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Microalgae growth in effluents from olive oil industry for biomass production and decreasing phenolics content of wastewater

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ABSTRACT

The present study investigates the possibility of growing microalgae in wastewater generated from olive production factory. Wastewater samples were collected from the three stages of olive oil production as well as from the final effluent of the factory. The analysis results of the effluent revealed that it contains: nitrogen (0.31%), phosphorus (0.59%), potassium (0.70%), calcium (741ppm), iron (59.78 ppm), magnesium (301 ppm), cupper (5.45 ppm), sulfur (314 ppm) and high amount of phenolic compounds (2999.4 mg /L). *Nostoc muscorum*, *Anabaena oryzae* and *Spirulina platensis* were chosen as three blue-green microalgae and were cultivated in standard media and two media from olive oil industry effluents. The three microalgae were cultivated over 30 days period on olive effluent (OWW) and diluted effluent (OWW 50%). The growth of the three strains in diluted media OWW50% showed better results as expressed from the total chlorophyll, optical density and biomass dry weight. The total chlorophyll content values of *Spirulina*, *Anabaena* and *Nostoc* genera were 2.3, 1.87 and 1.87µg/L, respectively. Dry weights obtained in diluted OWW50% were 1.72, 1.45 and 1.28g/L for *Spirulina*, *Anabaena* and *Nostoc*, respectively. The values of optical density were 0.38 for *Spirulina*, 0.29 for *Anabena* and 0.25 in case of *Nostoc*. During algae cultivation, the initial phenols concentration was reduced by 50-60% in 15 days, and more than 80% of phenols were removed by day 30. Thus blue-green microalgae could be grown on olive oil wastewater for biomass production and wastewater treatment of phenolic compounds.

INTRODUCTION

Wastewater treatment and reuse can be considered a promising alternate solution for water conservation as the problem of water scarcity is increasing all over the world. Agro-food industries are major contributors to worldwide industrial pollution. The effluents resulting from different agro-food industries contain high load of organic pollutants which need appropriate management(Raposo *et al*., 2010).

Since several decades, olive oil extraction sector has been one of the important industries in the Mediterranean countries (Gholamzadeh *et al*., 2016).

At present in Egypt; olive mill wastewater is usually discharged into open environment with no suitable method applied for its treatment, thus producing disturbance of biological activities, soil surface and underground water pollution (Omer, 2012). It is thus very important to find out efficient as well as economic methods for olive mill wastewater treatment.

Olive mill wastewater is characterized by its intense purple-brown to black color, odor of olive oil, acidic pH , high salinity , rich in mineral nutrients, organic matter and phenolic compounds (Tekaya *et al*., 2018; Meftah *et al*., 2019).

The use of microalgae to treat contaminated wastewater is an economic and environmental friendly method with no secondary pollution as the resulting biomass is reused and allows proficient nutrient recycling (Rawat *et al*., 2011).

Microalgae are favorable for use in treating wastewaters due to their ability to take up nutrients and convert them into biomass. Further, the obtained biomass from microalgae cultivation in wastewater can be used as a commercial value added product such as biodiesel (El Shimi and Mostafa, 2016), pharmaceuticals, food, animal feed, chemicals and cosmetics (Labbé *et al*., 2017) and biofertilizers (Rashad *et al*., 2019).

The aim of the present work investigates the development of microalgae cultures in olive oil industry wastewater and the effect of microalgae on decreasing phenolic content of wastewater.

MATERIALS AND METHODS

Wastewater collection and analysis

olive oil industry wastewater (OWW) generated by the three stages of phases olive-oil production process was obtained from ALJAZEERA local factory an olive oil factory, at Ismailia, (Egypt).The samples were collected in tightly closed bottles then filtered and the supernatant was collected to be used in further experiments.

Table 1 shows the wastewater quality characteristics e.g. pH, electrical conductivity value (EC) (µs/cm), salinity (ppt) and total suspended solids (TSS) (mg/L) were determined by following the recommended standard procedures (APHA, 2005).

