Egyptian J. of Phycol. Vol. 4(2), 2003

ROLE OF VITAMINS IN AMELIORATING THE BIOLOGICAL WASTEWATER TREATMENT BY ALGAE

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Abstract

This study evaluated the effect of different concentrations of vitamins, B_{12} and C in the process of wastewater treatment by the blue – green algae *Synechocystis aquatilis* and *Chroococcus schizodermaticus*. *C. schizodermaticus* was more efficient than *S. aquatilis* in removing phosphorus by using both vitamins at all concentrations, while maximum nitrate removal was attained after 20 days using vitamin C at concentration 25 mg/l in case of C. *schizodermaticus* reactor. Vitamin B_{12} caused the accumulation of nitrate in both algal reactors after 6 days at concentration 50 mg/l, while that accumulation began after 8 days with the use of concentration 75 mg/l. Concentration 25 mg/l of vitamin B_{12} caused accumulation after 6 days in C. *schizodermaticus* reactor and after 12 days at *S. aquatilis* reactor. Ammonia was completely disappeared from wastewater treated by C. *schizodermaticus* using vitamin B_{12} (concentration 75 mg/l) and vitamin C at its three concentrations in C. *schizodermaticus* reactors and only at concentration 25 mg/l in case of *S. aquatilis* reactor.

Key words: Wastewater treatment, vitamins, *Synechocystis aquatilis*, *Chroococcus schizodermaticus*.

Introduction

Microalgae have a vast industrial and economic potential as valuable sources for pharmaceuticals, health food, carotenoids, dyes, fine chemicals, biofuels and other applications (Faulkner 1986). The history of the commercial use of algal cultures spans about 50 years resulting in various applications as valuable sources for pharmaceuticals, health foods, carotenoids, dyes, fine chemicals and biofuels (Radmer 1996, and Borowitzka, 1999). Furthermore, they may serve as a solution to emerging environmental problems such as greenhouse effect and waste treatments (Nagase *et al.*, 1998 and Hirata 1996) because they can fix carbon dioxide by photosynthesis and remove excess nutrients efficiently at a minimal cost (Murakami and Lkenouchi 1997, Akimoto *et al.*, 1998 and Hirano *et al.*, 1997).

Many wastewaters are lacking in the nutrients required for microbial growth. A number of nutrients are essential for metabolic activity. A wide range of micronutrients including vitamins are required by activated sludge (Lind *et al.*, 1994, Lemmer *et al.*, 1998). Sufficient micro-nutrition is needed to support all the genera required for activated sludge to treat wastewater.

(ISSN: 1110-8649)

Many factors affect the process of wastewater treatment like organic nitrogen sources (Lau *et al.*, 1994), micro-nutrients (Burgess *et al.*, 1999), light (Lee & Lee 2001), vitamins (He and Hader, 2002) and aeration (Lee & Lee 2002).

Vitamin supplementation is advertised in wastewater treatment to compensate for a deficiency of growth factors and thereby increase sludge activity and purification efficiency. Addittion of vitamins of the B- complex was tested with activated sludge by Lemmer *et al.* (1998). This work was performed to throw light on the role of vitamin B_{12} and vitamin C on the process of wastewater treatment by two cyanobacterial species.

Material and Methods

Cyanobacterial growth and culture conditions

Two cyanobacterial taxa were used in this study, *Synechocystis aquatilis* Sauvageau and *Chroococcus schizodermaticus* Nageli. They were obtained from Trepon Culture Collection at Czech Republic. Both algae were inoculated in 500 ml culture flasks in BGII medium (Vonshak, 1986). The cultures were incubated at 35 ºC and continuous light intensity of 3000 Lux.

Wastewater treatment

Primary treatment carried out through the preliminary sieving step at station to get-rid of the large suspended solids. Wastewater sample was supplied from Wastewater Treatment Station at Abees district, Alexandria City, Egypt.

