AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2012, 4(1):56-65

Notulae Scientia Biologicae

Cultivating Microalgae in Domestic Wastewater for Biodiesel Production

Soha S.M. MOSTAFA[1], Emad A. SHALABY[2*], Ghada I. MAHMOUD[2]

[1]*Agricultural Research Centre, Soils, Water and Environmental Research Institute, Microbiology Department, Giza, Egypt*

[2]*Cairo University, Faculty of Agriculture, Biochemistry Department, Giza, Egypt; dremad2009@yahoo.com (*corresponding author)*

Abstract

The objective of this study was to evaluate the growth of nine species of microalgae (green and blue green microalgae) on domestic wastewater obtained from Zenein Wastewater Treatment Plant (ZWWTP), Giza governorate, Egypt. The species were cultivated in different wastewater treatments namely: without treatment; after sterilization; with nutrients with sterilization and with nutrients without sterilization. The experiment was conducted in triplicate and cultures were incubated at 25±1°C under continuous shaking (150 rpm) and illumination (2000 Lux) for 15 days. Algal growth parameters i.e., pH, electric conductivity (EC), optical density (OD), dry weight (DW) and chlorophyll-a (Ch-a) were measured at zero-time and at the end of the experimental period; while, the percentages of total lipids, biodiesel and the residual sediments (glycerine, pigments, etc) were determined in the harvested algal biomass. The data revealed that domestic wastewater with nutrients and with sterilization (T_3) was promising for cultivating five algal species as compared to the synthetic media. Moreover, the sterilized-domestic wastewater (T_2) was the selective medium for cultivating *Oscillatoria* sp. and *Phormedium* sp; however, T_1 medium (wastewater without treatment) was the promising medium for cultivating *Nostoc humifusum*. Biodiesel production from the algal species cultivated in synthetic media was ranging between 3.90% (*Wollea saccata*) and 12.52% (*Nostoc muscorum*). On the other hand, the highest biodiesel production from algal biomass cultivated in wastewater was obtained by *Nostoc humifusum* (11.80%) when cultivated in wastewater without treatment (T_1) and the lowest (3.80%) was recorded by *Oscillatoria* sp. when cultivated on the sterilized-domestic wastewater (T_2). The results of this study suggest that cultivating microalgae on domestic wastewater combines nutrients removal and algal lipid production for potential use as a biodiesel feedstock. Additionally, using the domestic wastewater, as nutrient media for microalgae cultivation, is suitable and non-expensive method as compared to the conventional cultivation methods for sustainable biodiesel and glycerol production.

Keywords: biodiesel production, domestic wastewater, growth parameters, microalgae

Introduction

The need of energy is increasing continuously, because of increases in industrialization and population. The basic sources of this energy are petroleum, natural gas, coal, hydro and nuclear (Kulkarni and Dalai, 2006). Renewable, carbon neutral transportation fuels are necessary for environmental and economic sustainability and biofuel from biomass is one of the most promising alternatives to petroleum fuels. (U.S. DOE, 2010).

Bioenergy is one of the most important components to mitigate greenhouse gas emissions and substitute of fossil fuels (Goldemberg, 2000). Biomass is one of the better sources of energy (Kulkarni and Dalai, 2006). Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economic (Turkenburg, 2000). Biodiesel (monoalkyl esters) is one of such alternative fuel, which is obtained by the transesterification of triglyceride oil with monohydric alcohols. It has been well-reported that biodiesel obtained from canola and soybean, palm, sunflower oil, algal oil as a diesel fuel substitute (Lang *et al.*, 2002; Spolaore *et al.*, 2006).

Biodiesel is a nontoxic and biodegradable alternative fuel that is obtained from renewable sources. Biodiesel fuel can be prepared from waste cooking oil, such as palm, soybean, canola, rice bran, sunflower, coconut, corn oil, fish oil, chicken fat and algae (Sharif *et al.*, 2007) which would partly decrease the dependency on petroleum-based fuel. Biodiesel from oil crops are being produced in increasing amounts as renewable biofuels, but their production in large quantities is not sustainable (Chisti, 2008).

An alternative is offered by microalgae (Chisti and Yan, 2011). Microalgae are photosynthetic microorganisms that are able to rapidly generate biomass from solar energy, CO_2 and nutrients in bodies of water. This biomass consists of important primary metabolites such as sugars, oils and lipids, for which process path-ways exist for the production of high-value products including human and animal feed supplements, transport fuels, industrial chemicals and pharmaceuticals. Algae are capable of producing 30 times the amount of oils and lipids per unit area of land as compared to terrestrial oilseed crops (Sheehan *et al.*, 1998).

Many microalgae are exceedingly rich in oil which can be converted to biodiesel using existing technology. More

Received 07 November 2011; accepted 11 February 2012

than 50% of their biomass as lipids, sometimes even up to 80%, and oil levels of 20-50% are quite common (Chisti, 2007). Lipids production and biodiesel extraction from algae depend on algal species and extraction solvent system (Afify *et al.*, 2010).

There is a unique opportunity to both treat wastewater and provide nutrients to algae using nutrient-rich effluent streams. By cultivating microalgae, which consume polluting nutrients in municipal wastewater, and abstracting and processing this resource, then the goals of sustainable fuel production and wastewater treatment can be combined (Andersen, 2005). Treated wastewater is rich in nitrogen and phosphorus, which if left to flow into waterways, can spawn unwanted algae blooms and result in eutrophication (Sebnem Aslan, 2006). These nutrients can instead be utilized by algae, which provide the co-benefit of producing biofuels and removing nitrogen and phosphorus as well as organic carbon (Mostafa and Ali, 2009). Wastewater treatment using algae has many advantages. It offers the feasibility to recycle these nutrients into algae biomass as a fertilizer and thus can offset treatment cost. Oxygen rich effluent is released into water bodies after wastewater treatment using algae (Becker, 2004).

