Bioscience and Bioengineering
Vol. 1, No. 3, 2015, pp. 48-56
http://www.aiscience.org/journal/bio

Role of Cloacal Algae in the Treatment of Wastewater and Their Biotechnological Applications

Ahmed A. Issa[1, 2, *], Farag A. Shaieb[3], Reem M. Al-Sefat[3]

[1]Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia
[2]Botany Department, Faculty of Science, Assiut University, Assiut, Egypt
[3]Microbiology Department, Faculty of Life Sciences, Omar Almukhtar University, El-Beida, Libya

Abstract

This work aimed to cultivate of cloacal algae in wastewater for nutrient and heavy metals removal, resulting biomass can be used for bioactive compounds. *Scenedesmus*, *Ankistrodesmus* and *Oscillatoria* cultivated in Al-Garega wastewater reduced nitrogen to about 90% from the medium. At the same time, phosphorus was also reduced to 91 % form Al-Garega and /or Shahat wastewater types by *Scenedesmus*, *Ankistrodesmus* and *Lyngbya* . Also, *Nitzschia, Oscillatoria* and *Lyngbya* reduced TIC about 75% using Shahat wastewater. But ,in case of *Euglena* and *Oscillatoria* about 65% reduction using Al-Garega wastes . *Oscillatoria* and *Lyngbya* were grown in Al-Garega wastewater were reduced Cd to 88 %, Zn to 78% and Pb to 75% from the wastes. Using the same algae in Shahat waste water, the maximum Cd reduction (72%) was recorded by *Scenedesmus* and *Euglena,* while, 80 % reduction of Pb was recorded by *Lyngbya* and 87 % of Zn was observed by *Ankistrodesmus* . Both aqueous and ethanol extracts of most algae exhibited significant activity against *Bacillus cereus* and *Escherichia coli* ,while low zone of inhibition was observed against, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The inhibitory effect of algae on bacterial strains calculated by inhibition zone was in the following order : *O. rubscens* > *L .muscula* >Mixture > *N. Palea* > *E .gracils* > S. Acutus > *A .monoraphids.*

Keywords

Cloacal Algae, Nutrient Removal, Antibacterial

Received: April 29, 2015 / Accepted: May 24, 2015 / Published online: July 7, 2015

1. Background

Cloacal algae play a remarkable role in the treatment of municipal wastewater. This is universal and may be much more efficient in tropical regions where temperature is warm and sunlight is optimum. Algae has indispensable role in oxygen/carbon dioxide recycling with a net sequestration of CO_2. Algae have also a potential for nitrogen and phosphorous uptake into the cells; thus acting as fertilizers by mineral recycling. Moreover, algae also remove pathogens from the domestic wastewater (Lau *et al.*, 1994; Issa *et al* ,1995 , El-Enany and Issa,2000, Luz *et al.,* 2004, Muñioz and Guieysse, 2006, Sengar *et al*,2011).Therefore, algae exert multiple effects in cleansing sewage effluents. Oxidative algal ponds do not require any external source of oxygen as it produces its own oxygen simultaneously with consumption of CO_2 produced by bacteria, thus reducing the carbon load on environment. In oxidation ponds, algae have a symbiotic relationship with bacteria. They provide the necessary oxygen to aerobic bacteria to biodegrade the organic contaminants of wastewater while in turn they utilize carbon dioxide from bacteria and energy from the sunlight

* Corresponding author
Email address: aaissaeg@yahoo.com (A. A. Issa), aaissaeg@gmail.com (A. A. Issa), faragshaieb@yahoo.co.uk (F. A. Shaieb), salehbofarowa@ yahoo.com (R. M. Al-Sefat)

for the synthesis of carbohydrates .But algae do not limit their relation of interaction to a simple oxygen and carbon dioxide exchange (Luz *et al.,* 2004; Muñioz and Guieysse, 2006; Shi *et al.,* 2007),algae has harmful effects on the activity of bacteria by increasing the dissolved oxygen, pH or even by the secretion of inhibitory metabolites. The latter impact is beneficial in killing anaerobic bacteria which are mostly pathogenic and/or organic and inorganic substances which were released into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution. The normal primary and secondary treatment processes of these wastewaters have been introduced in a growing number of places, in order to eliminate the easily settled materials and to oxidize the organic material present in wastewater. The final result is a clear, apparently clean effluent which is discharged into natural water bodies(Hammouda,*et.al.,*1994).This secondary effluent is, however, loaded with nitrogen and phosphorus and causes eutrophication and more long-term problems because of refractory organics and heavy metals that are discharged(Oliver and Ganf, 2000).Using algae for wastewater treatment offers some interesting advantages over conventional wastewater treatment. The advantages of algae-based treatment include: cost effective treatment, low energy requirement, reduction in sludge formation as well as production of algal biomass(Chu ,2012) . In some cases, algae also play a role in the removal of pathogens in the tertiary treatment stage. Several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity, which is basically an effect of assimilation of nutrients as the algae grow, but other nutrient stripping phenomena also occur e.g. ammonia volatilization and phosphorus precipitation as a result of the high pH induced by the algae (Karin ,2006) . In the current study we will highlight on the role of six micro-algae in the laboratory for nutrient and heavy metals removal of two wastewater plants (Al-Garegaa and Shahat) at EL-Bida ,Libya, resulting biomass can be used for bioactive compound extraction.