Sampling and analyses

Water samples were analyzed for NH^{+}_{4}-N and NO^{-}_{3}-N following the methods described by Markus *et al*. (1982), while PO^{3-}_{4}-P was analysed according to the method of APHA (1985). The recorded results represent the mean values of three replicates of determinations**.**

Statistical analysis:

Data obtained in the present investigation were statistically analyzed using the Least Significant Difference test (LSD) at 1% and 5% levels of probability (Snedecor and Cochran, 1967).

Results

Table 1 shows the effect of different concentrations of vitamin B_{12} on the process of removing ammonium-nitrogen from wastewater. It is clear that concentration 75 mg/l gave the maximum ammonium-nitrogen removal (100%) by *Chroococcus schizodermaticus* after only 18 days. *Synechocystis aquatilis* caused 97.75% ammonium-nitrogen removing after 20 days.

Statistically, no significant difference between the two algal reactors in ammonium-nitrogen removal by the three concentrations of vitamin B_{12}, while high significant difference between each of the algal reactor and the control reactor in case of concentrations 50 and 75 mg/l. Concentration 25 mg/l gave significant difference between *C. schizodermaticus* and the control reactor and unsignificant difference between *S. aquatilis* and the control reactor.

The data in table 2 shows that nitrate-nitrogen concentration increased by addition of vitamin B_{12} at the three used concentrations and concentration 25 mg/l caused the highest increasing of nitrate concentration in *S. aquatilis* reactor, while concentration 75 mg/l caused the highest nitrate concentration in *C. schizodermaticus* reactor after 20 days.

No significant difference in nitrate-nitrogen removal between the two algal reactors by using the three concentrations of vitamin B_{12}, while significant difference appeared only between *C. schizodermaticus* and the control reactor in case of using concentration 25 mg/l (L.S.D. at 1% and 5% levels were 50.7 and 37.2, respectively,). High significant difference was also present between the control and each of the algal reactors in case of concentration 50 and 75 mg/l of vitamin B_{12}.

Results in table 3 show the high efficiency of both algae in removing phosphorus by addition of vitamin B_{12}. Concentration 25 mg/l caused maximum removing of phosphorus (99.13%) by *C. schizodermaticus*, while concentration 75 mg/l caused maximum phosphorus removing (97.63%) by *C. schizodermaticus* after 20 days.

In the three concentrations of vitamin B_{12}, the difference in phosphorus removal between the three reactors is not significant (L.S.D. was 3.2 and 2.4 at 1% and at 5% levels respectively, by using concentration 25 mg/l. Concentration 50 mg/l showed L.S.D. 2.9 and 2.2 at 1% and 5% levels, respectively, while concentration 75 mg/l showed L.S.D. 3.1 and 2.3 at 1 and 5% levels, respectively.

Data in table 4 show the effect of different concentrations of vitamin C on the ammonium-nitrogen removal from wastewater. Concentration 25 mg/l of vitamin C caused the highest removal of ammonium- nitrogen in both algal reactors (100%) after 12 days in case of *C. schizodermaticus* reactor and after 16 days in case of *S. aquatilis* reactor, while concentration 50% caused that highest percentage of removing only in case of *C. schizodermaticus* reactor after 16 days. 100% ammonium- nitrogen removal appeared at *C. schizodermaticus* reactor after 18 days by using 75 mg/l of vitamin C. Concentration 25 mg/l of vitamin C is considered the best one for the complete removing of ammonium- nitrogen by *C. schizodermaticus* after 12 day, while concentration 50 and 75 mg/l of vitamin B_{12} are the best for ammonium- nitrogen removal by *S. aquatilis* and caused a removal percentage of 97.75% after 20 days.

No significant difference between the three reactors in ammonium-nitrogen removing in the three used concentration of vitamin C. By using the concentration 25 mg/l of vitamin C, L.S.D. at 1% and 5% levels were 7.3 and 5.4, respectively. At concentration 50 mg/l, L.S.D. at 1% level was 6.5 and at 5% levels was 4.8 while at concentration 75 mg/l, L.S.D. at 1% level was 6.8 and at 5% level was 5.0.