Table-1: Chemical properties of OWW

Wastewater	OWW1*	OWW2**	OWW3***	OWW****
pH	4.60	4.53	4.73	4.51
EC; $\mu s.cm^{-1}$	12.7	15.9	30.5	14.85
Salinity ; ppt	7.2	9.2	18.7	8.41
TSS;mg/L	3636	99484	613800	44241
Total phenol ; mg /L	294.5	3797.4	4872.93	2999.4
NO_3 mg/l	10.97	1.69	4.69	5.12
NO_2 mg/l	977	8.69	21.46	10.47
N%	0.30	0.20	0.50	0.31
P%	0.05	0.70	1.1	0.59
K%	0.2	0.60	1.4	0.70
Ca ppm	616.7	318.8	1750	741
Fe ppm	68.19	48.18	76.40	59.78
Mg ppm	143.7	189.3	541.3	301
Cu ppm	5.1	6.22	6.46	5.45
S ppm	258	301	386	314

*OWW1: wastewater from 1st stage: washing step
**OWW2: wastewater from 2nd stage: olive milling
***OWW3: wastewater from 3rd stage: oil extraction and separation of oil from solid waste)
****OWW: wastewater from the end effluent of the factory

Nitrogen, nitrite, nitrate and mineral analyses were determined using the official methods described by AOAC (1990). Phenolic compounds were determined by Folin method (Singleton *et al*.,1999).

Microalgae strains

Three algae strains namely: *Nostoc muscorum*, *Anabaena oryzae* and *Spirulina platensis* were kindly supplied from Department of Microbiology; Soils, Water and Environment Research Institute (SWERI); Agricultural Research Center (ARC), Giza, Egypt.

Standard media were used for the culture of microalgae where Blue green algae (BG11) medium was used in case of *Nostoc muscorum* and *Anabaena oryzae* whereas Zarrouk medium was used in case of *Spirulina platensis*. The cyanobacteria strains were also cultured on media containing OWW and diluted OWW (50%). Culture suspensions at log phase were inoculated at the rate of 20% ($V_{inoculum}$/ V_{media}).Cultures were incubated in growth chamber under continuous shaking (150 rpm) and illumination (2000 lux) at 27± 2°C for 30 days to be used as inoculum for laboratory experiments. In case of *Spirulina platensis*, all dilutions were supplemented with 5 g/L $NaHCO_3$ to high alkalinity mandatory for the growth (Markou *et al*., 2012).The microalgae culture experiment consisted of three standards and six treatments as given in Table (2).

Table 2: Experimental design

Treatment	Strain + Culture media
S1	*Spirulina platensis*+ Zarrouk medium
S2	Anabaena oryzae+ BG11 medium
S3	*Nostoc muscorum* + BG11 medium
M1	*Spirulina platensis* + OWW 100%
M2	*Anabaena oryzaen*+ OWW 100%
M3	*Nostoc muscorum*+ OWW 100%
M4	*Spirulina platensis* + OWW 50%
M5	*Anabaena oryzaen* + OWW 50%
M6	*Nostoc muscorum*+ OWW 50%

Determination of microalgae growth in OWW

Algal biomass development was monitored on three days interval during the 30 days of experiment. The monitored parameters included: dry weight(g/L), optical density at 560nm (Mostafa *et al*., 2012). Total chlorophyll content (µg/L) was measured by spectrophotometer using the modified method of Arnon (Jia *et al*., 2017).

Effect of alga growth on phenols in wastewater

The effect of cyanobacteria culture on total phenols removal from OWW was evaluated by determining the phenolic content during growth experiment and calculating the removal percentage as follows:

Removal percentage (%) = 100 x (C_i-C_f/ C_i), where C_i is the initial concentration of phenols and C_f is the concentration of phenols at during growth experiment.

