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Potential Cultivation of Halophilic Oleaginous Microalgae on Industrial Wastewater

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MICROALGAE cultivation offers an effective solution for wastewater treatment, as they provide a tertiary bio-treatment coupled with the production of valuable biomass, which can be further used for different purposes. Using wastewater for microalgal cultivation is beneficial for minimizing the use of water, reducing the cost of nutrient addition and removing nitrogen and phosphorus from wastewater.

Lipids obtained from marine *Nannochloropsis oculata* and *Tetraselmis chuii* microalgae have received growing attention for the production of biofuels. In the present study, the effluent of El-Malyaa Company (EMC) and Salt and Soda Company (ESC) were used as growth media for the two tested oleaginous marine microalgae. Wastewater was used in different ratios with sterilized seawater, or mixed with synthetic medium (F/2). The growth was evaluated by optical density and dry weight, total lipids content and productivity were also determined.

The results showed that the tested species were capable of growing on the effluent wastewater of the two companies. In addition, dilution of the industrial wastewater with seawater or its mixing with synthetic culture medium (F/2) increased growth of the two tested marine microalgae. The maximum growth was recorded using a ratio of 25:75 of F/2 medium: the effluent of both companies. However, the highest lipid content and lipid productivity were recorded using a ratio of 75:25 of F/2 medium: the effluent of both companies. Therefore, this study suggests that it is possible to utilize a mixture of industrial wastewater and synthetic medium for potential biomass and lipid production from microalgae for biofuel production.

Keywords: Biomass, Lipid, Marine, Microalgae, Oleaginous, Wastewater.

Introduction

Rapid industrialization, population growth, and complete ignoring for environmental health have led to serious environmental pollution (Dash et al., 2013). Among all the environmental pollutions, Water pollution is a critical growing problem (Karuppaiah et al., 2015). Pollution is a man-made phenomenon, arising either when the concentrations of naturally occurring substances are increased or when synthetic compounds are released into the environment (Abdel-Raouf et al., 2012). Releasing of organic and inorganic substances into the environment as a result of industrial, agricultural and domestic water activities causing many organic and inorganic pollution (Mouchet 1986 and Lim et al., 2010). Wastewater is essentially the end product generated by industrial, municipal, agricultural, and domestic sources (Ellis, 2011). These waste water resources can be used as an alternative to the use of synthetic fertilizers, which are rich in organic and inorganic pollutants such as nitrogen and phosphorus (Sutherland et al., 2014),

Simultaneously, with the cultivation of microalgae using wastes and wastewaters for biomass production these pollutants could be removed from the aquatic environment. Compared to physical and chemical water treatment processes, algae biotreatment can potentially achieve organic wastes removal in a less expensive and enviromentally safer way with the added benefits of resource recovery and recycling (Oswald, 2003). Microalgae assimilate a potenial amount of nutrients because they require

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high amounts of phosphorus and nitrogen for the synthesis of 45–60 % proteins of microalgal dry weight, nucleic acids and phospholipids. Nutrient removal can also be increased by NH_3 removing or phosphorus precipitation due to the increasing of the pH associated with photosynthesis (Oswald, 1988).

Recent researches indicated significance mass production of algal biomass for biofuel and other applications using wastewaters and lipids obtained from microalgae have received more attention during the last decade for the production of biofuels (Zhou, 2011). Biofuels are considered to be one of the potenial alternative energy sources, as they are renewable, and environment-friendly. Microalgae were discussed to be the promising source for biodiesel due to their simplicity structure, higher growth rate and higher lipid content than terrestrial plants, moreover, most of microalgae can complete an entire growing cycle every few days, which results in higher oil productivity than other oil crops (Stephens et al., 2010). The yield of oil from algae is over 200 times the yield of soybean oils (Gouveia & Oliverira, 2009). Furthermore, microalgae can also be grown on non-arable lands (e.g. desert, seashore, rocky and sandy lands) which do not compete with food crops and can use saline water instead of fresh water of irrigation and drinking. Microalgae are able to produce various forms of biofuels including microalgal lipid-derived biodiesel (Scott et al., 2010), bioethanol by fermentation of carbohydrate (Dismukes et al., 2008), and biohydrogen from photosynthesis or fermentation (Hemschemeier et al., 2009).

In earlier 2000s, there were less or no researches were carried out in such a way which integrates the treatment of industrial wastewater with biomass productivity (Umamaheswari & Shanthakumar, 2016). In Egypt, Many factories drain there industrial wastes in the river and the canal causing serious environmental problems, affecting negatively the stability of many aquatic ecosystems and can also cause adverse effects to human health and the environment directly or indirectly. In our study we focused on two companies, Salt and Soda and Elmalyaa in Kafr El-zayat that drain their wastes directly to the Ganag drain for mass production and lipid content of two marine microalgal species.