Table (1): Ammonium-nitrogen (NH_4^+) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin B12.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	NH_4^+ Conc.	Removal %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %
0	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00
2	14.17	3.41	13.33	9.13	14.17	3. 41	13.33	9.13	9.67	34.08	9.17	37.49	11.83	19.36	8.50	42.06	10.83	26.18
4	13.50	7.98	12.83	12.54	13.83	5.73	12.50	14.79	6.00	59.10	5.83	60.26	10.67	27.27	5.00	65.92	7.50	48.88
6	13.17	10.22	12.50	14.79	13.83	5.73	11.83	19.36	5.67	61.35	4.50	69.33	9.33	36.40	4.83	67.08	5.33	63.67
8	32.17	-	11.67	20.45	13.00	11.38	12.67	13.63	4.17	71.57	3.00	79.55	10.17	30.67	4.50	69.33	4.17	71.57
10	32.17	-	10.00	31.83	12.67	13.63	14.67	0.00	2.50	82.96	2.67	81.80	13.50	7.98	3.50	76.14	2.67	81.80
12	41.67	-	8.00	45.47	12.50	14.79	23.33	-	2.17	85.21	2.17	85.21	27.67	-	2.17	85.21	2.33	84.12
14	10.83	26.18	6.67	54.53	10.00	31.83	6.33	56.85	2.17	85.21	1.00	93.18	4.67	68.17	2.00	86.37	1.17	92.02
16	9.67	34.08	5.33	63.67	8.67	40.90	3.83	73.89	1.50	89.78	0.83	94.34	2.50	82.96	1.67	88.62	0.33	97.75
18	8.67	40.90	4.17	71.57	7.83	46.63	2.67	81.80	1.00	93.18	0.50	96.59	1.33	90.93	1.50	89.78	0.00	100.00
20	8.00	45.47	4.02	72.60	6.67	54.53	2.00	86.37	0.33	97.75	0.33	97.75	1.00	93.18	0.67	95.43	0.00	100.00

Table (2): Nitrate-nitrogen (NO_3^-) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin B12.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	NO_3^- Conc.	Removal %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %
0	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0
2	4.36	3.11	4.40	2.22	4.22	6.22	3.99	11.33	4.27	5.11	4.33	3.78	4.16	7.56	4.21	6.44	3.83	14.89
4	4.06	9.78	4.27	5.11	4.05	10.00	3.85	14.44	3.79	15.78	4.21	6.44	3.79	15.78	4.00	11.11	3.16	29.78
6	3.99	11.33	6.06	-	5.74	-	4.21	6.44	4.69	-	4.21	6.44	4.63	-	4.57	-	4.17	7.33
8	3.99	11.33	6.82	-	15.20	-	4.37	2.89	6.20	-	8.08	-	4.92	-	5.52	-	4.95	-
10	3.84	14.67	16.42	-	25.20	-	4.45	1.11	6.95	-	7.52	-	5.79	-	6.00	-	6.51	-
12	28.26	-	38.74	-	39.45	-	9.66	-	11.53	-	25.35	-	20.78	-	7.14	-	12.06	-
14	79.39	-	33.16	-	40.11	-	22.26	-	24.40	-	17.12	-	18.32	-	20.61	-	11.47	-
16	100.00	-	36.29	-	37.75	-	48.84	-	29.22	-	27.43	-	13.21	-	18.42	-	14.27	-
18	129.58	-	39.75	-	36.80	-	65.47	-	13.09	-	31.20	-	9.26	-	17.22	-	12.21	-
20	191.58		46.75	-	31.68	-	90.52	-	8.94	-	36.84	-	7.89	-	11.73	-	23.68	-

