

Circulation of Quaron Lake Wastes I- Solidification of Magnesium Salts and Complementary Demineralization by the Green Alga *Scenedesmus* sp.

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Abstract Magnesium rich waste was used to partially extract and solidify the initial soluble content of Mg ions from HCO_3 , CO_3 , Cl and SO_4 salts. Solidification was performed by nitrification the oily reddish wastes at high pH value. After extraction the remainder water rich in mineral content was used to grow the green alga *Scenedesmus* sp. The obtained Mg yielded was 153 g.l^{-1} on the form of ammonium magnesium chloride hexa hydrate ($\text{NH}_4\text{MgCl}_3 \cdot 6\text{H}_2\text{O}$). Less than 0.005% of impurities were confirmed by X-Ray crystallography of such salt. Processes of extraction not fully purified the used wastewater, thus it was used for algal growth at 0.0, 25, 50, 75 and 100 ml.l^{-1} to meet the bio-demineralization. The complementary nutrition of the remainder water was done by nitrogen on nitric acid and phosphorous on phosphoric acid-form. Dry weight, total chlorophyll and total carotenoids were the measured parameters. Data showed that growth rate (on the average and maximum) was highly affected by the levels of remained water supplied and 50 ml.l^{-1} of the remained water could serve as the most appropriated treatment to gain the maximum biomass, while higher remained water supply increase the rate of carotenoids accumulation. [Journal of American Science 2010; 6(9):870-875]. (ISSN: 1545-1003).

Key words: Quaron Lake; Mg Wastes; *Scenedesmus*; X-Ray crystallography, Growth Profile.

Introduction

Two main technologies are standing the demineralization of wastewater. Chemical methods seem to be more efficient with concentrated solution, while biological treatment is more useful for the minor quantities. The selective extraction of metals from industrial wastes both diminishes disposal problems and opens up new possibilities of recycling this refuse by use of pyro-metallurgical methods. A promising technique in this field is leaching metals or contaminants by microorganisms. With this technique both energy requirements and environmental damage are kept low. The use of autotrophic organisms is advantageous because no organic carbon source is needed for their growth. On the other hand, heterotrophic bacteria and fungi can be used with higher pH (alkaline, acid-consuming materials). A further advantage of leaching with heterotrophic microorganisms is the formation of complexes between metals and metabolites. Precipitations can be avoided when high concentrations of metals and alkaline pH are present. At the same time complex formation often reduces the toxicity of metal ions (Avakyan and Rabotnova. 1971 & Babich and Stotzky 1980). The oily waste (water soluble with high mineral content) not demonstrated to be clarifying by microorganisms. Mg wastes were early utilized by the green alga *Scenedesmus* sp. at the maximum of 50 ml.l^{-1} of culture volume (El-Sayed, 2004a).

The production of secondary metabolites especially by actinomycetes and fungi imperfection leads to a series

of unusual compounds. The use of microorganisms in wastewater treatment (especially bio-elimination of chemicals and heavy metals) is also very relevant. Interest is the use of microalgae in biotechnology due to some aspects of their metabolism. Microalgal biotechnology also has become one of the most studied fields within biological sciences research ;for instance; *Dunaliella salina*, was the best natural source of β -carotene, (Avron and Ben-Amotz, 1992). Another key point in algal biotechnology was the utilization of *Spirulina* as the source of healthy food products (Ciferri, 1983). The other alga; *Scenedesmus* sp. was early used successfully to utilize the initial waste of Quaron Lake (El-Sayed, 2004a&2005).

On the other hand, the ability of microalgae to assimilate inorganic nitrogen into biomass could be very effective for nitrogen compound detoxification. Florencio and Vega (1983) described the use of nitrate, nitrite, and ammonium by *Chlamydomonas reinhardtii*. Vflchez and Vega (1995) reported that alginate entrapped *Chlamydomonas reinhardtii* cells provide a stable and functional system to remove nitrogenous contaminants from wastewaters. Since the most important contaminants in wastewaters are inorganic nitrogen and metals, studies concerning the removal of other toxic compounds using microalgae are still few in number. Chevalier and De la Nou'e (1985) also used immobilized hyper concentrated algae for orthophosphate removal from wastewaters.