Materials and Methods

Culture media

The F/2 medium contained ($mg.L^{-1}$) $NaNO_3$, 75; $NaH_2PO4.H_20$, 5; Na_2 EDTA. H_20, 4.16; $FeC1_3.6H_20$, 3.15; $CuSO_4.5H_20$, 0.01; $ZnSO_4.7H_20$, 0.022; $CoC1_2.6H_20$, 0.01; $MnC1_2.4H_20$, 0.18; $Na_2MoO4.2H_20$, 0.006; Vitamin B_{12}, 0.0005; Vitamin B_1, 0.1; and Bi-tin, 0.0005 (Guillard & Ryther, 1962). The seawater used in the study was obtained from Alexandria Beach (Egypt). The wastewater used as a nutrient source was collected from Ganag drain in Kafr El-zayat City El-Gharbya Governorate, Egypt, that receives the effluent of Salt and Soda (ESC), which produce oil, soap and fodder and El-malyaa Company (EMC) which produce chemicals.

Physico-chemical characteristics of water samples

The initial physico-chemical analysis of wastewater samples was made before inoculation of tested microalgal species. Total dissolved solids (T.D.S) were measured using YSI Model 33 (yellow springs) S-C-T meter, pH: using a pocket pH meter (Research model 201/ Digital pH meter), Nitrate–nitrogen according to (Strickland & Parsons, 1965), Ammonia–nitrogen according to Jackson (1960) and Allen et al. (1974) and Phosphate according to Strickland & Parsons (1972)

Tested microalgal species

The species selected for cultivation were a strain of *Tetraselmis chuii* and *Nannochloropsis oculata* were kindly provided from Invertebrate Lab., National Institute of Oceanography and Fisheries, Alexandria, Egypt. The microalgal species were chosen due to their high lipids contentaccording to literature screening.

Cultivation conditions

The overall ability of the two tested species to use the effluent of industrial wastewater of the two companies as a nutrient source was evaluated, then evaluating the growth of tested species on the diluted industrial wastewater with sterilized and filtered seawateror on mixed wastewater with synthetic F/2 (Guillard & Ryther, 1962) enriched medium with four different concentrations: (25%, 50%, 75% and 100%). Certain volume of exponentially growing pre cultured microalgae strains were inoculated to 650ml of each specific sterilized culture medium (F/2 enriched seawater medium) in 1L Erlenmeyer flasks used as a control. The same volume of exponentially

growing pre cultured microalgae strains were inoculated to 650ml of the mixture of wastewater (EMC and ESC) and sterilized and filtered seawater or with synthetic medium F/2 in 1L Erlenmeyer flasks (25%, 50%, 75% and 100%). Aeration was provided to culture through silicon pipes whose one end is inserted into the culture flask and other end to the aerator which helped to provide aeration for proper mixing of cells in the culture flasks. Algal culture flasks were then incubated under continuous fluorescent light of 80µmol m-2s^{-1} and temperature at 26ºC±1ºC.

Growth assay

Growth curves were evaluated by measuring the optical density at 680nm using spectrophotometer (SHIMADZU UV- 2401PC, Japan) and algal dry weight (g.L^{-1}). Dry weight was estimated by centrifugation of 40ml of the culture at 2000 round for 10min, and then cell pellets were washed twice using distilled water, and then dried in the oven at 80°C until constant weight. The biomass productivity BP (g.$L^{-1}d^{-1}$) was calculated by equation: BP = (WBF –WB0)/t, where WB0 and WBF are the weights of dry biomass at the beginning and the end of a batch run and t is the overall culture time.

Estimation of total lipids

Extraction of lipids was done using chloroform: methanol (2:1) according to the method described by Folch et al. (1957). The pre-weighted glass vials containing the lipid extracts were dried at 80°C for 30 min, cooled in a desiccator and weighed. Lipids productivity PL was calculated by the equation: PL = (WLF –WL0)/t, where WL0 and WLF are the weights of lipids content at the beginning and the end of a batch run and "t" is the overall culture time.

Statistical analysis

Results are presented as mean±standard deviation of the mean, n=3. The statistical analyses were carried out using the SPSS program, SPSS 10.0 Software (SPSS, Richmond, VA, USA) as described by Dytham (1999). Data were analyzed to determine the degree of significance between treatments using one way analysis of variance (ANOVA) with Duncan's multiple range tests for comparison of the significance level between values at $P<0.05$ level of significance.

Results and Discussion

Wastewater analysis

The wastewater used in the experiments that was collected from Ganagdrain was analyzed to estimate its different characters and the results are shown in Table 1. Before cultivation of tested microalgal species we sterilized the effluent of the two companies in order to kill any microbial contaminants as well as any pathogen. The chemical composition including essential nutrients of wastewater may be even changed under the effect of sterilization due to high pressure and temperature. Where, the stability of compounds and the affinity between different elements decreased, so more nutrients will be released such as NO_3 and NH_3 and others will be decomposed such as organic matter while the values of TDS and PO_4 decreased due to precipitation. So, the values of TDS and PO_4 decreased but values of pH, NO_3 and NH_4 increased and these results were in agreement with the results obtained by El-Sheekh et al. (2005) and Stover et al. (1976) who mentioned that the nutrients and metals are presented in complex organic form in sludge and are rather stable under normal conditions due to retention mechanisms including ion exchange, sorption, chelating and precipitation.

TABLE 1. Characterization of the wastewater of El- Malyaa Company (EMC) and Salt and Soda Company (ESC) before and after sterilization.