Table (3): Phosphorus (P) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin B12.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)						Vitamin B_{12} conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	P Conc.	Removal %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %
0	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
2	7.45	6.88	6.97	12.88	6.89	13.88	5.15	35.61	4.27	46.63	4.79	40.13	6.09	23.88	3.76	53.04	4.34	45.75
4	7.26	9.26	5.64	29.45	6.43	19.63	4.59	42.63	2.01	74.88	1.82	77.26	5.05	36.89	1.58	80.24	1.94	75.75
6	6.99	12.66	1.58	80.24	6.12	23.50	4.10	48.75	1.94	75.75	1.07	86.63	4.59	42.63	0.98	87.75	1.38	82.75
8	6.07	24.14	1.53	80.88	5.97	25.38	3.45	56.88	1.16	85.50	0.61	92.38	2.99	62.63	0.97	87.89	1.00	87.50
10	5.05	36.89	1.16	85.50	1.73	78.33	1.05	86.88	0.97	78.89	0.60	92.50	1.97	75.35	0.92	88.50	0.70	91.25
12	3.37	57.88	1.14	85.76	1.16	85.50	0.70	91.29	0.83	89.63	0.49	93.88	0.49	93.88	0.70	91.25	0.53	93.38
14	1.94	75.75	1.12	85.98	1.07	86.63	0.54	93.25	0.73	90.88	0.44	94.50	0.37	95.38	0.54	93.25	0.43	94.63
16	1.89	76.38	1.11	86.13	0.99	87.63	0.54	93.25	0.70	91.25	0.34	95.75	0.19	97.63	0.52	93.50	0.41	94.88
18	1.12	86.00	0.73	90.88	0.66	91.75	0.50	93.75	0.44	94.37	0.27	96.63	0.09	98.88	0.24	97.00	0.34	95.75
20	1.00	87.50	0.65	91.88	0.58	92.75	0.44	94.50	0.27	96.63	0.26	96.75	0.07	99.13	0.14	98.25	0.19	97.63

Table (4): Ammonium-nitrogen (NH_4^+) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin C.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	NH_4^+ Conc.	Removal %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %	NH_4^+ Conc.	Reomval %
0	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00	14.67	0.00
2	14.50	1.16	14.17	3.41	14.50	1.16	13.33	9.13	12.50	14.79	13.33	9.13	14.17	3.41	11.67	20.45	14.17	3.41
4	13.67	6.82	13.83	5.73	12.83	12.54	12.50	14.79	9.00	38.65	11.83	19.36	13.83	5.73	11.17	23.86	13.33	9.13
6	10.17	30.68	8.17	44.31	12.33	15.95	5.33	63.67	7.17	51.12	7.50	48.88	9.00	38.65	10.00	31.83	9.83	32.99
8	2.50	82.96	6.50	55.69	2.00	86.37	3.00	79.55	3.17	78.39	5.83	60.26	1.50	89.78	2.33	84.12	4.00	72.73
10	2.50	82.96	2.00	86.37	1.83	87.53	2.17	85.21	2.33	84.12	5.67	61.35	1.50	89.78	2.33	84.12	3.33	77.30
12	1.50	89.78	1.67	88.62	1.50	89.78	1.67	88.62	1.67	88.62	3.83	73.89	0.00	100.00	2.17	85.21	3.33	77.30
14	1.50	89.78	1.50	89.78	1.35	90.80	0.83	94.34	1.67	88.62	2.67	81.80	0.00	100.00	2.00	86.37	1.83	87.53
16	1.50	89.78	1.47	89.98	1.17	92.03	0.00	100.00	1.50	89.78	2.00	86.37	0.00	100.00	0.00	100.00	1.67	88.62
18	1.17	92.02	1.00	93.18	1.00	93.18	0.00	100.00	1.50	89.78	1.67	88.62	0.00	100.00	0.00	100.00	0.00	100.00
20	1.10	92.50	1.00	93.18	1.00	93.18	0.00	100.00	1.20	91.82	1.50	89.78	0.00	100.00	0.00	100.00	0.00	100.00

Table 5 shows that vitamin C at its three concentrations caused no removing of nitrate- nitrogen from wastewater in both algal reactors and nitrate concentration increased.