2. Results and Discussions

Wastewater is the spent or used water containing dissolved or suspended solids such as pollutants and microbes, including nutrients. The wastewater discharged into the water bodies are hazardous to environment and cause various health problems in human beings. Eutrophication is one such major environmental problem caused due to the discharge of nutrient rich wastewater into the nearby water bodies. Several methods, commonly chemical treatment and conventional biological methods are used to remove nutrients (phosphorus and nitrogen) from wastewater. However, high cost and more sludge production, are the major disadvantages limiting its use and hence researchers are now focusing on microalgae for nutrient removal from wastewater as it is less expensive and results in less sludge production. Table 1 shows the physico-chemical composition of the collected wastewater used as a cultivation medium, Total nitrogen (TN), Total phosphate (TP), Total inorganic carbon (TIC) , Total organic carbon (TOC) and heavy metals were analyzed for the influent and effluents. Their concentrations are found to be higher in the influent than the effluents.

Table (1). List of physico-chemical compositions of Al-Garegaa and Shahat Plants at EL-Bida Libya throughout the study period,(data are means of 3 replicates).

Items	Al-Gharegaa		Shahat	
	Influent	**Secondary effluent**	**Influent**	**Secondary effluent**
pH	7.9	7.7	7.9	7.5
Color	Brownish	Yellow	Black	Brownish
Total nitrogen (TN, mg/L)	17	15	18	22
Total phosphate (TP, mg/L)	3.5	2.2	3.5	3.6
Total inorganic carbon (TIC, mg/L)	6.9	5.4	9.7	6.9
Total organic carbon (TOC, mg/L)	4	2	5	7
BOD (mg/L)	540	380	620	430
Al (mg/L)	0.04	0.02	0.04	0.03
B (mg/L)	0.37	0.2	0.36	0.36
Ca (mg/L)	28.9	31.0	28.0	25.9
Cd (mg/L)	0.09	0.08	1.1	1.0
Pb (mg/L)	1.2	1.2	1.7	1.65
Zn (mg/L)	0.05	0.05	0.09	0.09
Cu (mg/L)	0.02	0.01	0.02	0.02
Fe (mg/L)	0.11	0.04	0.11	0.11
K (mg/L)	8.75	11.48	8.75	9.90
Mg (mg/L)	4	4.73	4	4.9
Na (mg/L)	25.69	23.06	22.69	20.9

A total of 13microalgal species were identified (Table 2), including 5 taxa of Chlorophyta, 4 of Cyanophyta, taxa of Bacillariophyta and two for Euglenophyta in Al-Garegaa and Shaat plants at EL-Bida , Libya throughout the summer season. The frequency of occurrence of algae differed between sites .The dominant algae which high frequency of occurrence were *Ankistrodesmus, Nitzschia* and *Euglena*, (100%) followed by *Scenedesmus* (83 %) , *Oscillatoria* and *Phacus* (66 %) .The algal taxa *Chlamydomonas ,Chlorella* , *Lyngbya* and *Navicula* were encountered as moderate frequency (50%),while the others were low and rare occurrence. In this respect, algae isolated from wastewater treatment plant sites can usually adapt to, and grow better in, culture conditions. In microalgae based wastewater treatment, several species were commonly isolated or used, which include members of the genera *Scenedesmus ,Chlamydomonas*, *Chlorella, Micractinium,* and *Actinastrum* (Park *et al.,* 2011). These microalgae are well known to increase biomass and lipid production when they are cultured under mixotrophic conditions . (Ogbonna *et al.*, 1998 and Wan *et a*l., 2011).

Table (2). List of algal taxa identified throughout the study period in Al-Agarega and Shahat Plants at EL-Bida Libya.

Algal taxa	Al-Garegaa Plant			Shaat Plant			Frequency %
	1	2	3	4	5	6	
Ankistrodesmus	+	+	+	+	+	+	100
Chlamydomonas	+		+		+		50
Chlorella	+	+	+				50
Scenedesmus	+	+	+	+	+		83
Ulothrix			+	+			33
Osillatoria	+	+	+	+			66
Phormidlum	+		+				33
Lyngbya	+	+		+			50
Spirullina	+		+				33
Navicula	+				+	+	50
Nitzschia	+	+	+	+	+	+	100
Euglena	+	+	+	+	+	+	100
Phacus	+	+			+	+	66

Microalgae represent an exceptionally diverse, but highly specialized, group of microorganisms adapted to various ecological habitats. The results show that the growth of microalgae was slightly higher in the wastewater and depends on species tested, *Scenedesmus acutus* was the best alga grown in wastewater medium for 10 days calculated on either O,D or chlorophyll-a or dry weight ,followed by *Ankistrodesmus monoraphids* , *Nitzchiapalea ,Euglena gracilas, Oscillato riarubscens and Lyngbya muscular* whatever the wastewater used. After 10 days *Scenedesmus acutus*, *Ankistrodesmus monoraphids* and *Nitzschia palea,* were flourished at Al-Garega plant and having algal biomass 29.9 ,23.2 and 21.7 mg dry weight / L , respectively. Also, in Shahat wastewater, the maximum algal biomass were recorded in *Nitzschia palea* (51.7 mg/L) *,Ankistrodesmus monoraphids* (43.2 mg/L) , *Scenedesmus acutus* (39.9 mg/L) (Table,3) .