Water sample	pH		TDS mg.L^{-1}		PO_4 mg.L^{-1}		NO_3 mg.L^{-1}		NH_3 mg.L^{-1}	
	ESC	EMC	ESC	EMC	ESC	EMC	ESC	EMC	ESC	EMC
Raw waste water	6.5± 0.025	7.12± 0.033	827± 2	734± 1.5	5.06± 0.034	10.05± 0.045	3.87± 0.005	4.95± 0.045	0.105± 0.001	0.09± 0.001
Waste water after sterilization (control)	7.1± 0.027	7.6± 0.031	683± 2.4	543± 1.4	4.1± 0.033	8.65± 0.055	4.9± 0.033	5.8± 0.045	0.142± 0.001	0.129± 0.001

Cultivation of tested microalgal species on a different dilution of treated industrial wastewater (ESC or EMC) and sterilized and filtered seawater

Growth curve

The first test was carried out in order to evaluate whether the selected *N oculata* and *T. chuii* could be able to utilize nutrients from industrial wastewater. Results showed that both of the tested species could grow using wastewater of the two companies and also dilution increased their growth efficiency. The highest value of optical density was reached at 10th day of incubation on control of F/2 enriched medium that was 0.661 O.D. with *T. chuii* and 0.563 O.D. with *N. oculata* while, 75% EMC showed the highest growth of the diluted samples with 0.302 O.D. in case of *T. chuii* and with 0.252 O.D. with *N oculata.* 100% ESC showed the lowest growth values that were (0.088 and 0.155 O.D.) with *N oculata* and *T. chuii*, respectively as apparent in Fig.1 and 2.

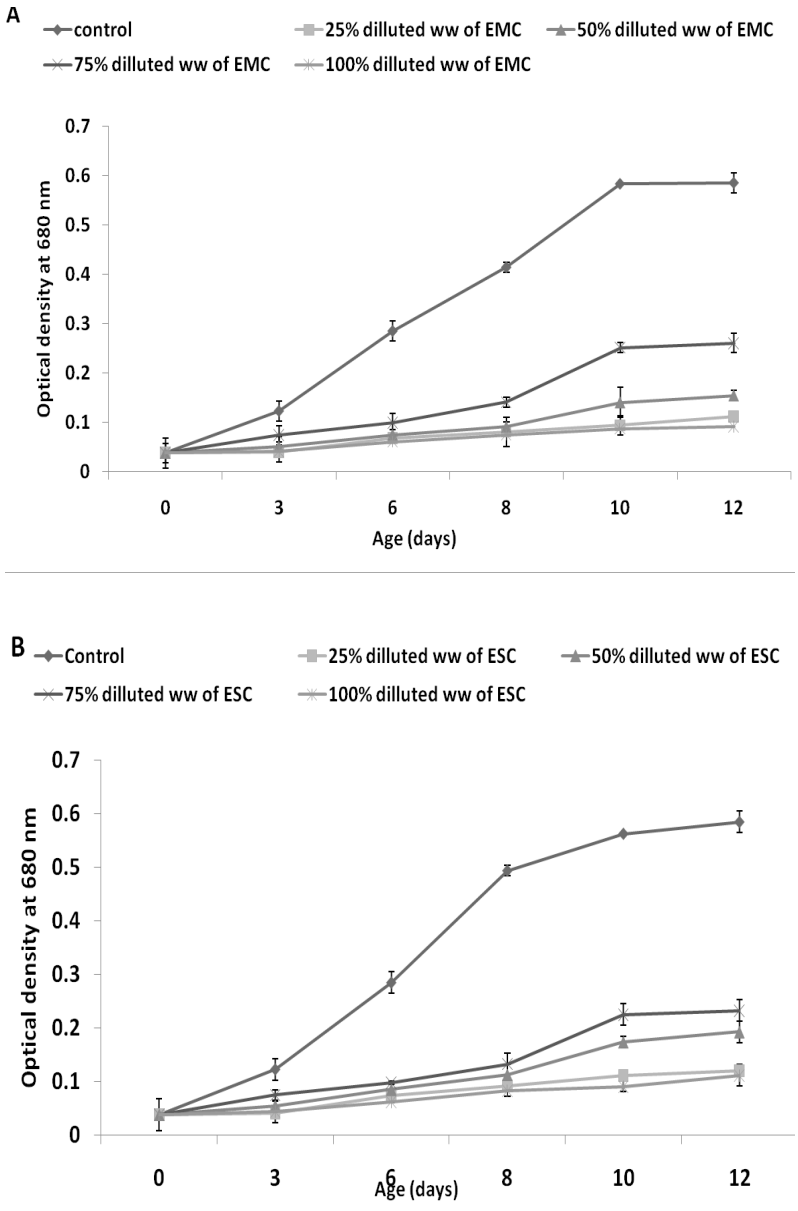

Fig. 1. Growth curves of *N. oculataon* diluted treated industrial wastewater of (A) El- Malyaa Company (EMC), (B) Salt and soda Company (ESC) with seawater measured as optical density at 680nm. Each point represents the mean value of three replicates; bars indicate standard deviations.