The difference between the two algal reactors in nitrate-nitrogen removal is not significant by using the concentration 50 and 75 mg/l of vitamin C but was significant at concentration 25 mg/l. The reactors of *C. schizodermaticus* showed high significant difference with the control reactor at the three concentrations, while *S. aquatilis* reactor showed not significant difference compared to the reactor by using the concentrations 25 and 50 mg/l and showed high significant difference with control at concentration 75 mg/l.

Table 6 proves that concentration 25 mg/l of vitamin C was the most suitable concentration for removing phosphorus by *C. schizodermaticus*, while concentration 75 mg/l caused maximum removal of phosphorus by *S. aquatilis* after 20 days. It must be mentioned that vitamin B_{12} is more effective for the process of removing phosphorus by both algae than vitamin C, while vitamin C concentration 25 mg/l caused the highest ammonium removal by both algae after 12 days in case of *C.schizodermaticus* and after 16 days in case of *S. aquatilis*.

High significant difference appeared between each of the algal reactor and the control but no significant difference between the two algal reactors in phosphorus removal by addition of the three concentrations of vitamin C. At concentration 25 mg/l of vitamin C, L.S.D. at 1% and 5% levels were 2.5 and 1.8, respectively, while they were 2.3 and 1.7 respectively, at concentration 50 mg/l. Concentration 75 mg/l of vitamin C, L.S.D. at 1% level was 1.8 and at 5% level was 2.4.

Discussion

Concerning the fate of vitamins inside the cells of both algae, vitamin B_{12} was proved to be converted to coenzymes form of B_{12} in *Chlamydowonas reinhardti*i (Watanabe *et al.,* 1991) and *Pleurochrysis carterae* (Miyamoto *et al.,* 2002).

The increase of activity of both algae in uptaking ammonium nitrogen and phosphorus may return to the increase in the growth of both algae. Addition of vitamins to the algal cultures was proved to increase growth significantly (Zhang *et al.,* 1997).

Optical density of the alga *Chattonella antique* showed a significant increase by addition of vitamin B_2 to the cultured medium. The increase seemed to be due to an accumulation of reduced ferrodoxin caused by the inhibition of ferrodoxin NADP reductase by the vitamin (Furuki and Tachibana, 1986). Ascorbic acid was found by El-Naggar and El- Sheekh (1998) to accelerate the growth and chlorophyll content of *Chlorella vulgaris* and the enhancement is a dose dependent.

Table (5): nitrate-nitrogen (NO_3^-) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin C.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	NO_3^- Conc.	Removal %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %	NO_3^- Conc.	Reomval %
0	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00
2	4.11	8.67	4.50	0.00	4.42	1.78	3.47	22.89	3.16	29.78	4.21	6.44	3.68	18.22	4.50	0.00	3.97	11.78
4	2.63	41.56	3.08	31.56	4.21	6.44	2.43	46.00	2.95	34.44	3.25	27.78	3.46	23.11	4.11	8.67	3.89	13.56
6	4.79	-	6.79	-	4.92	-	2.33	48.22	2.69	40.22	3.07	31.78	3.16	29.79	3.40	24.44	3.89	13.56
8	13.21	-	8.95	-	14.89	-	5.16	-	3.82	15.11	2.87	36.22	2.74	39.11	4.42	1.78	3.68	18.22
10	17.11	-	13.21	-	19.84	-	6.95	-	8.42	-	7.26	-	3.60	20.00	4.42	1.78	3.26	27.56
12	19.05	-	17.32	-	20.53	-	7.58	-	9.63	-	6.32	-	4.20	6.67	4.40	2.22	3.16	29.78
14	19.79	-	25.79	-	21.04	-	10.53	-	9.58	-	9.47	-	4.84	-	4.40	2.22	3.16	29.78
16	19.16	-	26.02	-	20.00	-	15.79	-	15.47	-	9.89	-	7.68	-	4.20	6.67	3.05	32.22
18	18.95	-	26.32	-	18.95	-	19.39	-	19.16	-	11.69	-	3.16	29.78	4.10	8.89	2.95	34.44
20	18.95	-	18.84	-	18.53	-	19.79	-	19.47	-	15.89	-	1.63	63.78	3.89	13.56	2.87	36.22