This work aimed to evaluate the laboratory cultivation of nine algal strains belonging to *Nostocales* and *Chlorellales* in secondary treated municipal domestic wastewater for biomass and biodiesel production.

Materials and methods

Chemicals and reagents

Pure chloroform, ether, acetone and methanol were purchased from E. Merch Co. (Germany), and dis¬tilled before use.

Preparation of algal inoculums

Cyanobacteria strains (*Anabaena flous aquae, Anabaena oryza*e, *Nostoc humifusum*, *Nostoc muscorum*, *Oscillatoria* sp., *Spirulina platensis, Phormedium fragile* and *Wollea saccata*) and the green alga strain *Chlorella vulgaris* were obtained from the Microbiology Department, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Centre (ARC), Giza-Egypt. Cyanobacteria strains were maintained in BG11 medium (Rippka *et al.*, 1979) except *Spirulina platensis* which was cultivated in Zarrouk medium (Zarrouk, 1966). While, Bold medium (Nichols and Bold, 1965) was used for the green alga *Chlorella vulgaris*. Cultures were incubated in a growth chamber under continuous shaking (150 rpm) and illumination (2000 Lux) at 25±1°C for 30 days. Tab. 1 shows the characterization (OD, DW, chlorophyll a and pH) of the algal inoculums APHA (1998).

Characterization of wastewater sample

The effluent of the secondary treated sewage wastewater from Zenien Wastewater Treatment Plant (ZW-WTP), Giza Governorate, Egypt was used, after filtered using glass microfiber filter to remove large particles and indigenous bacteria, for algal cultivation experiment. The biological and physio-chemical analyses of the initial water sample were carried out following the methods described by APHA (1998) as shown in Tab. 2.

Experimental design

Algae were inoculated at 20% ($V_{inoculation}$ /V_{media}) in 500 ml Erlenmeyer flasks containing 200 ml liquid media (100% wastewater effluent supplemented with/without nutrients and with/without sterilization) and the treatments were as follows:

T_1: wastewater without nutrients or sterilization

T_2: wastewater with sterilization

T_3: wastewater + nutrients with sterilization

T_4: wastewater + nutrients without sterilization

Wastewater effluent was supplemented with nutrients of $NaNO_3$, K_2HPO_4 and $FeSO_4.7H_2O$ in amounts equal to those of the standard BG11, Bold and Zarrouk to be used as basal media while, the synthetic media of BG11, Bold and Zarrouk were used as control. The cultures were incubated at 25°C±1°C, under continuous shaking (150 rpm) and illumination (2000 Lux) for 15 days.

The experiment was carried out in triplicate and average values were recorded.

Algal growth analyses

The analyses of algal growth parameters i.e., pH, electric conductivity (EC), optical density (OD), dry weight (DW) and chlorophyll-a (Ch-a) were measured at zero-time and at the end of the experimental period (stationary phase of each one).

Tab. 1. The growth parameters of the microalgae cultures

Parameters	*Nostoc muscorum*	*Anabaena flous aquae*	*Chlorella vulgaris*	*Oscillatoria* sp.	*Spirulina platensis*	*Anabaena oryzae*	*Wollea saccata*	*Nostoc humifusum*	*Phormedium fragile*
	Cultures growth parameters								
pH	7.63	6.61	8.11	5.82	10.48	6.27	6.11	8.7	9.33
Optical density at 650 (nm)	1.163	1.155	1.611	0.219	2.50	1.755	1.981	1.20	1.66
Ch-a (mg l^{-1})	4.335	1.776	5.876	4.8	23.45	5.874	14.25	5.23	2.86
Dry weight (mg l^{-1})	744.32	727.65	1052.16	140.16	2622.4	1123.20	1267.84	760.80	1062.4

Tab. 2. Chemical analyses of the initial domestic wastewater comparing with the algal synthetic media (Zarrouk, BG11 and Bold)

Parameters	Wastewater	Standard synthetic media		
		Zarrouk	BG11	Bold
TS (mg l^{-1})	425	-	-	-
TSS (mg l^{-1})	18	-	-	-
BOD (mg l^{-1})	15	-	-	-
COD (mg l^{-1})	50	-	-	-
Physical parameter				
EC (dsm^{-1})	0.55	19.89	0.55	1.14
pH	6.8	10	7.5	6.6
Chemical parameters (mg l^{-1})				
N	3.78	410	240	1640
K	10.95	471.14	1257.14	2236.96
P	0.224	110.71	885.71	2487.94
B	0.154	50.02	50.02	0.0
Cd	0.0	0.0	0.0	0.0
Co	0.0	14.17	22.69	99.34
Cr	0.002	20.0	0.0	0.0
Cu	0.027	22.93	22.93	0.0
Fe	0.014	3.68	120	1003.16
Mn	0.014	790.07	228.5	181.81
Mo	0.0	9.96	7.13	331.33
Ni	0.0	0.0	0.0	0.0
Pb	0.0	0.0	0.0	0.0
Zn	1.146	90.18	90.18	90.18

TS: Total solids; TSS Total suspended solids; COD: Chemical oxygen demand; BOD: Biological oxygen demand

Cultures concentration was determined as optical density (OD) by spectrophotometer at 560 nm (Leduy and Therien, 1977). Chlorophyll-a was determined spectrophotometrically after extraction by absolute methanol as reported by Vonshak and Richmond (1988), pH values and algal dry weight were estimated according to Vonshak (1986). Electric conductivity (EC) was measured by conductivity meter (systronics 304) (Kar *et al*., 2008)

Lipids extraction

Dry extraction procedure according to Zhu *et al.* (2002) as a modification of the wet extraction method by Bligh and Dyer (1959) was used to extract the lipid in microalgae cells. Typically, cells were harvested by centrifugation at 8500 rpm for 5 min and washed once with distilled water. After drying the samples using freeze drier, the samples were pulverized in a mortar and extracted using mixture of chloroform:methanol (2:1, v/v). About 50 ml of solvents were used for every gram of dried sample in each extraction step. After stirring the sample using magnetic stirrer bar for 5 hr and ultrasonicated for 30 min., the samples were centrifuged at 3000 rpm for 10 min. The solid phase was separated carefully using filter paper (Advantec filter paper, no. 1, Japan) in which two pieces of filter papers were applied twice to provide complete separation. The extracted oil was evaporated under vacuum to release the solvent mixture solutions using rotary evaporator at 40-45°C.