Nitrogen is an important macro element in microalgae as a component of the biomass and it can range from 1% to more than 10% of the total mass, it is also a critical factor for regulating the algal cell lipid content (Chisti, 2008). The result of this study showed that the microalgae cultures were able to grow in both types of wastewater effluents. *Scenedesmus*, *Ankistrodesmus* and *Oscillatoria* cultivated in Al-Garega wastewater reduced nitrogen to about 90% from the medium. On the other hand, *Oscillatoria, Lyngbya, Euglena and Scenedesmus* grown in Shahat wastewater were highly reduced nitrogen than the other algae tested after 10 days (Table,4&5).

Table (3). Algal growth in wastewater of Al-Garega and Shahat plants for 10 days under controlled conditions,(data are means of 3 replicates).

Algae	Al-Garega plant						Shahat plant					
	Optical Density		Chlorophyll-a (mg/L)		Dry weight (mg/L)		Optical Density		Chlorophyll-a (mg/L)		Dry weight (mg/L)	
	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days	0 day	10 days
A.monoraphids	0.03	0.3	0.6	3.9	0.5	23.2	0.03	0.5	0.6	7.9	0.5	43.2
S. acutus	0.03	0.28	0.6	4.9	0.51	29.9	0.03	0.38	0.6	5.9	0.51	39.9
E.gracils	0.03	0.27	0.55	2.1	0.6	15.1	0.03	0.31	0.55	3.1	0.6	35.1
N. palea	0.03	0.4	0.3	2.9	0.6	21.7	0.03	0.49	0.3	3.9	0.6	51.7
O. rubscens	0.03	0.6	0.2	1.9	0.3	13.1	0.03	0.69	0.2	2.9	0.3	33.1
L muscula	0.03	0.36	0.2	2.1	0.3	14.0	0.03	0.64	0.2	2.3	0.3	24.0
Mixture	0.03	0.65	0.6	3.1	0.6	21.5	0.03	0.75	0.6	4.1	0.6	41.5

Table (4). Effect of algae addition to post treated wastewater after 10 days , on the contents of , phosphorus , nitrogen ,BOD and Total inorganic carbon (TIC) at Al-Garega station in El-Bida, Libya. ,(data are means of 3 replicates).

Algae	Phosphorus (mg/L)			Nitrogen (mg/L)			BOD (mg/L)			TIC (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction	0 Day	10 days	% reduction
A.monoraphids	0.12	0.01	91	1.1	0.1	90	600	210	65	6.9	3.3	5.2
S. acutus	0.12	0.01	91	1.1	0.11	90	458	180	60	6.9	3.6	47
E.gracils	0.12	0.02	83	1.1	0.2	81	560	120	78	6.9	2.1	69
N. palea	0.12	0.03	75	1.1	0.3	70	610	195	68	6.9	3.7	46
O. rubscens	0.12	0.04	66	1.1	0.1	90	480	110	77	6.9	2.3	66
L muscula	0.12	0.01	91	1.1	0.4	63	390	125	67	6.9	2.6	62
Mixture	0.12	0.01	91	1.1	0.09	91	590	110	78	6.9	1.9	72

Table (5). Effect of algae addition to post treated wastewater after 10 days , on the contents of, phosphorus , nitrogen ,BOD and Total inorganic carbon (TIC) at Shahat station in El-Bida, Libya,(data are means of 3 replicates).

Algae	Phosphorus (mg/L)			Nitrogen (mg/L)			BOD (mg/L)			TIC (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction
A.monoraphids	0.12	0.01	91	1.3	0.3	76	620	260	58	9.7	3.9	63
S. acutus	0.12	0.01	91	1.3	0.2	84	550	287	47	9.7	4.1	57
E.gracils	0.12	0.02	83	1.3	0.11	91	562	213	58	9.7	4.6	52
N. palea	0.12	0.03	75	1.3	0.3	76	475	189	60	9.7	2.3	76
O. rubscens	0.12	0.04	66	1.3	0.1	92	610	178	70	9.7	2.4	75
L muscula	0.12	0.01	91	1.3	0.12	90	560	120	78	9.7	2.6	73
Mixture	0.12	0.01	91	1.3	0.2	84	630	100	84	9.7	2.1	78

At the same time, phosphorus was also reduced to 91 % form Al-Garega and / or Shahat wastewater types by *Scenedesmus*, *Ankistrodesmus* and *Lyngbya* (Table,4&5). Being phosphorus an essential element for growth and other mechanisms including energy transfer and biosynthesis of DNAs (Richmond, 2004), its precipitation and assimilation into biomass and the intracellular polyphosphate dynamics have been proposed as mechanisms of P removal especially at high pH values (de Godos *et al*., 2010).The primary mechanism for the TN and TP removal is assumed to be a biomass uptake. Therefore, the influent, which shows a higher growth rate, has a high ratio of the removed TN and TP (Table 4&5). Since nitrogen (N) is an essential constituent of all structural and functional proteins in algal cells and phosphorus (P) is a major macronutrient that plays an important role in the cellular metabolic processes by forming many structural and functional components required for normal growth and development of microalgae, an increase in the algal biomass was found under N and P rich conditions (BBM) when the N concentration was 41.22 mg/ L and the P concentration was 53 mg/L. In wastewater conditions, a decrease in algal biomass was observed due to the low concentrations of N and P compared to the BBM. Secondary municipal wastewater supported rates of biomass productivity for 11 of the tested microalgal isolates between 21 and 33 mg/L/day with *Scenedesmus* sp. AMDD being the most productive (de-Godos *et al.,* 2010 and Park *et al.,* 2011)