Table (6): Phosphorus (P) concentration (mg/l) and removal efficiency in wastewater during 20 days treatment in control, *Synechocytis aquatilis* and Chroococcus *Chroococcus schizodermaticus* reactors using 25, 50 and 75mg/l of vitamin C.

Time (days)	Wastewater (Control)						*Synechocytis aquatilis*						*Chroococcus schizodermaticus*					
	Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)						Vitamin C conc. (mg/l)					
	25		50		75		25		50		75		25		50		75	
	P Conc.	Removal %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %	P Conc.	Reomval %
0	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
2	6.89	13.88	7.16	10.50	7.48	6.50	5.32	33.49	5.24	34.50	5.20	35.00	4.17	47.88	2.75	65.36	2.14	73.25
4	6.55	18.13	6.89	13.88	7.12	11.00	5.25	34.38	5.13	35.88	4.06	49.25	2.84	64.50	2.23	72.13	1.85	76.88
6	6.55	18.13	6.82	14.75	6.68	16.50	1.90	76.25	3.84	52.00	1.34	83.25	2.35	70.63	2.01	74.88	1.34	83.25
8	6.00	25.00	6.55	18.13	6.38	20.25	0.83	89.63	2.64	67.00	1.11	86.13	2.07	74.13	1.38	82.75	1.09	86.38
10	5.87	26.63	6.27	21.63	6.27	21.63	0.54	93.25	1.09	86.38	0.92	88.50	1.85	76.84	1.05	86.88	0.90	88.75
12	5.80	27.50	6.21	22.38	5.87	26.63	0.34	95.75	1.04	87.00	0.90	88.75	1.45	81.88	0.97	87.88	0.83	89.63
14	5.51	31.13	6.10	23.75	5.58	30.25	0.97	87.89	0.97	87.88	0.87	89.13	0.95	88.13	0.92	88.50	0.77	90.38
16	5.24	34.50	5.95	25.63	5.30	33.75	0.97	87.89	0.95	88.13	0.83	89.63	0.70	91.25	0.63	92.13	0.73	90.88
18	4.95	38.13	5.64	29.50	4.69	41.38	0.94	88.25	0.94	88.25	0.83	89.63	0.58	92.75	0.41	94.88	0.70	91.25
20	4.83	39.63	5.51	31.13	4.34	45.75	0.90	88.75	0.94	88.25	0.77	90.38	0.17	97.88	0.31	96.13	0.63	92.13

Increasing the organic carbon (like vitamins) content in the culture accelerates the growth and metabolic processes of phytoplankton by reducing the cellular uptake of heavy metals (Sudhakar *et al.*, 1991).

The high activity for nutrients removal by both algae may return to reverse the harmful effect of wastewater. Gowrinathan and Rao (1992) attributed the reversal of heavy metal toxicity by ascorbic acid in diatoms to two possible mechanisms 1- ascorbic acid may act as a reducing agent, protecting the oxidation of the SH group by photosystem 11 or 2- ascorbic acid may bind metals thereby affecting their movement across biological membranes. Rai and Raizada (1988) proved that *Nostoc muscorum* could be protected from heavy metal toxicity by supplementing ascorbic acid. They postulated that ascorbic acid can protect against metal toxicity possibly by providing a reducing power which protects the enzymes from undergoing oxidation.

The presence of vitamin C was found to protect the algae from chlorophyll bleaching and damage of photosynthetic apparatus (He & Hader, 2002). Ascorbic acid can reverse mercury, chromium and lead toxicities in algal cells (Gowrinathan & Rao, 1992 and El-Naggar & El-Sheekh, 1998).