Transesterification and biodiesel production

Lipids extract was mixed with a mixture of catalyst (0.25 g NaOH) and 24 ml methanol, a process called transesterification, with stirring properly for 20 min. The Mixture was kept for 3 hrs in electric shaker at 3000 rpm. (National Biodiesel Board, 2002). After shaking the solution was kept for 16 hr to settle the biodiesel and the sediment layers clearly. The biodiesel layer was separated from sedimentation by flask separator carefully. Quantity of sediments (glycerine, pigments, etc) was measured. Biodiesel was washed by 5% water many times until it becomes clear then biodiesel was dried by using dryer and finally kept under the running fan for 12 hr the produced biodiesel was measured (using measuring cylinder), pH was measured and stored for analysis.

Statistical analysis

Data were subjected to an analysis of variance, and the means were compared using the Least Significant Difference (LSD) test at the 0.05 levels, as recommended by Snedecor and Cochran (1982).

Results and discussion

The main nutrients, nitrogen and phosphorus, required for microalgae growth can be utilized from various wastewaters generated by different sources (Becker, 2004). The chemical composition of the secondary treated sewage wastewater from Zenien (Egypt) revealed that the initial sample has a great deficiency in all the required nutrients for algal optimal growth comparing with their synthetic media (Tab. 2).

Algal growth parameters

pH

The pH values of microalgal cultures were changed by the time from initial phase up to stationary phase depending on the algal species and the growth media. Changes in pH values of the algal cultures were ranged between 6.02 and 9.30 (Tab. 3). However, the highest values (pH>9) at the stationary phase were observed with *Spirulina platensis*. Variation in pH can affect metabolism and growth of algae in a number of ways, including altering the equilibrium of inorganic carbon (C) species, changing availability of nutrients, and, at extremes, directly affecting cell physiology. Most algae have pH optima for growth and photosynthesis in the neutral to alkaline pH range, but species may be found growing in acid conditions as low as pH 1±0 (Albertano *et al*., 1971; Langworthy, 1978; Raven, 1990).

Azov (1982) found that biomass production by the green algae *Scenedesmus obliquus* and *Chlorella vulgaris* in intensive laboratory continuous cultures was considerably

affected by the pH at which the cultures were maintained. Goldman *et al.* (1981) suggested that pH tolerance limits of the algae are governed either by chemical influence on the growth medium or by metabolic effects on the cells. In addition it was concluded that the maximum tolerable pH is not influenced by the availability of inorganic carbon. However, pH is the major determinant of the relative concentrations of the carbonaceous system species in water and could affect the availability of carbon for algal photosynthesis in intensive cultures.

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes (Reactive oxygen species (ROS), fluoride and phenols accumulation) in closed systems. The decreased algal production which followed pH increase in continuous algal cultures was due mainly to decreasing the free carbon dioxide concentration in the medium (Azov, 1982).

The decrease of pH value in some treatment may be due to the ability of species for utilization the alkaline elements and this led to decrease the concentration of these elements in culture media or formation of acids compounds eg: carbonic acid during cultivation and led to decrease of pH value as reported by Richmond (1986).

High pH may affect cellular enzyme function and change the speciation of metals (McKnight *et al*., 2001). Since enzyme activities are pH-dependent, changes in extracellular pH are likely to affect intracellular pH and hence enzymatic activities and enzyme structure in algae. The enhanced ROS production observed in *Chattonella marina* under high pH (9.0 and 9.5) may possibly be attributable to an increase in the activities of enzymes regulating ROS production. The possibility also exists that elevated pH may affect metal speciation and reduce the bioavailability of iron, which may in turn enhance ROS production (Liu *et al*., 2007).

Microalgal growth rate and species composition may also be affected by pH. As an example, Fontes *et al.* (1987) found that optimal productivity of the cyanobacterium *Anabaena variabilis* were obtained at pH 8.2-8.4, being slightly lower at 7.4-7.8, decreasing significantly above pH 9, and at pH 9.7-9.9 the cells were unable to thrive (Mostert and Grobbelaar, 1987). However, many algal species accept higher pH values than that. In algal cultures, pH usually increases due to the photosynthetic CO_2 assimilation (Borowitzka, 1998; Chevalier *et al.*, 2000). During algal growth, fluxes of inorganic and organic species occur between different compartments inside the cells and between the cells and the surrounding medium. In a closed system, a steady decrease in nutrients is accompanied by a change in pH and alkalinity. Changes in pH and alkalinity are the results of net fluxes of H^+ across the plasma membrane as well as influx of different dissociation states of weak electrolytes such as CO_2/HCO_3^- and NH_4^+/NH_3 (Raven and De Michelis, 1979).