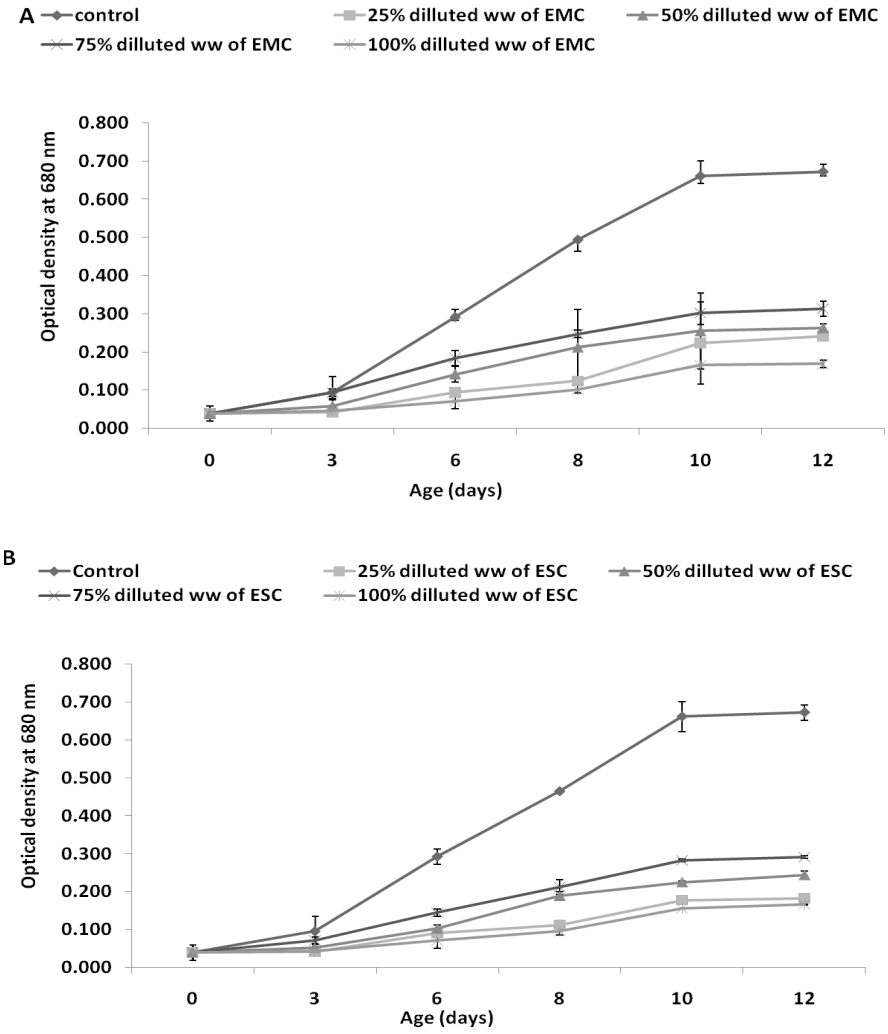

Fig. 2. Growth curves of *T. chuii* on diluted treated industrial wastewater of (A) El- Malyaa Company (EMC), (B) Salt and Soda Company (ESC) with seawater measured as optical density at 680nm. Each point represents the mean value of three replicates; bars indicate standard deviations.

Dry weight and biomass productivity

The amount of dry weight and biomass productivity obtained from each culture medium at the end of the experiment is shown in Table 2. *T. chuii* showed higher dry weight and biomass productivity than *N. oculata* and the highest values (the most significant) of dry weight and biomass productivity were observed at cultivation of tested species on F/2 medium only and these results were in agree with Sousa et al. (2014) who mentioned that the cell density of synthetic medium was higher than the cell concentrations in the experiments with wastewater addition as pure synthetic culture medium provide algae with their all required nutrients showing the best growth rates. Regarding to different dilution concentrations, 75% WW concentration showed the highest growth relative to the other dilutions. While 25% ESC, showed the lowest values (0.22 $g.L^{-1}$ with *N. oculata* and 0.25 $g.L^{-1}$ with *T. chuii*) and the slowest growth rates were found in case of using 100% effluent of wastewater and it was in accordance with Wang et al. (2013) who tried to determine the optimum conditions for *Chlorella* sp. through diluting the municipal wastewaters in to four different concentrations (25, 50, 75 and 100%) and found 50% diluted effluent of wastewater recorded that the highest biomass (0.258 $g.L^{-1}$), but like our results they found that the lowest and slowest growth rates were found in 100% effluent of wastewater due to high concentrations of different constituents of wastewater that strongly reduce microalgal growth.

TABLE 2. Dry weight and biomass productivity of tested microalgae on diluted industrial wastewater of A, El-Malyaa Company (EMC); B, Salt and Soda Company (ESC) with seawater at the end of exponential phase. Each point represents the mean value of three replicates. Different letters represent significant differences at P<0.05 (Duncans).