The high salinized water of Quaron Lake (34 g.l^{-1}) of total dissolved salts commercially used to produce

the table salt (NaCl 99.5%) and anhydrous sodium sulphate (Na₂SO₄ 99.5%). Both of the produced salts were separated depending on their physical characteristics. Process steps including the precipitation of the above mentioned salts within different ponds ended by the accumulation of magnesium salts at a final 520 g.l⁻¹ of total dissolved salts mainly magnesium chloride 435.34 g.l⁻¹ and magnesium sulphate 45.249 g.l⁻¹ (Tables 1).

The previous study (El-Sayed, 2004a) indicated the ability of the green alga *Scenedesmus* sp. to grow under different concentrations (10-50 ml.l⁻¹) of such brain solution. More than one problem generated from these residuals because of the limiting store area on the expense of agricultural area and the environmental balancing factors where there is no permission to discard this solution again to the Lake. Many studies concerning the beneficial uses of such solution for industrial and agricultural purposes are in demand.

Table 1. Chemical analysis (g.l⁻¹) of bittern solution

HCO ₃ ⁻¹	CO ₃ ⁻²	SO ₄ ⁻²	Cl ⁻¹
2.928	1.92	36.114	334.695
Ca ⁺²	Mg ⁺²	Na ⁺	K ⁺
0.0	121.15	5.179	2.6
Mg(HCO ₃) ₂	MgCO ₃	MgSO ₄	MgCl ₂
3.511	2.697	45.249	435.34
CaCO ₃	Na ₂ SO ₄	NaCl	KCl
0.0	0.0	13.171	4.967
T.D.S	S.G (g.cm ⁻³)	Others (g.l ⁻¹)	
520	1.36	15.064	

To overcome these problems the present work was achieved aiming at the chemical precipitation of Mg salts at the bench scale technique and use the remainder solution for algae cultivation. The main target is to chemical conversion of different soluble Mg salts to the solid nitrogenous form (NH₄MgCl₃.6H₂O).

2. Materials and Methods

2.1. Mg salts extraction

The oily reddish samples obtained from Mg pond were silted and cooled to precipitate the residual content of sodium bisulphate salt and then filtered under vacuum because of their higher density (520 g.l⁻¹). Following to this the clear filtrate was nitrified at pH 9.5 in presence of 1-2 fold of tape water til the completely white color using separator funnel. The received volume was vortexed and the jelly precipitate was centrifuged after neutralization. The obtained white precipitate was water washed two times and then after oven dried at 105°C/30minutes.

2.2. Analysis and crystallography

All of the obtained salt and filtrates were analyzed. Concerning salt, X-ray detection was preformed to define crystals according to ICCD1998 JCPDS International Center for Diffraction. Detection conditions were Rad.:Cukal λ:1.54050, Filter: Mono d-sp: Debye-s. 114.6, Cut off: 50.0 Int. As for the remainder solution, mineral contents including N, P, K, Mg, Na, Ca, S, Fe, Cu, Zn, and Mn were determined based on the methods adopted by Chapmen and Pratt (1974). N was determined based on Kjeldahl technique. P was color-metrically determined by Vanedate methods. S was photo-metrically determined by barium chloride. K, Ca and Na were photo-metrically measured by flame emission technique while; Mg, Fe, Cu, Mn and Zn were measured by atomic absorption technique.

2.3. Alga, treatments and growth conditions

The complementary demineralization was operated by growing the green alga *Scenedesmus* sp. (El-Sayed, 2004) under different volumes of the remainder water after Mg salt precipitation. The applied volumes of the remained water ;based on their chemical composition; were 0.0, 25, 50, 75 and 100 ml.l⁻¹ added to the growth medium. Growth medium was composed of tap water enriched by HNO₃ (0.85 ml.l⁻¹) and H₃PO₄ (0.1 ml.l⁻¹) to increase the solubility of other nutrients and decrease the concentration of the accompanied ions. Control culture received 0.01 g.l⁻¹ of K₂SO₄ added to the amount of nitric and phosphoric acid. Concerning other nutrients (K, Na, Mg, Ca and micronutrients); they were found in adequate content that allow algal growth. Prior treatments, all cultures were grown in control conditions for 12 hours to allow the complete consumption of the initial nutrients by the same rate (El-Sayed, 1999). In addition, dense cultures were used in this action to overcome the osmosis effect.