EC (Electrical conductivity)

Electrical conductivity (EC) refers to the ability of an aqueous solution to carry electric current. This ability depends on the presence of ions, their total concentration, mobility, and valence, and the temperature of the solution. Most solutions composed of inorganic compounds are relatively good electrical conductors. Conversely, although some organic compounds dissociate in aqueous solutions which are very good electrical conductors, most molecules in organic compounds do not dissociate in aqueous solution (Yu *et al.*, 2005). All treatments reduced Electric conductivity (EC) values (dsm^{-1}) by the time from initial phase to stationary phase (Tab. 4) and this may be due to the ability of algal species to consume the nutrients during the algal growth. The important of EC as control parameters, agreed with that expressed by Oswald (1988), on the fact that total dissolved salt one among the decision factors that define the relationship culture medium species in the cultivation of microalgae. Earlier, Oswald and Gotaas (1957) studied the use of micro algae for tertiary treatment of municipal wastewater (phycoremediation) to

Tab. 3. Changes of pH values of different microalgal cultures in wastewater during the treatment period

Algal species	pH value									
	Control		T_1		T_2		T_3		T_4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nostoc muscorum	7.11	7.42	7.02	7.24	7.42	7.87	7.26	7.72	7.80	7.24
Anabaena flous aquae	7.61	7.32	7.18	8.03	7.54	7.53	7.00	7.90	7.63	7.95
Chlorella vulgaris	7.55	7.69	8.73	7.90	8.34	8.15	9.26	8.37	8.60	8.55
Oscillatoria sp	6.90	6.96	7.36	8.41	7.76	8.17	7.67	8.33	7.88	8.05
Spirulina platensis	9.24	9.13	9.00	8.13	8.90	8.32	9.20	9.26	9.30	9.24
Anabaena oryzae	7.02	8.24	7.34	7.22	7.30	8.28	7.56	8.85	7.72	7.50
Wollea saccata	6.39	7.40	8.38	8.05	8.27	7.98	8.20	8.13	7.84	8.39
Nostoc humifusum	7.53	6.02	7.36	7.99	8.40	7.90	7.43	8.02	8.42	7.11
Phormedium fragile	6.82	7.24	7.53	7.84	7.32	6.38	7.64	8.34	7.99	7.81
LSD		0.032		0.625		0.032		0.111		0.032

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range test

T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

Tab. 4. Changes of electric conductivity (dsm^{-1}) values of different microalgal cultures in wastewater during the treatment period

Algal species	Value									
	Control		T_1		T_2		T_3		T_4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nostoc muscorum	0.031	0.28	0.55	0.54	0.60	0.58	0.70	0.44	1.00	0.28
Anabaena flous aquae	0.56	0.18	0.55	0.27	0.65	0.18	1.42	1.26	1.45	1.36
Chlorella vulgaris	1.07	0.61	0.54	0.48	0.91	0.16	0.71	0.37	0.90	0.26
Oscillatoria sp	1.00	0.28	0.53	0.28	0.75	0.15	1.75	0.42	1.34	0.34
Spirulina platensis	23.00	19.00	1.00	1.68	2.00	3.23	5.80	4.20	5.75	4.36
Anabaena oryzae	0.70	0.59	0.61	0.19	0.65	0.22	0.72	0.62	0.75	0.50
Wollea saccata	0.75	0.41	0.63	0.36	0.70	0.26	0.78	0.28	0.84	0.63
Nostoc humifusum	0.47	0.36	0.50	0.26	0.60	0.25	0.66	0.38	0.80	0.46
Phormedium fragile	0.75	0.23	0.71	0.50	0.62	0.40	0.75	0.35	1.05	0.27
LSD		0.032		0.032		0.032		0.032		0.032

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range test
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

comprise several applications: (a) nutrient removal from municipal wastewater and effluents rich in organic matter; (b) nutrient and xenobiotic compounds removal with the aid of algae-based biosorbents; (c) treatment of acidic and metal wastewaters.

Experiments on municipal wastewater effluent resulted in 23% reduction in N and 82% reduction in P (Rectenwald and Drenner, 2000). Nitrogen removal by microalgae and cyanobacteria could be limited by several environmental factors, such as light, pH and availability of carbon source (Garbisu *et al*., 1992; Urrutia *et al*., 1995). Cation concentrations such as Ca^{2+}, Mg^{2+} and pH affected the removal efficiency of TP (Li *et al*., 2006).

Optical density (OD), dry weight (DW) and chlorophyll content

Tab. 5, 6 and 7 show optical density (OD), dry weight (DW) and chlorophyll content (mg l^{-1}) of the microalgal species cultivated in different wastewater treatments (T_1,T_2, T_3 andT_4).

Algal growth can be expressed as biomass yield. Yield, as an expression of organic production, is usually given in terms of dry weight of the organic mass produced over a period of time per unit volume. Optical density (OD) measurements can be translated into biomass yield in terms of dry weight or chlorophyll (Tadros, 1988).

Optical density measurement is an indication of cell growth; to obtain the exact cell mass at certain optical density dry mass test was performed. Biomass yield of algae in synthetic media (control) are generally much higher than those found in most of the treatments. *Nostoc muscorum*, *Oscillatoria* sp., *Spirulina platensis* and *Anabaena oryzae* reached the maximum values of OD (0.28, 0.80, 0.47 and 0.50 respectively), DW (181.12, 513.28, 303.36 and 320.0 mg l^{-1}, respectively) and chlorophyll content (1.05, 2.99, 1.77 and 1.86 mg l^{-1}respectively) when cultivated in synthetic media (control). However, *Anabaena flous aquae*, *Chlorella vulgaris* and *Wollea* sp. reached the highest values of OD, DW and chlorophyll content when cultivated in media contain the wastewater + nutrients with sterilization (T_3). Otherwise, *Nostoc humifusum* reached the

Tab. 5. Optical density of different microalgal species cultivated in different wastewater treatments

Algal species	Value									
	Control		T_1		T_2		T_3		T_4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nostoc muscorum	0.03	0.28	0.05	0.14	0.05	0.15	0.06	0.16	0.04	0.15
Anabaena flous aquae	0.05	0.34	0.02	0.13	0.09	0.15	0.07	0.37	0.05	0.35
Chlorella vulgaris	0.13	0.42	0.09	0.25	0.12	0.41	0.32	1.05	0.23	0.71
Oscillatoria sp	0.02	0.80	0.04	0.79	0.04	0.45	0.04	0.25	0.03	0.26
Spirulina platensis	0.04	0.47	0.01	0.09	0.05	0.32	0.04	0.43	0.09	0.26
Anabaena oryzae	0.03	0.50	0.02	0.30	0.10	0.37	0.08	0.36	0.03	0.37
Wollea saccata	0.07	0.16	0.04	0.11	0.06	0.25	0.08	1.09	0.04	1.03
Nostoc humifusum	0.07	0.63	0.02	0.90	0.05	0.76	0.05	0.14	0.03	0.46
Phormedium fragile	0.05	0.16	0.03	0.24	0.01	0.75	0.05	0.14	0.01	0.20
LSD		0.032		0.032		0.032		0.032		0.030