A high BOD indicates the presence of excess amounts of organic carbon. Oxygen depletion is a consequence of adding wastes with high BOD values to aquatic ecosystems. The higher the BOD of a source of wastes the higher the polluting power of that waste .The results obtained in this study are summarized in Tabs. 4 and 5 show that a relatively high removal of BOD in Al-Garega plant ,79% and 77% were recorded by *Euglena* and *Oscillatoria* cultivation in these wastes for 10 days. However, at Shahat plants the algae reduced BOD (about 74%) were *Oscillatoria* , *Lyngbya.* Growth of algal populations allows further decomposition of the organic matter by producing oxygen. The production of this oxygen replenishes the oxygen used by the heterotrophic bacteria. Oxidation ponds also tend to fill, due to the settling of the bacterial and algal cells formed during the decomposition of the sewage. Overall, oxidation ponds tend to be inefficient and require effluents containing the oxidized products need to be periodically removed from the ponds (Abdel-Raouf *et al*.,2014).

Similarly, Total inorganic carbon (TIC) exceeds more times in wastewater ,in this study microalgae can reduced TIC after 10 days growth, *Nitzschia, Oscillatoria* , *Lyngbya* reduced TIC about 75% using Shahat wastewater. But*, Euglena* and *Oscillatoria* reduced it about 65% using Al-Garega wastes (Table 4&5). The decrease in the TIC concentration after the cultivation of microalga indicates that even domestic wastewaters contain insufficient carbon to fully support optimal algal production and it also indicates that this can be a desirable point for discharging the wastewaters into natural environments. The consumption of CO_2 and HCO_3 during photosynthesis in algal cultures raises the pH values (Heubeck et al., 2007; Park *et al.,* 2011). The elevated pH can enhance the N removal from the pond liquid *via* ammonia volatilization and the P removal through phosphate precipitation (Craggs, 2005). Therefore, the pH can influence both the algal growth and the N and P removal efficiency in wastewater treatment.

The role of algae in waste water treatment and their affinity for heavy metal based on high negative surface change were also followed. Heavy metal uptake by microbial cell has been shown in laboratory cultures to be dependent on the free ions activity (Moffett and Brand, 1995).The data in tables (6& 7) show that, *Oscillatoria* and *Lyngbya* grown in Al-Garega wastewater were reduced Cd to 88 % , Zn to 78% and Pb to 75% from the wastes after 10 days. Also, *Euglena* can reduced the heavy metals Zn and Pb for 80 % and 75% , respectively. Using the same algae in Shahat waste water, the maximum Cd reduction (72%) was recorded by *Scenedesmus* , *Euglena,* while*,* 80 % reduction of Pb was recorded by *Lyngbya* and 87% of Zn was observed by *Ankistrodesmus*. It is worthy to mention that the mixture six algae was the best reducer either in nutrients or heavy metals.. In this respect, cyanobacterial cell wall is provided with amine, carboxylic, thiol, sulphydryl and phosphoric functional groups which can bind metal ions. However, the adsorption efficiency strongly depends on the type of metal ions, their number of charges and the affinity of the binding site for each metal (Converti *et al.,*2006).As reported by Xue*et al.* (1988) histidine which found on cell wall was able to bind Copper Cu+2 because furnishes a bidentate site. Amine and Carboxylic groups can also interact bidentately with copper. The presence of methionine as one of the amino acids is very significant, which may result in binding of the metals with sulphydryl groups. Further, the polysaccharides may act as chelators (Caire *et al*., 1997).Van and Clijsters (1999), proposed two possible mechanisms for the effect of heavy metals on algae: first, the displacement of an essential metal ion the central and functional part of the enzyme protein, and secondly, interference with sulphydryl (-SH) groups which often determine the secondary and tertiary structure of the proteins. However, Gupta and Agrawal (2007) found that heavy metals (Ni, Cu, Zn, Co, Fe and Hg) at (1-200 ppm) concentrations were affected the survival and motility of algae.

Table (6). Effect of algae addition to post treated wastewater after 10 days , on the contents of heavy metals (cadmium, lead and zink)at Al-Garegastation in El-Bida , Libya,(data are means of 3 replicates).

Algae	Cadmium (mg/L)			Lead (mg/L)			Zink (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction
A.monoraphids	0.09	0.03	66	1.2	0.7	41	0.5	0.2	60
S. acutus	0.09	0.05	44	1.2	0.5	58	0.5	0.3	40
E.gracils	0.09	0.04	55	1.2	0.3	75	0.5	0.1	80
N. palea	0.09	0.05	44	1.2	0.5	58	0.5	0.11	78
O. rubscens	0.09	0.01	88	1.2	0.3	75	0.5	0.13	74
L muscula	0.09	0.01	88	1.2	0.3	75	0.5	0.11	78
Mixture	0.09	0.02	77	1.2	0.3	75	0.5	0.11	78

Table (7). Effect of algae addition to post treated wastewater after 10 days , on the contents of heavy metals (cadmium, lead and zink) at Shahat plant in El-Bida, Libya,(data are means of 3 replicates).