2.4. Alga growth profile

Growth profile was determined as dry weight, total chlorophyll and carotenoids. 10ml were filtered over membrane filter (0.45µm) to achieve dry weight (g.l⁻¹). As for pigments, the precipitated algal biomass over membrane filters were soaked overnight into DMSO 95%, then after filtered by centrifugation. Chlorophyll absorbance was measured at 666nm. By the same, carotenoids were extracted after chlorophyll decomposition and measured at 466nm. Growth rate was determined according to the method described by Pirt 1973.

3. Results and discussion

3.1. Solidification and X-ray crystallography

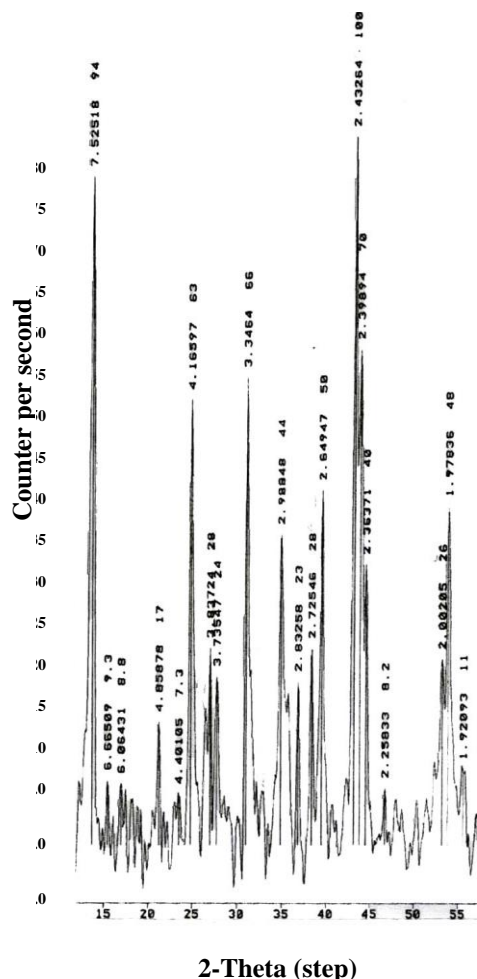
Different trails were employed to precipitate the initial amount of Mg salts from the bittern solution, however no available data were found in this action.

Table 2. X-ray crystallography and absorbance properties of the solidified Mg salt (NH₄MgCl₃.6H₂O)

Wavelength=1.54050									
D(A)	Int.	h	k	l	D(A)	Int.	h	k	l
6.66	25	0	2	0	2.014	8	0	6	2
5.97	<1	1	1	1	2.003	8	2	2	4
4.78	4	2	0	0	1.997	4	4	4	0
4.72	6	1	2	1	1.943	<1	4	3	2
3.887	40	2	2	0	1.920	4	0	4	4
3.818	40	0	2	2	1.909	<1	4	3	2
3.699	<1	1	3	1	1.893	4	0	4	4
3.332	100	0	4	0	1.875	<1	2	3	4
3.256	2	2	3	0	1.850	<1	5	0	1
3.018	2	3	0	1	1.829	<1	3	4	3
2.983	30	2	2	2	1.811	<1	1	0	5
2.953	6	1	0	3	1.804	<1	1	1	5
2.942	4	3	1	1	1.789	<1	5	2	1
2.884	4	1	1	3	1.780	<1	3	6	1
2.748	8	3	2	1	1.773	<1	4	5	0
2.735	2	2	4	0	1.728	2	2	4	4
2.710	4	0	4	2	1.710	<1	5	3	1
2.698	4	1	2	3	1.690	<1	3	5	3
2.668	4	2	3	2	1.668	<1	1	3	5
2.497	2	3	3	1	1.655	6	4	0	4
2.477	2	1	5	1	1.636	<1	4	1	4
2.461	2	1	3	3	1.617	<1	5	4	1
2.392	10	4	0	0	1.584	<1	5	1	3
2.358	25	2	4	2	1.564	<1	6	1	0
2.329	2	2	5	0	1.551	<	3	2	5
2.350	2	4	2	0	1.536	2	6	2	0
2.235	6	3	4	1	1.524	4	4	6	2
2.211	<1	1	4	3	1.509	2	2	6	4
2.197	2	0	2	4	1.491	4	6	0	2
2.128	2	4	0	2	1.477	8	2	8	2
2.110	4	3	2	3	1.357	2	2	0	6
2.102	<1	2	5	2	1.366	2	6	4	6
2.085	2	2	1	4	1.350	2	4	8	0
2.068	16	4	2	2	1.335	2	2	4	6
2.026	8	2	6	0					
M.Wt: 256.79 Volume [CD]:1188.30.					ICDD1998 JCPDS International Center for Diffraction Data, PCPDWIN V.2.01 <1505				
Rad.:Cukal Å:1.54050									
Filter: Mono d-sp: Debye-s. 114.6 Cut off: 50.0 Int. Film I/cor: 1.37									
Ref: Technisch Physische Dienst. Netherlands.ICDD Grant-in-Aid									
Sys.:Orthorhombic SG.:Bb2,m(36)									
a:9.570 b:13.330 c:9.315 A:0.7179 C:0.6988									
Ref:lbid. DX:1.435 Dm:1.456 SS/FOM:F30=68(0.0108,41)									
Color: Colorless Ap.a. chemical impurities<0.005%									
The unit cell of Andrass and Saffe, S.B, VII,117 (1939) is probably described on our b, 1/2(a+c). 1/2(a-c). PSC:108°C To replace 1-660.									