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range test
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

Tab. 6. Dry weight (mg l^{-1}) of different microalgal species cultivated in different wastewater treatments

Algal species	value									
	Control		T_1		T_2		T_3		T_4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nostoc muscorum	21.12	181.12	29.44	86.40	37.76	102.4	36.48	104.32	24.32	92.80
Anabaena flous aquae	30.08	216.32	11.52	83.20	54.40	97.28	44.16	236.80	12.80	89.54
Chlorella vulgaris	83.20	271.36	57.60	158.08	75.52	261.76	204.80	670.72	148.48	452.48
Oscillatoria sp	14.72	513.28	2.56	53.12	24.32	288.00	26.24	157.44	19.20	164.48
Spirulina platensis	25.60	303.36	6.40	57.60	32.00	202.24	25.60	272.00	57.60	167.68
Anabaena oryzae	19.84	320.00	14.72	192.00	60.80	236.80	51.20	232.96	18.56	238.08
Wollea saccata	44.80	103.68	22.40	71.68	39.40	156.80	51.20	698.88	24.96	656.64
Nostoc humifusum	47.36	400.00	10.88	572.80	33.92	487.68	29.44	90.88	21.76	295.04
Phormedium fragile	28.80	103.68	21.12	156.16	8.32	481.92	32.00	90.88	7.04	129.92
LSD		0.028		0.032		0.028		0.032		0.032

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.05 according to Duncan's multiple range test
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

Tab. 7. Chlorophyll-a (mg l^{-1}) of different microalgal species cultivated in different wastewater treatments

Algal species	value									
	Control		T_1		T_2		T_3		T_4	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nostoc muscorum	0.12	1.05	0.17	0.50	0.22	0.43	0.21	0.61	0.14	0.54
Anabaena flous aquae	0.07	1.26	0.03	0.48	0.13	0.57	0.11	1.38	0.03	0.34
Chlorella vulgaris	0.46	1.58	0.32	0.92	0.42	1.52	1.14	3.91	0.83	2.64
Oscillatoria sp	0.50	2.99	0.09	0.31	0.83	1.68	0.90	0.92	0.66	0.96
Spirulina platensis	0.23	1.77	0.06	0.34	0.29	1.18	0.23	1.58	0.52	0.98
Anabaena oryzae	0.10	1.86	0.08	1.12	0.32	1.38	0.27	1.36	0.10	1.39
Wollea saccata	0.50	0.60	0.25	0.42	0.44	0.91	0.58	4.07	0.28	3.82
Nostoc humifusum	0.26	2.33	0.07	3.34	0.23	2.84	0.20	0.53	0.15	1.72
Phormedium fragile	0.08	0.60	0.06	0.91	0.02	2.81	0.09	0.53	0.02	0.76
LSD		0.032		0.043		0.030		0.045		0.032

Each value is presented as mean of triplet treatments, LSD: Least different significantly at P ≤ 0.05 according to Duncan's multiple range tests
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

maximum values of these growth parameters in wastewater without any treatment (T_1) while, *Phormedium fragile* reached the maximum when cultivated in wastewater after sterilization (T_2) (Tab. 8).

This variation in microalgal growth depend on the ability of different species for grown in wastewater without any treatment (T_1) which have low concentration from nutrients as shown in Tab. 2, or cultivation in wastewater media after treatment by sterilization (T_2) to prevent microorganism grown or after enrichment with nutrients to increase the nutrient concentration (T_3 and T_4).

The results were in an agreement with the results obtained by Wang *et al.* (2009), who reported that, *Chlorella* sp. could adapt well in different wastewaters (municipal wastewater treatment plant, MWTP) with no lag phase observed.

It is well known that the developing of algal growth and nutrient removal efficiency could be increased depending upon many factors, including algal species, cell concentration, aeration, and retention time. Many authors reported that *Chlorella* is a common and effective species for the immobilization and nutrient removal purposes (Abdel Hameed, 2007; Lau *et al.*, 1997, 1998; Robinson *et al.*, 1988; Tam *et al.*, 1994).

Lipids and biodiesel

Nostoc species have the highest concentration of total lipid (16.8 and 14.8%) followed by *Chlorella vulgaris*, *Phormedium fragile, Spirulina platensis, Oscillatoria* sp., *Anabeana oryzae, Wollea saccata* and *Anabaena flous aquae* of 12.50, 12.20, 10.0, 8.0, 7.40, 6.30, and 5.50%, respectively (Tab. 9).

