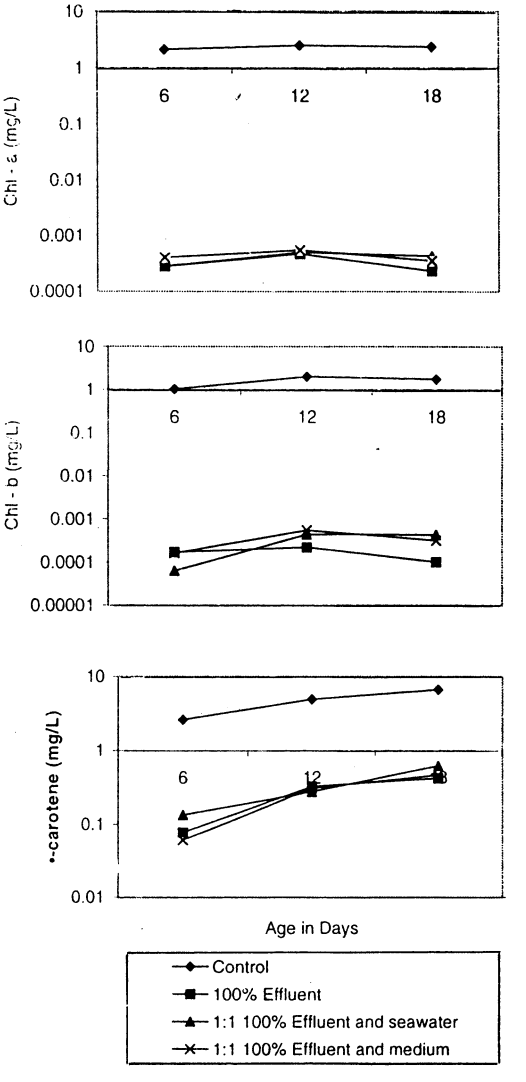

Fig. 1. Effect of 100% effluent, 1 : 1 100% effluent and seawater and 1 : 1 100% effluent and medium on *Dunaliella salina*

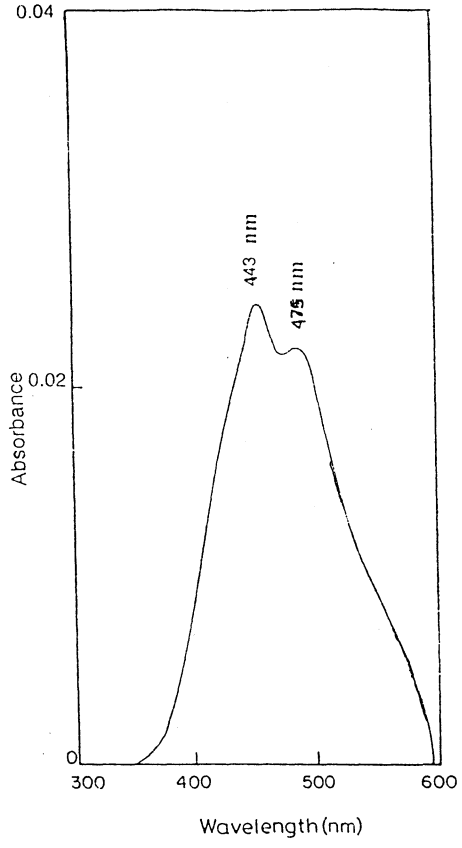

Fig. 2. UV absorption spectrum of β-carotene globules of *Dunaliella salina*

on (a) the level of toxicants in the water and (b) the physiological and genetic resistance of algae to heavy metals and other pollutants[4]. Algae serve as indicators of water pollution since they respond typically to many ions and toxicants[5]. *Dunaliella salina* is capable of surviving a range of NaCl concentrations from 0.1 M to 5.0 M. Moreover, no other eukaryotic alga can survive in the extreme saline conditions except *D. salina*[2].

The growth of aquatic plants may increase the hardness of water whereas vigorous algal growth may reduce hardness[6]. In the present study the hardness ($CaCO_3$) of 100% effluent and the other two mixtures was found decreased due to the growth of *D. salina*. Chloride is generally considered as one of the major pollutants, which is found difficult to remove by the conventional treatments. The alga, *D. salina,* was capable of reducing the Cl level up to 59.0% in 100% effluent.

Sulphate is widely distributed in nature and plays an important role in the formation of chlorophyll. It is also an important constituent of proteins and organic acids. *Dunaliella salina* was able to reduce the level of SO_4 in salt refinery effluent and its mixtures indicating the possible utilization of SO_4 for chlorophyll, protein and organic acids syntheses. Further, the major nutrients *viz.*, NO_3—N,

NO_2—N, NH_3—N, PO_4—P and K were found decreased due to the possible utilization of these for its growth.

Chemical Oxygen Demand (COD) is generally considered as a major indicator of organic pollution in the effluent. The use of acclimatized algal cultures for reducing BOD and COD from different types of wastewaters has been reported[7]. In this present investigation, *D. salina* was found to reduce the levels of BOD and COD considerably from the effluent and its mixtures indicating the oxygenation of effluent due to its photosynthetic activity. Among the three different conditions, the concentration in algal pigments was found maximum when the organism was grown in salt refinery effluent followed by the effluent and seawater mixture and effluent and medium mixture.

The reduction of BOD, COD and nutrients by *D. salina* in the salt refinery effluent clearly indicates that the alga possesses certain inherent abilities. These include the incredible metabolic and physiological versatility of the alga that allows it to inhabit the hostile environment and to exploit the energy sources, not suitable for other organisms. The ecological adaptation and flexibility make *D. salina* a promising candidate in the treatment of salt refinery effluent. The UV absorption spectrum of the 80% acetone extract shows (Fig. 2) two peaks 443 nm and 475 nm confirming the presence of two isomers all-*trans* and 9-*cis*-β-carotene isomers[8, 9].

ACKNOWLEDGEMENT

We are very grateful to Prof. D. Lalithakumari, Former Director, CAS in Botany, University of Madras, Guindy Campus, Chennai-600 025, India for providing laboratory facilities.

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(*Received*: 27 September 2003; *Accepted*: 31 December 2003) AJC-3321