Microalgae	*N. oculata*		*T. chuii*	
Treatments	Dry weight ($g.L^{-1}$)	Biomass productivity ($g.L^{-1}.d^{-1}$)	Dry weight ($g.L^{-1}$)	Biomass productivity ($g.L^{-1}.d^{-1}$)
Control	0.55 ± 0.01^{A}	0.054 ± 0.002^{A}	0.68 ± 0.02^{A}	0.073 ± 0.003^{A}
25% ESC	0.22 ± 0.02^{H}	0.017 ± 0.001^{BCD}	0.25 ± 0.01^{E}	0.026 ± 0.004^{D}
50% ESC	0.3 ± 0.03^{D}	0.026 ± 0.003^{BC}	0.32 ± 0.03^{C}	0.034 ± 0.001^{BC}
75% ESC	0.39 ± 0.03^{B}	0.033 ± 0.001^{BC}	0.41 ± 0.01^{B}	0.043 ± 0.001^{B}
100% ESC	0.24 ± 0.02^{G}	0.015 ± 0.002^{CD}	0.28 ± 0.02^{D}	0.027 ± 0.002^{D}
25% EMC	0.25 ± 0.02^{F}	0.018 ± 0.004^{D}	0.28 ± 0.02^{D}	0.028 ± 0.004^{C}
50% EMC	0.32 ± 0.01^{C}	0.026 ± 0.005^{B}	0.41 ± 0.03^{B}	0.038 ± 0.005^{B}
75% EMC	0.39 ± 0.01^{B}	0.034 ± 0.001^{BC}	0.30 ± 0.01^{C}	0.032 ± 0.001^{BC}
100% EMC	0.29 ± 0.02^{E}	0.019 ± 0.002^{D}	0.32 ± 0.02^{C}	0.032 ± 0.003^{BC}
F value	506***	450**	670***	607***

** Significant at $P \leq 0.05$,*** Significant at $P \leq 0.005$ and (ns) Non-significant at $P \leq 0.01$ using one way analysis of variance (ANOVA).

Total lipids content and lipids productivity of tested microalgae

It could be observed that increasing in seawater concentration for dilution of wastewater did not effect on lipids content of tested species and pure wastewater showed the highest lipids content and productivity although it recorded the lowest growth rates and it was in accordance with Karpagam et al. (2015) who found that the stress created that decreased biomass, causing an increasing in lipid content and also Davis et al. (2016) mentioned that, decreased growth rate leads to lipid accumulation. Both of tested microalgal species recorded the highest lipids content and productivity on pure wastewater of El-Malyaa Company that was $0.121 g.L^{-1}$ $0.012 g.L^{-1}.d^{-1}$ for *T. chuii* and , 0.114 $g.L^{-1}$, 0.008 $g.L^{-1}.d^{-1}$ for *N. oculata*, respectively. Cultivation of tested species on waste water elevated % of lipids of dry weight than the control by 20% and 17% with *T. chuii* and 17% and 10% with *N. oculata* in case of cultivation on ESC and EMC, respectively (Fig. 3).

Cultivation of tested microalgal species on a mixture of different concentrations of treated industrial wastewater (ESC or EMC) and F/2 enriched medium

Growth curve

It was clearly observed that mixing wastewater with synthetic medium caused significant increase in microalgal growth than its dilution with seawater and it was in agree with Onalo et al. (2014) who mentioned that incase of dilution the available nutrients in diluted wastewater are not sufficient enough for microalgal growth and lower than that available in case of normal artificial media that increase availability of nutrients and supply microalgal cells with different nutrients that are not available in wastewater for enhancing of their growth. 75% ESC was the best mixture recording the highest OD value that was (0.455 and 0.352) in case of *N. oculata* and *T. chuii*, while 100% ESC showed the minimum values with tested species as apparent in Fig. 4 and 5.

Dry weight and biomass productivity of tested microalgae

The results indicated that the microalgal growth increased by increasing the concentration of wastewater in the mixture and the highest dry weight and biomass productivity were recorded in case of mixing 75% of industrial wastewater with 25% of enriched F/2 medium regarding other mixtures and the obtained results were relatively close to that of the tested species cultivated on pure synthetic medium only, while cultivation on pure wastewater showed the lowest growth. The obtained results were in accordance with Sousa et al. (2014) who found that increasing wastewater concentration addition 22% did not obviously increased algal growth, that might be due to the relatively small increase in wastewater concentration. But when they added 44%, cell growth was almost two times higher the addition of 19 and 22% of the wastewater. *T. chuii* showed higher dry weight and biomass productivity (0.611 $g.L^{-1}$ and $0.057 g.L^{-1}.d^{-1}$) than *N. oculata* (0.587 $g.L^{-1}$ and $0.043 g.L^{-1}.d^{-1}$) on 75% EMC. It was observed a highly significant effect of the changes of concentrations and companies on dry weight and biomass productivity and there interactionin the two-way ANOVA at $P < 0.05$ (Table 3).

Total lipids content and lipids productivity of tested microalgae

In case of mixing with artificial media, 25% wastewater: 75% artificial media (that showed the lowest growth) showed the highest total lipids content that was relatively close to that of 100% wastewater due to the created stress that decreased biomass, causing an increasing in lipid content. There was a clear significant increase in total lipids content and productivity of *N. oculata* when it was cultivated on 25% EMC than the control. *N. oculataa* showed higher lipids content and productivity than *T. chuii* (0.185g.L^{-1} and 0.02g.L^{-1}.d^{-1}) in case of 25% EMC and *T. chuii* showed its highest lipids content (0.140g.L^{-1}) with 25% ESC and (0.135g.L^{-1}) and the highest lipids productivity (0.015g.L^{-1}.d^{-1}) with 25 % EMC. Mixing of wastewater with synthetic medium caused an obvious increase in the percentage of total lipids of dry weight than the control by 30% and 20% with *N. oculata* in case of using 25% ESC and 25% EMC, respectively and by 20% and 8% with *T. chuii* in case of using 25% ESC and 25% EMC, respectively (Fig. 6).