However, cultivation of these microalgae in different wastewater treatments (T_1,T_2, T_3 and T_4), the lipid concentration fluctuated (decrease or increase) e.g: the lipid content in *Nostoc muscorum* decreased from 16.8% to 12.5% when cultivated in synthetic media (control) and wastewater + nutrients with sterilization (T_3), respectively. However, total lipid concentration of *Anabeana flous aquae* increased from 5.5 to 7.4% when cultivated in the same treatments. This may be due to the variation between the algal species in a biotic stress response and the great deficiency in all required elements in secondary treated sew-

Tab. 8. Correlation coefficient (r) among the growth parameters of different microalgae cultivated under various conditions (T_1-T_4)

Algal species	Characters correlated	pH	Chlorophyll a	Optical density	Dry weight	Electric conductivity
Nostoc muscorum	pH		-0.1951	-0.07974	0.020213	0.496449
	Chl			0.976242	0.954805	-0.67887
	OD				0.994692	-0.59702
	DW					0.10136
	EC					
Anabaena flous aquae	pH		0.41313	-0.08303	-0.37778	0.584855
	Chl			0.559706	0.988021	0.03519
	OD				0.680841	0.684073
	DW					0.22523
	EC					
Chlorella vulgaris	pH		0.92404	0.702432	-0.18554	-0.7376
	Chl			0.999998	0.562144	0.23027
	OD				0.562582	0.23108
	DW					0.18526
	EC					
Oscillatoria sp.	pH		0.92404	-0.46043	-0.92436	0.121074
	Chl			0.334642	0.999999	-0.31533
	OD				0.335554	-0.40504
	DW					0.365214
	EC					
Spirulina platensis	pH		0.698941	0.693391	0.699514	0.448424
	Chl			0.999551	0.999987	0.690408
	OD				0.999674	0.350117
	DW					0.147821
	EC					
Anabaena oryzae	pH		0.43038	0.398767	0.412538	0.547184
	Chl			0.999607	0.999989	0.651766
	OD				0.999709	0.600186
	DW					0.589652
	EC					
Wollea saccata	pH		0.663606	0.665698	0.66315	0.316057
	Chl			0.999966	0.999999	0.342927
	OD				0.999974	0.345066
	DW					0.589664
	EC					
Nostoc humifusum	pH		-0.02162	-0.96558	-0.02206	-0.42882
	Chl			0.01137	0.999999	-0.7065
	OD				-0.01077	0.498899
	DW					0.15236
	EC					
Phormedium fragile	pH		-0.85288	-0.85439	-0.8539	0.004374
	Chl			0.999989	0.999997	0.352767
	OD				0.999995	0.350117
	DW					0.289652
	EC					

*EC: Electric conductivity; OD: Optical density; Chl: Chlorophyll; DW: Dry weight

age water especially nitrogen; led to increase accumulation of carbon skeleton compounds e.g. lipids (Becker, 2004).

Also, the present results were in agreement with those of Shalaby *et al*. (2010), who reported that under salt stress conditions (with normal nitrate concentration in culture media) the algal metabolism was altered with over production of carbon skeleton which were partly directed towards the production of substances with beneficial role in algal tolerance or defence mechanism as polyols, carbohydrate, methylated amino acids and protein in addition to the nitrogenous compounds and partly to form lipids and biodiesel. The data obtained in this investigation were in good agreement with results published by Widjaja (2009), who reported that the green microalgae *Chlorella vulgaris* accumulated high lipid content when cultivated in nitrogen depletion condition (0.02 mg/l nitrate). The present results also went parallel with those obtained by Lardon *et al.* (2009) who found that the control of nitrogen stress during the culture and optimization of wet extraction led

Tab. 9. Total lipids, biodiesel, glycerine+pigments percentages, color and pH of biodiesel from different microalgal species cultivated in standard synthetic media

Algal species	Total lipids	Biodiesel content	Glycerin + pigments	Color	pH
Nostoc muscorum	16.80±3.62	12.52±1.74	4.28±1.74	Brown	7.4±0.33
Anabaena flous aquae	5.50±0.58	4.00±0.41	1.50±0.41	Red	6.9±0.95
Chlorella vulgaris	12.50±1.20	8.8±0.16	3.70±0.16	Green	8.1±1.0
Oscillatoria sp	8.00±0.58	4.30±0.32	3.70±0.32	Yellow	7.5±0.85
Spirulina platensis	10.0±0.11	7.80±0.17	2.20±0.17	Light green	8.0±0.32
Anabaena oryzae	7.40±0.90	4.50±0.10	2.90±0.10	Orange	7.3±0.96
Wollea saccata	6.30±1.31	3.90±0.60	2.40±0.60	Yellow	7.8±0.35
Nostoc humifusum	14.80±2.40	10.20±1.30	4.6±1.30	Yellowish brown	7.5±0.50
Phormedium fragile	12.20±1.66	10.10±1.50	2.10±1.50	Dark brown	7.1±0.0
LSD	0.159	0.151	0.151		1.659

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range tests
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

Tab. 10. Total lipids, biodiesel and glycerine+pigments percentages of different microalgal species cultivated in different wastewater treatments

Algal species	Optimal wastewater treatment	Total lipids	Biodiesel	Glycerin + pigments
Nostoc muscorum	T_3	12.50±2.65	7.40±0.74	5.10±0.74
Anabaena flous aquae	T_3	7.40±0.95	5.00±0.61	2.40±0.61
Chlorella vulgaris	T_3	13.20±1.87	8.50±1.74	4.70±1.74
Oscillatoria sp	T_2	6.80±0.65	3.80±0.32	3.00±0.32
Spirulina platensis	T_3	7.30±0.44	5.00±0.51	2.30±0.51
Anabaena oryzae	T_4	8.00±0.16	4.70±0.12	3.30±0.12
Wollea saccata	T_3	7.20±1.32	4.00±0.22	3.23±0.22
Nostoc humifusum	T_1	15.50±1.65	11.80±1.52	3.70±1.52
Phormedium fragile	T_2	11.60±0.88	8.40±0.65	3.20±0.65
LSD		0.159	0.159	0.152

Each value is presented as mean of triplet treatments, LSD: Least different significantly at $P \leq 0.05$ according to Duncan's multiple range tests.
T_1: wastewater without treatment; T_2: wastewater after sterilization; T_3: wastewater+ nutrients with sterilization T_4: wastewater+ nutrients without sterilization

to maximum biodiesel production from the microalgal culture *Chlorella vulgaris*.