RESULTS AND DISCUSSION

Microalgae growth in OWW

The characteristics of the final effluent OWW as given in Table (1) shows that it contains nitrogen, phosphorus and other micronutrients in suitable levels that may sustain microalgae growth. Previous studies showed that wastewater containing nutrients was rich media for cultivating algae (Arbib *et al*., 2014; Mukherjee *et al*., 2016;Labbé *et al*., 2017).

Spirulina, *Anabeana* and *Nostoc* were cultured in olive industry wastewater and their growth parameters including dry weight, total chlorophyll and optical density were monitored on three days basis during the 30 days of culture. Figures 1-3 show the chlorophyll content, optical density, dry weight of *Spirulina*, *Anabaena* and *Nostoc* cultivated on standard media and different concentrations of OWW media either 100% or diluted to 50%. Results revealed that the chlorophyll content in both OWW50% and OWW100% increased with increasing the cultivation period. At the end of the experiment the chlorophyll content values of *Spirulina*, *Anabena* and *Nostoc* were 2.3, 1.87 and 1.87µg/L, respectively. During the growth experimental period it was noticed that the cyanobacteria biomass grown on media containing OWW50% had increased chlorophyll content compared to the biomass cultivated on OWW100% media. Chlorophyll provides an important tool related to the growth of phototrophic organisms. There is considerable evidence that supports the fact that the amount of chlorophyll is positively correlated with cell density or biomass (Loaiza *et al*., 2016).

It was also noticed that different media supported growth of the three investigated strains in different rates; however better results of dry weight were obtained in diluted OWW50% as follows 1.72, 1.45 and 1.28g/L for *Spirulina*, *Anabena* and *Nostoc*, respectively.

The optical densities of the different microalgae biomass were determined to give an indication of cell growth. The optical density of algae in OWW 50% was generally much higher than those found in most of the treatments. The maximum values of optical density were 0.38 for *Spirulina*, 0.29 for *Anabena* and 0.25 in case of *Nostoc* at the 30th day of experiment in OWW 50%.

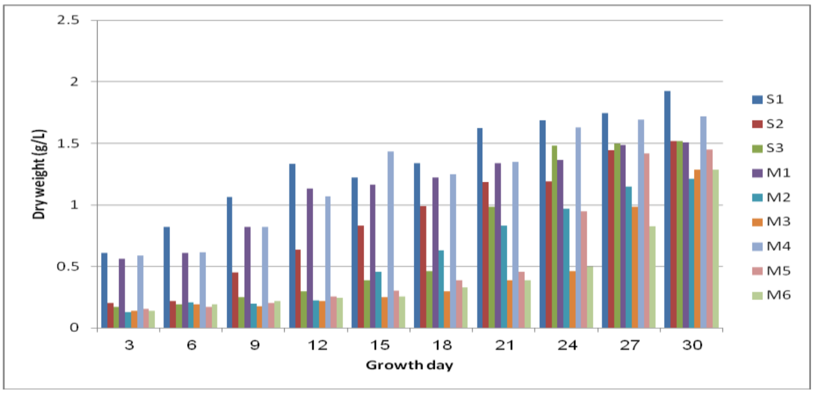

Fig. 1: Dry weight of *Spirulina*, *Anabaena* and *Nostoc* cultivated on different media