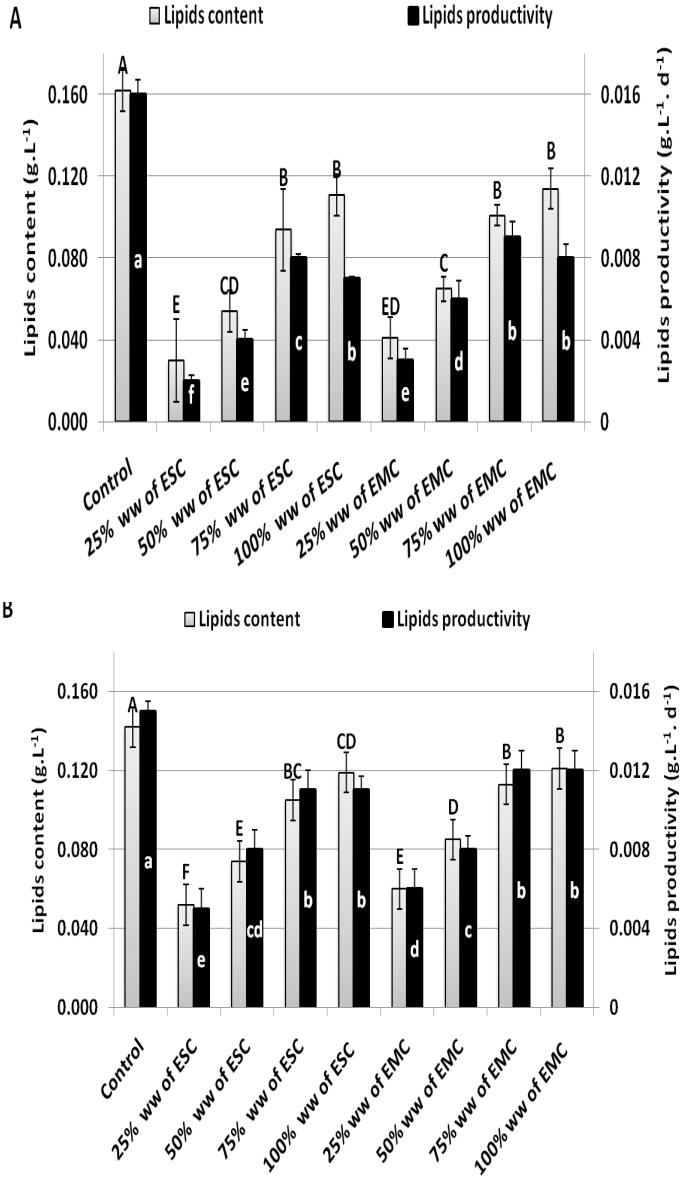

Fig. 3. Total lipids content and lipids productivity of A, *N. oculata* and B, *T. chuii* on diluted industrial wastewater (ESC) or (EMC) and seawater during the end of exponential phase. Each point represents the mean value of three replicates; bars indicate standard deviations. Different letters represent significant differences at P < 0.05 (Duncans).

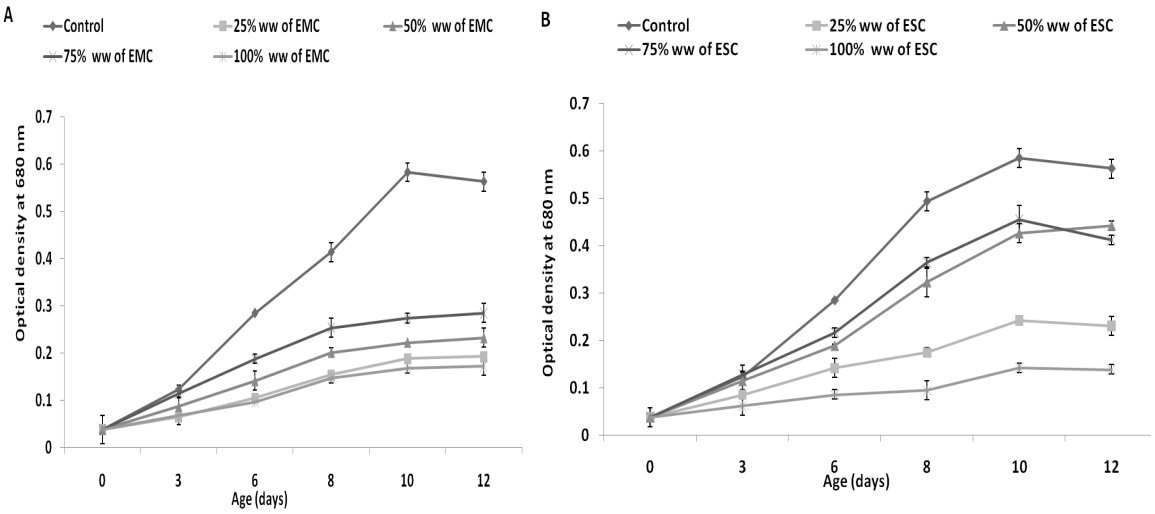

Fig. 4. Growth curves of *N. oculata* on industrial wastewater of A, El- Malyaa Company (EMC); B, Salt and Soda Company (ESC) and F/2 medium as optical density at 680nm. Each point represents the mean value of three replicates; bars indicate standard deviations.