At the same time complex formation often reduces the toxicity of metal ions. Four magnesium salts of the bittern solution are present (HCO₃, CO₃, SO₄ and Cl). Thus, the effect of ammonia addition might affect the electric charges of the initial salts to precipitate the Mg mainly from HCO₃, CO₃ and sulphate sources and partially from Cl source. This finding could be supported by the analysis of remained water, where no

detected amount of CO₃ and HCO₃. Otherwise, the remainder sulfate was found in Ammonium sulfate form. The net obtained weight of chloride salt was 153g.l⁻¹ of the initial bittern solution. This amount could be account as 13.48% of the total Mg content. As shown in Table 2 and Fig. 1, X-ray crystallography of the obtained salt showed their higher purity of the named salt ammonium magnesium chloride hexahydrate (NH₄MgCl₃.6H₂O).

**Fig. 1. X-Ray crystallography of Mg solidified salt**

This finding could be supported by the analysis of remained water, where no detected amount of CO₃ and HCO₃. Otherwise, the remainder sulfate was found in ammonium sulfate form. The net obtained weight of chloride salt was 153g.l⁻¹ of the initial bittern solution. This amount could be account as 13.48% of the total Mg content. As shown in Table2 and Fig. 1, X-ray crystallography of the obtained salt showed their higher purity of the named salt ammonium magnesium chloride hexahydrate (NH₄MgCl₃.6H₂O).

On the average, per each liter of treated bittern solution 153 g containing 16.337 g of Mg ion was obtained. However this salt is non water soluble, it is advised to re-acid soluble to make it more soluble and to take the high initial chloride away. Thus, advanced studies in this action should be considered. The main chemical and physical properties of salt are colorless with chemical impurities 0.005% and M.Wt: 256.79. The base employed here is to feed bittern solution by extra amount of ammonia to raise the pH value which in turn allows the precipitation, however it was in contrast with the hypothesis of Avakyan and Rabotnova. 1971 & Babich and Stotzky 1980. They claimed that precipitations can be avoided when high concentrations of metals and alkaline pH are present.

3.2. Remained water quality

Analysis of the remained water showed the non complete demineralization of Mg salts away from the treated sample. The rise of pH value from 6.51 of the initial to 9.54 after precipitation was return to the presence of extra ammonium content. On the other hand, electric conductivity (E.C) was severely downed from 16.25 to 11.47 dS.m^{-1} . Chloride was approximately disappeared, where the remained water was found to contain less than 1.0g.l^{-1} (825 ppm) as compared with the initial (334.695g.l^{-1}).

3.3. Complementary demineralization

To maximize the utilization efficiency of Mg ion and other related ions, the green alga *Scenedesmus* sp. was incubated as mentioned before under different concentrations of the remained water. Growth profile of this alga could be monitored and summarized as follows:

3.3.1. Dry weight

Dry weight of *Scenedesmus* sp. was severely differed due to the given level of remained water. In general, dry weight was markedly increased as alga was incubated at lower and moderate levels of remained water, but slight increases were observed with other over remained water levels. The lower growth observed under control treatment could be attributed to the competition effect due to the high initial biomass at zero time. The enhancement effect could be attributed to the fast penetration of nutrients into algal cells which in turn fasting the growth rate. The growth decline under higher levels might ascribe to the salting effect and high pH value of initial remained water. As shown in Fig. 2, miserable variation between growth rate (on the average and maximum) was observed.