Algae	Cadmium (mg/L)			Lead (mg/L)			Zink (mg/L)		
	0 day	10 days	% reduction	0 day	10 days	% reduction	0 day	10 days	% reduction
A.monoraphids	1.1	0.7	63	1.7	0.8	52	0.9	0.11	87
S. acutus	1.1	0.3	72	1.7	0.9	47	0.9	0.18	80
E.gracils	1.1	0.3	72	1.7	0.5	70	0.9	0.23	74
N. palea	1.1	0.5	54	1.7	0.7	58	0.9	0.35	61
O. rubscens	1.1	0.8	27	1.7	0.5	70	0.9	0.37	58
L muscula	1.1	0.7	63	1.7	0.3	82	0.9	0.32	64
Mixture	1.1	0.3	72	1.7	0.3	82	0.9	0.12	88

In vitro antibacterial activity of aqueous and ethanol extracts of each of *A .monoraphids* , *S. acutus*, *E .gracils, N. palea, O. rubscens, L muscular* and mixture enriched with waste water from Al-Garega and Shahat Plants were evaluated against two Gram-positive bacteria (*Staphylococcus aureus,* and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli, and Pseudomonas aeruginosa*). Both aqueous and ethanol extracts of most algae exhibited significant activity against *Bacillus cereus* and *Escherichia coli* ,while whereas low zone of inhibition was observed against, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Figure 1&2). Ethanolic extracts of all algae more effective on bacterial tested than aqueous extract in both Al-Garega and Shahat plants. The inhibitory effect of algae on bacterial strains calculated by inhibition zone was in the following order : *O.* rubscens, > L muscular >Mixture > N. palea> E .gracils> S. acutus> A *.monoraphids*, that means the secretion of antibacterial agents may be related to the algal species and algal nutritions. Microalgae represent a major untapped resource of genetic potential for valuable bioactive agents and fine biochemicals. Screening studies should reveal the existence of new molecules potentially interesting for their biological activities. From the basic point of view, the mechanisms of action of the already marketed products should be better established. For instance, it has been reported that, beyond ϖ-3 and antioxidants, fish oil also contain bioactive peptides (Mimouni *et al*.,2012). Moreover, Cynobacteria produce a wide variety of bioactive compounds, which include lipopeptides, amino acids, fatty acids, macrolides and amides (Issa,1999 , Kumar *et al*,2012, Issa *et al*., 2013 and Shaieb *et,al*.,2014). Many of them have an interest for health and pharmaceutical industries.

3. Materials and Methods

3.1. Wastewater Sampling for Microalgae Isolates

For isolation of microalgal cultures, water samples were collected during the summer season from municipal wastewater mixed with agricultural drainage for Al-Garegaa and Shahat plants , at El-Bida ,Libya , which was supporting an obvious algal bloom. After collection, samples were stored in 10 L plastic bottles and kept in dark on ice. On return to the laboratory, the pH of the wastewater was measured using pH meter (Orion 290A) and it was between 7.5 and 7.9 and the temperature was between 18 °C and 20 °C. All samples were examined microscopically for the presence of new species. Identification was based on description of algae by Desikachary (1959) Prescott (1962), Anagnostidis and Komarek (1990) and John *et. al* (2003) . The second step toward successful isolation involves the elimination of contaminants, especially those that can out-compete the target species as the zooplankters that feed upon the algae. Techniques of dilution, single-cell isolation by micropipette, and agar streaking were used.

3.2. Wastewater Sampling for Cultivation of Microalgae Strains

An influent and two different effluents samples of domestic wastewater were collected in sequence from Al-Garegaa and Shahat plants , at El-Bida ,Libya. The samples were collected in sterilized sampling bottles. The influent was automatically sediment, the secondary effluent was treated using an aeration process, and the tertiary effluent was treated with UV-radiation. The physico-chemical characterization of the wastewater was evaluated and analyzed using different methods explained in the next paragraphs. Water samples were immediately filtered using a 0.2 µm nylon membrane filter to remove microorganisms and suspended solids. The algal suspension was centrifuged and washed three times with sterilized distilled H_2O in order to investigate the potential of the selected microalgal speciesfor integrated wastewater treatment and antibacterial production. The initial optical density (OD) of the algal suspension was adjusted to 1.5 and 500 mL of the algal suspension was used as the initial inoculums. The selected microalgal species was cultivated at 27^{o}C in 10 L conical flask photobioreactors containing wastewater, with illuminated (50 µmol photons m-2 s-1) under cool fluorescent lights of 12:12 L:D cycle. The culture media were bubbled with 0.3% CO_2-enriched air. The algal growth was measured by alternative days' changes in the optical density at 680 nm with a spectrophotometer (HACH, DR/4000v, USA).

3.3. Evaluation of Nutrient Removal

Microalgal cells were harvested by centrifugation and the supernatant was filtered by micro-syringe filter (0.2 µm). The filtrated solution was used for measuring total nitrogen (TN), total phosphorus (TP) and total inorganic carbon (TIC). TN and TP were measured in the wastewater samples using the Persulfate Digestion and Ascorbate methods, respectively (APHA, 2000).