On the contrary chloroform:methanol (2:1, v/v) system extracted greater percentage of total lipid (non-polar and polar lipids) and consequently to higher biodiesel yields by transesterification. The lowest biodiesel production in the synthetic media was observed in W*ollea saccata, Anabeana flous aquae, Oscillatoria* sp. and *Anabeana oryzae* (3.9, 4.0, 4.3 and 4.5%, respectively), while, the green microalga (*Chlorella vulgaris*) and the cyanobacterium *Spirulina platensis* produced comparable biodiesel percentages (8.8 and 7.8 % respectively).

The greatest yields of biodiesel in synthetic media (Tab. 9) were archived by *Nostoc muscorum, Nostoc humifusum* and *Phormedium fragile* (12.52, 10.2 and 10.1% respectively). The produced biodiesel have slightly alkaline pH values ranged between 6.9 and 8.1 in all preparations. The present results were in agreement with those of Afify *et al.* (2010) and Hossain and Salleh (2008).

The highest biodiesel production from *Nostoc species* (*Nostoc muscorum* and *Nostoc humifusum*) observed in this investigation was in good agreement with data reported by Chisti (2007) and Shalaby (2011) who demonstrated that the biodiesel from microalgae seems to be the only renewable bio-fuel that has the potential to completely displace petroleum derived transport fuels. The author added that oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops.

The maximum accumulation of biodiesel after cultivating the microalgae in domestic wastewater (Tab. 10) was obtained by *Nostoc humifusum* (11.80%) when grown in wastewater without any treatment (T_1). However, this value was higher than that obtained when the same species was cultivated in the synthetic medium (control). These results may due to the deficiency of nitrogen concentration in waste water media than the control media and this led to accumulation of lipid and biodiesel. These results are in agreement with the results obtained by Afify *et al.* (2010).

Conclusions

This study provided a proof-of-concept for a wastewater treatment process that combines nutrient removal and algal lipid production for potential use as a biodiesel feedstock. This study also contributed data on cultivation nine algal strains on secondary treated domestic wastewater for lipids production, in addition, the suitability of

the microalgae lipids for biodiesel production by transesterification. Overall, it could be concluded that using the domestic wastewater, as nutrient media for microalgae cultivation, is suitable and non-expensive methods when compared with the conventional cultivation methods for sustainable biodiesel and glycerol production.

References

Abdel Hameed MS (2007). Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal. Afr J Biotechnol 6(10):1185-1191.

Afify AMR, Shalaby EA, Shanab SMM (2010). Enhancement of biodiesel production from different species of algae. Grasas Y Aceites 61(4):416-422.

Albertano P, Pinto G, Santisi S (1971). *Spermatozopsis acidophila* Kalina (*Chlorophyta, Volvocales*), a little-known alga from highly acidic environments. G Bot Ital 115:65-76.

Andersen RA (2005). Algal Culturing Techniques. San Diego, Elsevier Inc, 678 p.

APHA (1998). Standard methods for the examination of water and wastewater, 20 ed., American Public Health Association Washington, DC.

Azov Y (1982). Effect of pH on Inorganic Carbon Uptake in Algal Cultures. Appl Environ Microbiol 43:1300-1306.

Becker EW (2004). Microalgae in human and animal nutrition. In: Richmond A (Ed). Handbook of Microalgae Culture. Biotechnology and Applied Phycology. Oxford: Blackwell Science.

Bligh EG, Dayer WJ (1959). A rapid method for total lipid extraction and purification. Can J Biochem Physiol 37:911-917.

Borowitzka MA (1998). Limits to growth, p. 203-226. In: Wong Y-S, Tam NFY(Eds.). Wastewater treatment with algae, Springer Verlag.

Chevalier P, Proulx D, Lessard P, Vincent WF, De la Noüe J (2000). Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment. J Appl Phycol 12:105-112.

Chisti Y (2007). Biodiesel from microalgae. Biotechnol Adv 25(3):294-306.

Chisti Y (2008). Biodiesel from microalgae beats bio-ethanol. Trends Biotechnol 26:126-131.

Chisti Y, Yan J (2011). Energy from algae: Current status and future trends Algal biofuels-A status report. Appl Energy 88:3277-3279.

Fontes AG, Vargas MA, Moreno J, Guerrero MG, Losada M (1987). Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. Biomass 13:33-43.

Garbisu C, Hall DO, Serra JL (1992). Nitrate and nitrite uptake by free-living and immobilized N-started cells of Phormidium laminosum. J Appl Phycol 4:139-148.

Goldemberg J (2000). World Energy Assessment, Preface. United Nations Development Programme, New York, NY, USA.

Goldman JC, Azov Y, Riley CB, Dennett MR (1981). The effect of pH in intensive cultures. I. Biomass regulation. J Exp Mar Biol Ecol 57:1-13.

Hossain ABM, Salleh A (2008). Biodiesel fuel production from algae as renewable energy. Am J Biochem Biotechnol 4:250-254.

Kar D, Sur P, Mandal SK, Saha T, Kole RK (2008). Assessement of heavy metal pollution in surface water. Int J Environ Sci Tech 5(1):119-124.

Kulkarni MG, Dalai AK (2006). Waste cooking oil-an economical source for biodiesel: A review. Ind Eng Chem Res 45:2901-2913.

Lang X, Dalai AK, Bakhshi NN, Reaney N, Hertz PB (2002). Preparation and characterization of biodiesels from various Bio-Oils. Bioresour Technol 80:53-62.

Langworthy TA (1978). Microbial life at extreme pH values, p. 279-315. In: Kushner DJ (Ed.). Microbial Life in Extreme Environments , Academic Press, New York.

Lardon L, Helias AQ, Sialve B, Steyer J-P, Bernard O (2009). Life-cycle assessement of biodiesel production from microalgae. Environ Sci Technol 3:1-6.

Lau PS, Tam NFY, Wong YS (1997). Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris*. Biores Technol 18:945-951.

Lau PS, Tam NFY, Wong YS (1998). Effect of Carrageenan immobilization on the physiological activities of *Chlorella vulgaris*. Biores Technol 63:115-121.