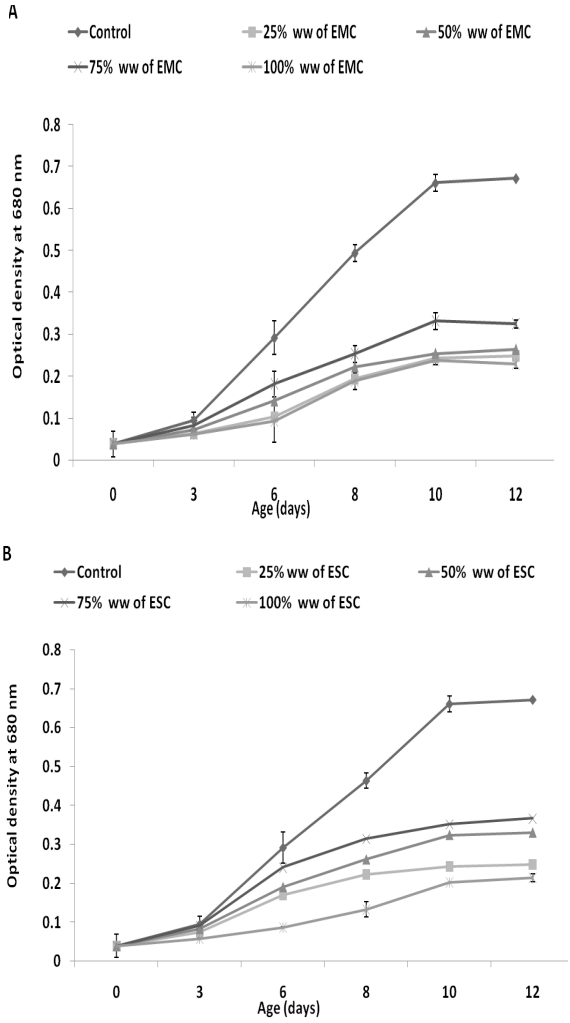

Fig. 5. Growth curves of *T. chuii* on industrial wastewater of A, El- Malyaa Company (EMC); B, Salt and Soda Company (ESC) and F/2 medium as optical density at 680nm. Each point represents the mean value of three replicates; bars indicate standard deviations.

TABLE 3. Dry weight and biomass productivity of tested microalgae on industrial wastewater of A, El- Malyaa Company (EMC); B, Salt and Soda Company (ESC) and F/2 medium at the end of exponential phase. Each point represents the mean value of three replicates. Different letters represent significant differences at P < 0.05 (Duncans).

Microalgae	*N. oculata*		*T. chuii*	
Treatments	**Dry weight ($g.L^{-1}$)**	**Biomass productivity ($g.L^{-1}.d^{-1}$)**	**Dry weight ($g.L^{-1}$)**	**Biomass productivity ($g.L^{-1}.d^{-1}$)**
Control	0.59 ± 0.01^{A}	0.058 ± 0.001^{A}	0.722 ± 0.05^{A}	0.077 ± 0.003^{A}
25% ESC	0.311 ± 0.03^{E}	0.025 ± 0.001^{G}	0.352 ± 0.01^{G}	0.03 ± 0.001^{H}
50% ESC	0.383 ± 0.04^{D}	0.035 ± 0.002^{D}	0.411 ± 0.02^{F}	0.038 ± 0.002^{F}
75% ESC	0.455 ± 0.04^{B}	0.041 ± 0.001^{B}	0.514 ± 0.03^{C}	0.048 ± 0.002^{C}
100% ESC	0.235 ± 0.01^{G}	0.017 ± 0.001^{I}	0.315 ± 0.03^{H}	0.028 ± 0.001^{I}
25% EMC	0.385 ± 0.02^{D}	0.032 ± 0.001^{F}	0.488 ± 0.03^{E}	0.045 ± 0.003^{E}
50% EMC	0.435 ± 0.02^{C}	0.033 ± 0.002^{E}	0.515 ± 0.02^{C}	0.046 ± 0.001^{D}
75% EMC	0.587 ± 0.04^{A}	0.043 ± 0.002^{B}	0.611 ± 0.03^{B}	0.057 ± 0.002^{B}
100% EMC	0.305 ± 0.01^{F}	0.023 ± 0.001^{H}	0.335 ± 0.01^{H}	0.031 ± 0.002^{G}
F value	6016***	1516***	165367***	2176***

** Significant at P ≤ 0.05 ,*** Significant at P ≤ 0.005 and (ns) Non-significant at P ≤ 0. 01 using one way analysis of variance (ANOVA).