For measuring TN, 20 gm of NaOH was dissolved in 500 mL DW, then 15 gm of Potassium Persulfate ($K_2S_2O_8$) was added to it and mixed well (Persulfate solution). 1 mL of Persulfate solution was added to 5 mL of sample and put in kit tube and autoclaved at 120°C for 30 min. The kit tubes were cooled in room temperature. 5 mL were taken from autoclaved kit tube solution and added to it 1mLHCl (1:16 DW). The absorbance of the final solution was measured at 220 nm using spectrophotometer "CARY®, 50 UV-VIS". TN concentration was calculated after an extensive data analysis of the standard curve and using the following equation (OD = 0.215 × TN concentration)

For measuring TP, 6 gm of Ammonium molybdate.4 H_2O was dissolved in 300 mL DW, 0.24 g of Potassium antimonyl tartrate was added, 120 mL of H_2SO_4 {2 : 1 (80 ml of H_2SO_4+ 40 ml DW)} was added, 5 g of Ammonium Sulfamate ($NH_4OSO_2NH_2$) was added and finally the solution was completed over to 500 mL by DW. The solution was mixed with another one composed of 7.2 gm of Ascorbic Acid dissolved in 100 mL DW. 0.5 mL of the mixed solution was taken and added to 5 mL of sample. Spectrophotometer "HACH, DR/4000v," was used to measure the sample at 880 nm. TP concentration was calculated after an extensive data analysis of the standard curve and using the following equation (OD = 0.7317 × TP concentration)

Total inorganic carbon (TIC) concentrations were determined using the TOC analyzer TOC-VCPH (Shimadzu) at days 0 and 10. 20 mL of filtered sample were used for measuring TIC.

Metals were analyzed using an ELAN DRC II Inductively Coupled Plasma–Mass Spectrophotometer (ICP) (PerkinElmer SCIEX, USA). While the pH was measured by a pH meter (Orion 290A). The percentage of nutrient removal rates were calculated by dividing the difference between the first day and the final day concentrations by the first day concentration, and then multiplying the result by 100. The experiments were performed in triplicate and the data are expressed as means. Blank experiments (wastewater without inoculums) were placed in the same condition as the cultures.

3.4. Algal Biomass Measurement

The microalgae biomass was measured as dry weight at 0 and 10 days after growth.

3.5. Estimation of Chlorophyll-a Content

The growth of algal can be determined by observing the expression of pigments in a period of time. The extraction of pigments from the algal cell can be done by using acetone extraction. The absorption (A) reading of the pigment extract at particular wavelength against a solvent blank in a spectrophotometer is defined as chlorophyll-a concentration (Marker ,1972)

3.6. Antibacterial Activity

One gram of dried biomass of the algae was extracted by water (aqueous) and /or with ethanol in a mortar pestle and kept overnight at 4°C for complete extraction. The supernatant was collected after the centrifugation at 10000×g at 10 min. The solvent extracts were concentrated under reduced pressure at 40°C. Dry residue was redissolved in dimethyl sulfoxide (DMSO) and kept at 4°C until use for bioassay. The antibacterial activities of micro-algae extracts were evaluated by agar plate diffusion test (*Escherichia coli, Pseudomonas aeruginosa, , Staphylococcus aureus, Bacillus cereus*) .Filter paper disks (5 mm) were saturated with 20 µl of 1 mg ml-1 test solution, dried, and placed on nutrient agar plates with a lawn of the test microorganisms. Plates were incubated at 37°C and inhibition zones were measured (Thummajitsakul*et.al*.,2012 and Shaieb*et.al*.,2014).

4. Conclusions

Cloacal algae can be used as nutrient and heavy metals removal, as feed for animals and as antibacterial. This dual process has several advantages such as less cost ,less energy input, and less greenhouse gas emission .There is indeed a wide range of applications of microalgae in biotechnology. In terms of applications in medical biotechnology, microalgae are potential sources of high-value products, including nutraceuticals, and bioactive molecules that may lead to the discovery of new drugs. The use of microalgae as a biological tool for monitoring and assessment of environmental toxicants is another application that has attracted much interest. The present study indicates that microalgae can be used for cleaning wastewater from nutrient and heavy metals. The levels of investigated metals were below these limits as in sewage sludge as in pig slurry solids except for cadmium .The risk of contamination of soil with Cd resulting from application of sludges and pig slurry solids may be further increased in industrially polluted areas where plants and animals may become affected and cause contamination of the entire food chain. The antibacterial property of the six algal species against the selected strains of human pathogenic bacteria varies depending upon solvent used for extraction. Further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extracts against bacteria.

Authors' Contributions

1) carried out the isolation and identification of algae in wastewater and cultivation processes , nutrient and heavy metal removal and helped design the whole study.
2) carried out analysis of algal biomass extraction and helped in manuscript preparation.
3) helped in algae as antibacterial .

All authors read and approved the final manuscript.