On the average, growth rate was found higher with the second treatment (T2) as culture received 50ml.l^{-1}

of the remained water; however the lowest maximum growth rate was also observed under this treatment.

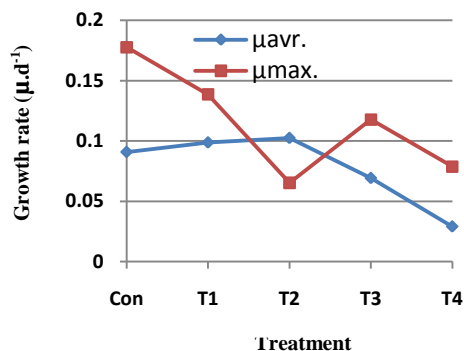


Fig. 2. Maximum (μ_{max}) and average (μ_{avr}) growth rate of *Scenedesmus* sp. dry weight under different levels of remained water. Con = control; T1=25; T2=50; T3=75 and T4=100 ml.l^{-1} of remained water after Mg solidification and isolation.

The variation might be attributed to the most suitability of media chemical composition and high potential adaptation of the used alga against such treatment. Cultures or treatments that represented high maximum growth rate mostly represented low average growth rate due to the high initial biomass at zero time with low next growth. Thus 50ml.l^{-1} of Remained water could be serving as the most appropriate treatment to meet the high growth.

3.3.2. Total chlorophyll

Unlike dry weight, total chlorophyll was more affected by remained water. Except for the lower remained water level used (25ml.l^{-1}) that exhibited no inhibitory effect on chlorophyll accumulation. Other remained water levels ($50, 75$ and 100ml.l^{-1}) resulted in different chlorophyll decomposition degree. The rate of chlorophyll decomposition was found in association with the supplementary remained water level. The effect of high nutrients concentration (36.7) and high pH value (9.61) at the hyper remained water level (100ml.l^{-1}) might be accelerating the rate of chlorophyll decomposition. Growth rates ;on the average; were most affected rather than their maximum. The decline on the average growth rate was associated with the level supplied of remained water and claimed the inhibitory effect of initial salts on the accumulation of chlorophyll, however extra Mg concentration was found. On the contrary, maximum growth rates were slightly varied due to the time that exposes their value. Most of these values were exhibited at the early time of incubation due to the aforementioned reason as media became non-suitable due to high initial salts.

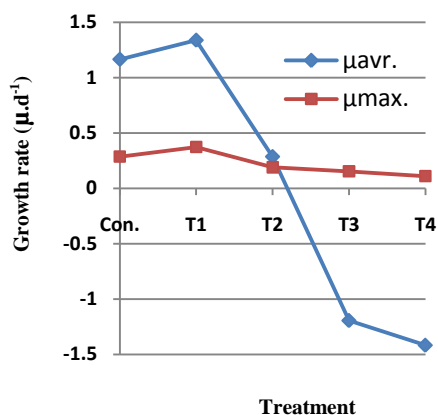


Fig. 3. Maximum ($\mu_{max.}$) and average ($\mu_{avr.}$) growth rate of *Scenedesmus* sp. total chlorophyll under different levels of remained water. Con = control; T1=25; T2=50; T3=75 and T4=100ml.l⁻¹ of remained water after Mg solidification and isolation.

3.3.2. Carotenoids

The completely opposite trend as compared with both dry weight and total chlorophyll accumulation was observed in the case of carotenoids formation. Comparing the data obtained from dry weight and total chlorophyll showing the completely differed profile on both maximum and average growth rates.

Under all remained water levels growth rate as a maximum or on the average was increased except the first treatment (T1) that received only 25 ml.l⁻¹ of remained water. The increases on growth rate determined as carotenoids explain the effect of initial salts received from the remained water on carotenoids accumulation as well as dry weight decline and chlorophyll decomposition.