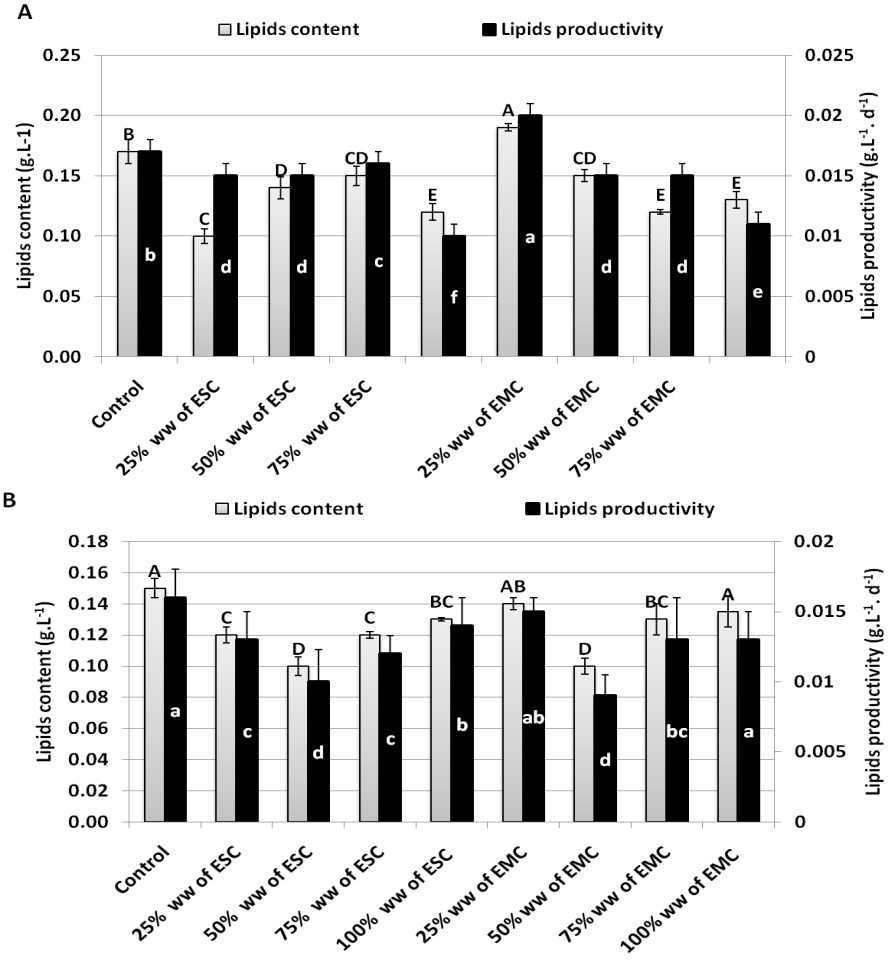

Fig. 6. Total lipids content and lipids productivity of of A, *N. oculata* and B, *T. chuii* on industrial wastewater (ESC) or (EMC) and F/2 medium during the end of exponential phase. Each point represents the mean value of three replicates; bars indicate standard deviations. Different letters represent significant differences at P < 0.05 (Duncans).

Conclusion

The possibilities of the economic importance of microalgal biomass in biodiesel production are well known. The results of this study suggest that cultivating microalgae on industrial wastewater combines nutrients removal and algal lipid production for potential use as a biodiesel feedstock. Additionally, using the industrial wastewater, as nutrient media for microalgae cultivation, is suitable and non-expensive method. Cultivation of tested strains on a mixture of industrial wastewater and synthetic medium increased their total lipids content and its percentage of dry weight than the cultivated species on synthetic medium only.

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امكانية زراعة الطحالب الزيتية المحبة للملوحة على مياه الصرف الصناعي

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زراعة الطحالب الدقيقة توفر حلاً فعالاً لمعالجة مياه الصرف الصحي، لأنها توفر المعالجة حيوية ثلاثية بالإضافة إلى انتاج كتلة الحيوية قيمة، والتي يمكن استخدامها بشكل كبير لأغراض مختلفة. فان استخدام مياه الصرف الصحي لزراعة الطحالب الدقيقة مفيد للحد من استخدام مياه الشرب، والحد من تكلفة إضافة العناصر الغذائية وباضافة إلى تنقية مياه الصرف الصحي من النيتروجين والفوسفور.

فقد اثبتت النتائج أن الدهون التي تم الحصول عليها من كل من *Nannochloropsis oculata* و *Tetraselmis chuii* اهتماما متزايدا لإنتاج الوقود الحيوي. وقد تم استخدام مياه الصرف الصناعى لشركة المالية (EMC) وشركة الملح والصودا (ESC) كوسط نمو للطحالب البحرية الزيتية التي تم اختبارها. كما استُخدمت بنسب مختلفة مع مياه البحر المعقمة، أو بإضافتها إلى وسط غذائي صناعى (F/2). وتم تقدير النمو من خلال الكثافة البصرية والوزن الجاف، كما تم تحديد محتوى الدهون الكلي والإنتاجية الكيلة للدهون.

وأظهرت النتائج أن الأنواع التي تم اختبارها كانت قادرة على النمو على مياه الصرف الصحي من الشركتين. بالإضافة إلى ذلك، فقد أدى تخفيف مياه الصرف الصناعية بمياه البحر أو اضافتها مع وسط الاستزراع الاصطناعي (F/2) إلى زيادة نمو الطحالب البحرية التي تم اختبارها. وتم تسجيل الحد الأقصى للنمو باستخدام نسبة 25:75 من متوسط F/2: مياه الصرف الصناعى للشركتين. ومع ذلك، تم تسجيل أعلى محتوى للدهون وإنتاجية الدهون باستخدام نسبة 75:25 من وسط F/2: مياه الصرف الصناعى للشركتين. ولذلك، تقترح هذه الدراسة أنه من الممكن استخدام خليط من مياه الصرف الصناعي والوسيط الصناعي لإنتاج الكتلة الحيوية والدهون من الطحالب الدقيقة لإنتاج الوقود الحيوي.