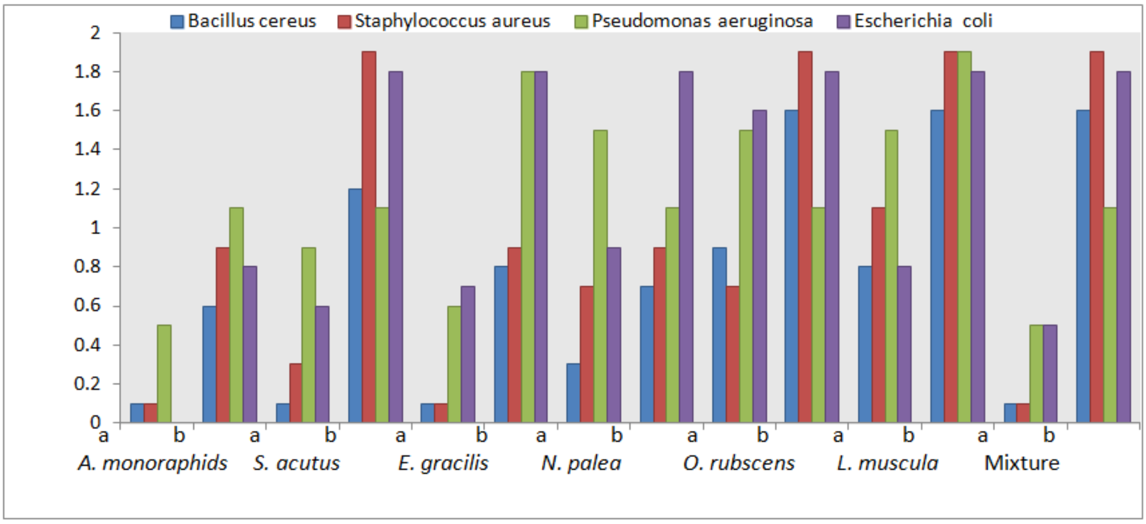

Figure (1). Antibacterial effect of algae (a-aqueous extract and b-ethanol extract) grown for 10 days in wastewater at Al-Garegaplant in El-Bida , Liby.

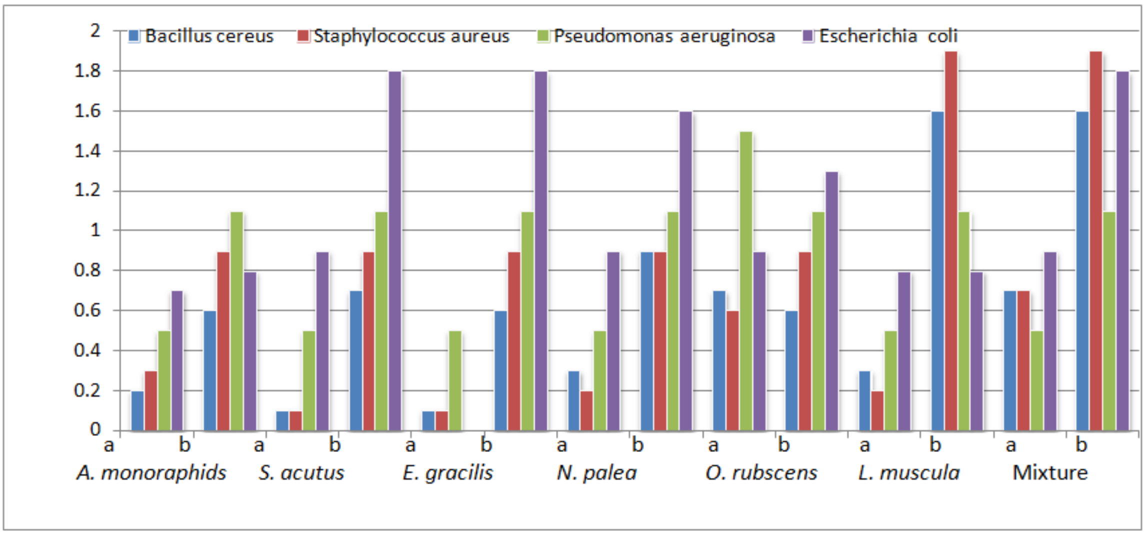

Figure (2). Antibacterial effect of algae (a-aqueous extract and b-ethanol extract) grown for 10 days in wastewater at Shahatplant in El-Bida , Liby.

References

[1] Abdel-Raouf, N., Ibraheem, I.B.M., Hammouda, O.(2014):Microalgae and wastewater treatment. Review Article Saudi Journal of Biological Sciences 19, 257–275.

[2] Anagnostidis K, Komarek J. (1990) :Modern approaches to the classification of cyanobacteria.Stigonematales. Arch Hydrobiol.; 4: 224-286.

[3] APHA.(2000) : Standard methods for the examination of water and wastewater . 21thed. American public Health Association Washington .D.C.

[4] Blackwell Science Ltd; 2004.

[5] Bulgariua L, Balana C, Bulgariubc D and Macoveanua M (2014):Valorisation of romanian peat for the removal of some heavy metals from aqueous media.Desalination and Water Treatment 52 (2014) 5891–5899.

[6] Caire, G.; Storni, De Cano, M. ;Zaccaro De Mule,M.C.; Palma,R.M. andColombo K. (1997): Exopolysaccharides of *Nostocmuscorum* (Cyanobacteria) in the aggregation of soil particles. J. Appl.Physiol., 9: 249-253.

[7] Chisti, Y(2007) : Biodiesel from microalgae. Biotechnol.Adv, 25, 294-306.

[8] Chu, W.L(2012) : Biotechnological applications of microalgae . Review Article IeJSME 6: 24-37 .

[9] Converti , A ; Lodi, A. ;Solisio , C. ; Soletto , D. ; Del Borghi , M. and Carvalho , J.C.M. (2006): *Spirulinaplatensis* biomass as adsorbent for copper removal. Cienc . Technol. Aliment., 5(2) : 85-88.

[10] Craggs, R.J., Sukias, J.P., Tanner, C.T., and Davies-Colley, R.J. (2004).“Advanced pond system for dairy-farm effluent treatment.” New Zealand Journal of Agricultural Research, 47, 449-460.

[11] de-Godos, I., V. A. Vargas, S. Blanco, M. C. Garcia-Gonzalez, R. Soto, P. A. Garcia- Encina, E. Becares and R. Muioz. 2010. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. Bioresour. Technol. 101: 5150–5158.

[12] Desikachary TV. (1959) ;Cyanophyta. Indian Council of Agricultural Research: New Delhi. pp. 686.