The high removal percentage in presence of vitamin C could be attributed to the action of vitamin C as antioxidant in plants and its pivotal role in the destruction of reactive oxygen species (Foyer & Ferrario, 1994, Padh, 1990, Liebler *et al.*, 1986 and He & Hader 2002).

Vitamins were proved by Burgess *et al.* (1999) to activate the process of wastewater treatment.

Nitrate was successfully reduced by *Chlorella kessleri* to below 2 mg from the initial nitrate concentration of 140 mg in 10 days (Lee & Lee, 2002). This disagreed with our results which showed that nitrate accumulated in the reactors.

Presence of large amounts of ammonium produced protons, reducing the pH. The low pH condition must have had a harmful effect on algal growth and thus nitrogen consumption (Lee & Lee, 2002). Syrett (1981) found that in presence of active nitrate reductase and NO^{-}_{3} uptake system, the addition of NH_4^+could lead to a rapid cessation of NO^{-}_{3} utilization. Vymazal (1995) pointed out that active nitrate reductase is not formed in the presence of NH^{+}_{4} nor in the NO_3^- uptake system.

The above mentioned facts could explain why in the *S. aquatilis* and *C. schizodermaticus* reactors, nitrate persisted and even accumulated in wastewater.

Ammonia oxidizing bacteria (AOB) cause the nitrification process which may be the cause of increase of nitrate concentration. AOB were found within and between different reactors of treating wastewater. They were most closely related to *Nitrosococcus mobilis* and *Nitrosomonas halophila* (Rowan *et al.*, 2003).

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دور الفيتامينات فى تحسين المعالجة البيولوجية لمياه المجارى بواسطة الطحالب.

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أوضحت هذه الدراسة تأثير بعض التركيزات لفيتامين ب$_{12}$ وفيتامين سى فى عملية معالجة مياه المجارى بواسطة طحلبين من الطحالب الخضراء المزرقة هما سينيكوسيستس أكواتيلز وكروكوكس شيزوديرماتيكس وذلك للتخلص من الفسفور والنيتروجين فى صورة الأمونيا والنترات.

بالنسبة للقدرة على نزع الأمونيا فلقد أختفت الأمونيا نهائياً من مياه المجارى المعالجة بطحلب الكروكوكس شيزوديرماتيكس باستخدام تركيز 75 ملى جرام/لتر من فيتامين ب$_{12}$ لمدة 18 يوماً وباستخدام الثلاثة تركيزات من فيتامين سى فى حالة المعالجة بطحلب كروكوكس شيزوديرماتيكس وفقط باستخدام تركيز 25 ملى جرام /لتر فى حالة المعالجة بطحلب سينيكوسيستس أكواتيلز لمدة 16 يوماً.

ولقد حصل على أعلى نسبة نزع للنترات بعد 20 يوماً باستخدام طحلب كروكوكس شيزوديرماتيكس بإضافة فيتامين سى بتركيز 25ملى جرام/لتر بينما سبب إضافة فيتامين ب$_{12}$ تراكم النترات باستخدام كلا الطحلبين بعد ستة أيام من بدء المعالجة فى حالة استخدام تركيز 50 ملى جرام/لتر وبعد ثمانية أيام باستخدام تركيز 75 ملى جرام/لتر أما تركيز 25 ملى جرام/لتر من فيتامين ب$_{12}$ فلقد سبب هذا التراكم للنترات بعد ستة أيام فى حالة كروكوكس شيزوديرماتيكس وبعد 12 يوماً فى حالة استخدام طحلب سينيكوسيستس أكواتيلز.

ولقد أثبتت الدراسة أن طحلب كروكوكس شيزوديرماتيكس كان أكثر كفاءة من الطحلب سينيكوسيستس أكواتيلز فى نزع الفسفور وذلك باستخدام كلا الفيتامينين بالثلاثة تركيزات المستخدمة من كلٍ منهما (25، 50، 75 ملى جرام/لتر).