In general, algal growth profile determined as any growth parameters even as cell growth metabolites are very growth conditions independent mainly nutritional status. Based on this hypothesis, algal growth for mass production purposes even for wastewaters treatments must be performed within defined and optimal nutritional status. Shifting of such optimal nutritional requirements led to a marked disturbance in growth metabolites. In the current case, the remained water after Mg solidification was found in higher concentration as compared with any fresh water medium, however it contains extra content of some major nutrients mainly carbon and magnesium. The excess of nutrients (react as salinity effect) led to the marked decline of growth dry weight and chlorophyll decomposition. On the other hand, a massive accumulation of primary metabolites including lipids as well as secondary metabolites including carotenoids took place. In this context, a lot of researches were done to meet the dual objectives. The first is to grow algae under stress conditions to accumulate such

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products. While the second is to use the high algal potential to growth under such conditions in industrial wastes utilization.

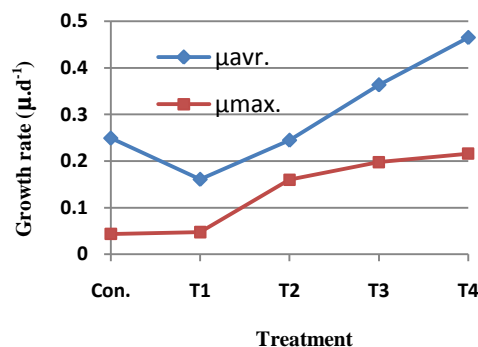


Fig. 4. Maximum ($\mu_{max.}$) and average ($\mu_{avr.}$) growth rate of *Scenedesmus* sp. total carotenoids under different levels of remained water. Con = control; T1=25; T2=50; T3=75 and T4=100ml.l⁻¹ of remained water after Mg solidification.

4. Conclusion

For wastewater treatment, the complementary demineralization is very advised and seems to be the more appropriate method to meet the fine absorbing of trace nutrients. However the current study not completely discarded the initial salts, a useful obtained mass was obtained by the moderate level of waste (50 ml.l⁻¹) of remained water and also more solidified effort should be done to obtain the maximum content of Mg ion.

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References

- Avakyan, Z. A., and I. L. Rabotnova. 1971. Comparative toxicity of free ions and complexes of copper with organic acids for *Candida utilis*. *Microbiology* 40:262-266.
- Avron, M. and Ben-Amotz, A. (1992). *Dunaliella*: Physiology, Biochemistry and Biotechnology. CRC Press, Boca Raton.
- Babich, H. and G. Stotzky. (1980). Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. *Crit. Rev. Microbiol.* 8:99-145.
- Chevalier, P. and De la Nou'e, J. (1985). Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme Microb. Technol.*, 7, 621-624
- Ciferri, O. (1983). *Spirulina*, the edible microorganism. *Microbial Rev.*, 47, 551-578.

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El-Sayed, A. B. (2004a). Circulation of Quaron Lake wastes. II-Growth of *Scenedesmus* sp. under Mg residences. Egypt. J. Biotechnol. 17, 477-485.

El-Sayed, A. B. (2004b). Screening and growth characterization of the green life stock of drill water from Jeddah, Saudi Arabia. I-Isolation and growth characterization of *Scenedesmus* sp. N. Egypt. J. Microbiol.; 8,376-385.

El-Sayed, A. B. (2005). Screening and growth characterizations of the green life stock of drill water from Jeddah. III- Enhancement of secondary carotenoids by oxidative stress in relation to medium composition. N. Egypt. J. Microbial.; 10, 232-241.

El-Sayed, A.B. (1999). Some physiological studies on green algae. *Ph.D. Thesis*, Faculty of Agric., Department of Agriculture Botany, Cairo University.

Florencio, F. J. and Vega, J. M. (1983). Utilization of nitrate, nitrite and ammonium by *Chlamydomonas reinhardtii*. *Planta*,158,288-293

Hall, D.O. and Rao, K.K. (1989). Immobilized photosynthetic membranes and cells for the production of fuels and chemicals. *Chimicaoggi*, 3,41-47.

Pirt, S.J. (Ed.) (1973). *Principle of Microbe and Cell Cultivation*. Blackwell ScientificPublication, pp: 4-7.

Vega, J.M.; Menacho, A. and Leo'n, J. (1991). Nitrate assimilation by microalgae. *Trends Photochem. Photobiol.*, 2, 69-111.

Vflchez, C. and Vega, J.M. (1995). Nitrate uptake by immobilized *Chlamydomonas reinhardtii* cells growing in airleft reactors. *Enzyme Microb. Technol.* 17, 386-390.

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