[13] El-EnanyA.E.and IssaA.A. (2000): Cyanobacteria as a biosorbent of heavy metals in sewage water. Enviromental Toxicology and Pharmacology.8, 95-101.

[14] Gupta, S. and Agrawal, S.C. (2007): Survival and motility of Diatoms *Naviculagrinmei* and *Nitzschiapalea* affected by some physical and chemical factors. Folia Microbiol..,52(2): 127-134.

[15] Hammouda, O., Gaber, A., and Abdel-Raouf, N. (1994) :Microalgae and wastewater treatment. Ecotoxicol. Environ. Saf. 31: p. 205–210.

[16] Heubeck, M., Aarvak, T., Isaksen, K., Johnsen, A., Petersen, I. K.and Anker-Nilssen,T.(2007):Mass mortality of adult Razorbills Alcatorda in the Skagerrak and

[17] IssaA.A.,Abdel-BassetR.andAdamM.S.(1995):Abolitionofheavymetaltoxicity on*Kirchneriellalunaris*(Chlorophyta)bycalcium.AnnalsofBotany 75:189-192.

[18] IssaA.A., Adam M.S. and Fawzy M.A. (2013): Alterations in some metabolic activities of *Scenedesmusquadricauda*and *Merismopediaglauca* in response to glyphosate herbicide. J Biol Earth Sci. 2013; 3(1): B17-B28..

[19] Issa AA. (1999): Antibiotic production by the cyanobacteria *Oscillatoriaangustissima*and *Calothrixparietina*. Environ ToxicolPharmacol.; 8: 33-37. http://dx.doi.org/10.1016/S1382-6689(99)00027-7

[20] John DM, Whitton BA, Brook AJ. (2003): The freshwater algal flora of the British isles, an identification guide to freshwater and terrestrial algae. Cambridge University Press, pp. 117-122.

[21] Karin L (2006):Wastewater treatment with microalgae –a literature review.VATTEN 62:31–38.

[22] Kumar, V., Bhatnagar, A.K., Srivastava, J.N., (2011): Antibacterial activity of crude extracts of *Spirulinaplatensis* and its structural elucidation of bioactive compound. J. Med. Pl. Res., 5, 7043-7048.

[23] Lau, P. S., N. F. Y. Tam, and Y. S. Wong (1994): Influence of organic-N sources on an algal wastewater treatment system.Resour. Conservation Recycling 11: 197-208.

[24] Luz, E., Bashan, D., Hernandez, J.P., Morey, T., and Bashan, Y. (2004): Microalgae growth-promoting bacteria as “helpers” for microalgae: A novel approach for removing ammonium and phosphorus from municipal wastewater. Water Res., 38: 466–474.

[25] Marker AFH. (1972) :The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. Fresh Water Biol.; 2: 361-385.

[26] Mimouni V, Ulmann L, Pasquet V, Mathieu M, Picot L, Bougaran G, Cadoret J, Morant-Manceau A and Schoefs B (2012): The Potential of Microalgae for the Production of Bioactive Molecules of Pharmaceutical Interest. 13 (15): 2733-2750.

[27] Můnioz, R. and Guieysse, B. (2006): Algal–bacterial processes for the treatment of hazardous contaminants: A review. Water Res., 40: 799–815.

[28] North Sea area .SEABIRD 24 (2011): 11–32.

[29] OgbonnaJC, Tomiyama S, Tanaka H (1998): Heterotrophic cultivation of Euglena gracilis z for efficient production of a-tocopherol. J. Appl. Phycol. 10: 67-74.

[30] Oliver, R.L. and Ganf, G.G. (2000): Freshwater blooms, in the ecology of cyanobacteria: their diversity in time and space B.A. Whitton and M. Potts, Editors. Kluwer: rdrecht p. 149–194.

[31] Park JBK, Craggs RJ, Shilton AN (2011): Wastewater treatment high rate algal ponds for biofuel production. BioresourTechnol, 102:35–42.

[32] Prescott GW. (1962): Algae of the Western Great Lake area. W.M.C. Dubuque, Brown Company, pp.543-551.

[33] Richmond A. Handbook of microalgal culture: biotechnology and applied phycology.

[34] SengarRMS, Singh KK and Surendra sigh (2011); Application of phycoremidiation technology in the treatments of sewage water to reduced pollution load. IndianJ,Sci.Res,2,33-39.

[35] Shaieb AF, Issa AA, Meragaa A (2014): Antimicrobial activity of crude extracts of cyanobacteria *Nostoc commune* and *Spirulinaplatensis* .Archives of Biomedical Sciences 2 (2): 34-41.

[36] Shi, J., Podola, B., Melkonian, M.(2007): Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. J. Appl. Phycol. 19 (5), 417–423.

[37] Thummajitsakul S, Silprasit K, Sittipraneed S. (2012): Antibacterial activity of crude extracts of cyanobacteria Phormidium and Microcoleus species. Afr J Microbiol Res; 6(10): 2574-2579. http://dx.doi.org/10.5897/ajmr12.152.

[38] Van Assche, F. and Clijsters (1999): Enzyme activity in plants. Plant cell environ, 13:195-206.

[39] Wan W, Chong Y , Ge L, Noh H , Douglas Stone A , Cao H (2011): Time-Reversed Lasing and Interferometric Control of Absorption. Science, 331, 6019: 889-892.DOI: 10.1126/science.1200735.

[40] Xue, H.B. ;Stum, W. and Sigg, L.(1988): The binding of heavy metals to algal surfaces. Water Research, 22 : 917-926.