Leduy A, Therien N (1977). An improved method for optical density measurement of semimicroscopic blue-green alga *Spirulina maxima*. Biotechnol Bioeng 19:1219-1224.

Li F, Xing LZ, Yan CL, An Y (2006). Influence of environmental factors on removal of nitrogen and phosphorus by microalgae. J Shandong Jianzhu Univ 21(3):268-272.

Liu W, Au DWT, Anderson DM, Lam PKS, Jack RJ, Wu RSS (2007). Effects of nutrients, salinity, pH and light:dark cycle on the production of reactive oxygen species in *Chattonella marina*. J Exper Mar Biol Ecol 346:76-86.

McKnight DM, Kimball BA, Runkel RL (2001). pH dependence of iron photoreduction in a rocky mountain stream affected by acid mine drainage. Hydrol Process 15:1979-1992.

Mostafa SM, Ali LKM (2009). Evaluation of humic substances on *Spirulina platensis* growth for preparation of biofertilizer. The 4th Conference on Recent Technologies in Agriculture: Challenges of Agriculture Modernization, Nov. 3rd-5th , Special Edition of Bull. Fac Agric, Cairo Univ 5:918-933.

Mostert ES, Grobbelaar JU (1987). The influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures. Biomass 13:219-233.

Nichols HW, Bold HC (1965). *Trichosarcina polymorpha* gen. et sp. nov. J Phycol 1:34-38.

National Biodiesel Board (2002). USA. Available in www.biodiesel.org.

Oswald W (1988). Large-scale algal culture (engineering aspects), p. 357-395. In: Borowitzka MA, Borowitzka YLJ (Eds.). Microalgal biotechnology Cambridge University Press.

Oswald WJ, Gotaas HB (1957). Photosynthesis in sewage treatment. Trans Am Soc Civ Engng 122:73-105.

Raven JA, De Michelis MI (1979). Plant Cell Environ 2:245-257.

Raven JA (1990). Sensing pH? Plant Cell Environ 13:721-729.

Rectenwald LL, Drenner RW (2000). Nutrient removal from wastewater effluent using an ecological water treatment system. Environ Sci Technol 34(3):522-526.

Richmond A (1986). CRC Handbook of microalgal mass culture Richmond, A. Ed. CRC Press, Inc, Florida.

Rippka R, Deruelles J, Waterbury J, Herdman M, Stanier R (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1-61.

Robinson PK, Reeve JO, Goulding KH (1988). Kinetics of phosphorus uptake by immobilized *Chlorella*. Biotechnol Lett 10:17-20.

Sebnem Aslan IKK (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Engineer 28:64-70.

Shalaby EA (2011). Algal Biomass and Biodiesel Production, Biodiesel-Feedstocks and Processing Technologies. x Stoytcheva M, Montero G (Ed.), InTech, Available from: http://www.intechopen.com/articles/show/title/algal-biomass-and-biodiesel-production.

Shalaby EA, Shanab SMM, Singh V (2010). Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. J Med Plants Res 4(24):2622-2632.

Sharif ABMH, Nasrulhaq AB, Majid HAM, Chandran S, Zuliana R (2007). Biodiesel production from waste cooking oil as environmental benefits and recycling process. A Review. Asia Biofuel Conference Book Dec. 11-13, Singapore.

Sheehan J, Dunahay T, Benemann J, Roessler P (1998). A look back at the US Department of Energy's aquatic species program-Biodiesel from algae. National Renewable Energy Laboratory (NREL) Report: NREL/TP-580-24190. Golden, CO.

Snedecor GW, Cochran WG (1982). Statistical Methods. The Iowa State Univ Press, Ames, Iowa, USA, 507 p.

Spolaore P, Joannis-Cassan C, Duran E, Isambert E (2006). Commercial applications of microalgae. J Biosci Bioeng 101:87-96.

Tadros G (1988). Characterization of *Spirulina* biomass for CFLSS diet potential. Alabama A and M University.

Tam NFY, Lau PS, Wong YS (1994). Wastewater inorganic N and P removal by immobilized *Chlorella vulgaris*. Wat Sci Tech 30(6):369-374.

Turkenburg WC (2000). Renewable energy technologies, p. 219-272. In: Goldemberg J (Ed.). World Energy Assessment, Preface. United Nations Development Programme, New York, USA.

Urrutia I, Sierra JL, Llama MJ (1995). Nitrate removal from water by *Scenedesmus obliquus* immobilized in polymeric foams. Enzyme Microb Technol 17:200-205.

U.S. DOE (2010). Biomass Program Multi-Year Technical Plan. U.S. Department of Energy. Available at: http://www1.eere.energy.gov/biomass/pdfs/mytp.pdf. Accessed 10 July 2010.

Vonshak A (1986). Laboratory techniques for the cultivation of microalgae. In: Richmond A (Ed.). Handbook of microalgal mass culture.

Vonshak A, Richmond A (1988). Mass production of the blue-green algae *Spirulina*. An overview. Biomass 15:233-247.

Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R (2009). Cultivation of green algae *Chlorella* sp. in different wastewater from municipal wastewater treatment plant. Appl Biochem Biotechnol 162(4):1174-86.

Widjaja A (2009). Lipid production from microalgae as a promising candidate for biodiesel production. Makara, Teknol 13:47-51.

Yu, IT, Su JJ, Wu JF, Lee SL, Ju CC, Yen HT (2005). Dietary modification for reducing electrical conductivity of piggery. Wastewater 18(9):1343-1347.

Zarrouk C (1966). Contribution á l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de *Spirulina maxima* (Setch. Et Gardner) Geitler. PhD. Thesis, University of Paris, France.

Zhu M, Zhou PP, Yu LJ (2002). Extraction of Lipids from *Mortierella alpina* and enrichment of arachidonic acid from the fungal lipids bioresour. Technol 84(1):93-95.