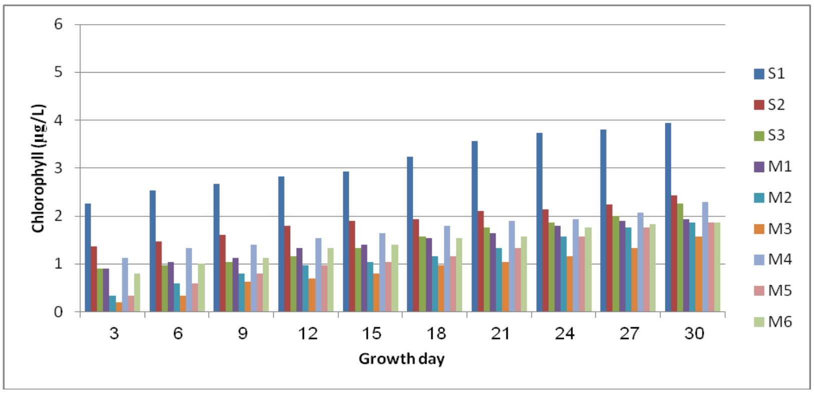

Fig. 2: Chlorophyll of *Spirulina*, *Anabaena* and *Nostoc* cultivated on different media

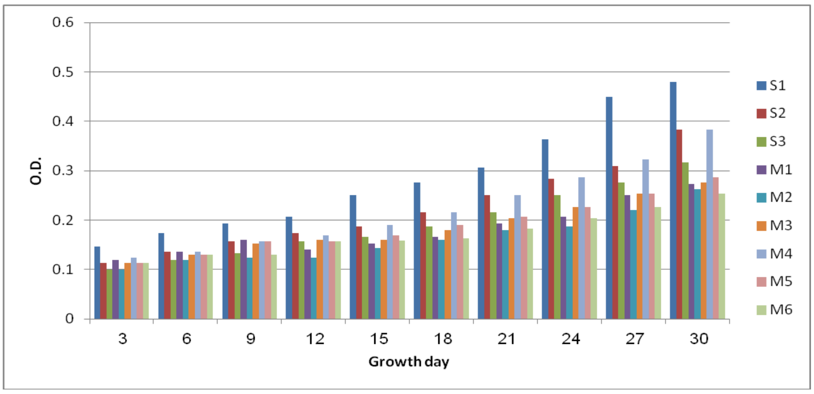

Fig. 3: Optical density of *Spirulina*, *Anabaena* and *Nostoc* cultivated on different media

Effect of microalgae cultures on phenolic compounds of OWW

The presence of high concentration of phenolic compounds in olive wastewater is posing high environmental risk as phenols are easily soluble in water and eventually may reach down to streams, rivers, lakes and soil and represent a serious ecological problem (Umran *et al*. 2008; Chatzisymeon *et al*. 2009;Bhattacharya *et al.* 2012).

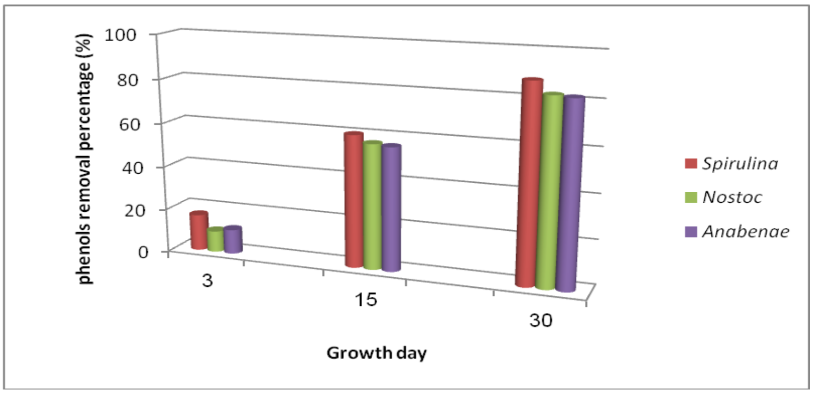

Fig. 4: Effect of *Spirulina*, *Anabaena* and *Nostoc* cultivation on phenols removal

The results of cyanobacteria growth showed that *Spirulina*, *Anabaena* and *Nostoc* cultures had better growth in OWW diluted to 50%.Thus the variation of total phenolic content of OWW was monitored at the 3rd, 15thand 30th day of the growth experiment (Figure 4). It can be observed that the initial phenols concentration was reduced by 50-60% in 15 days, and more than 80% of phenols were removed by Day 30.

Lee *et al*., (2015) suggested that the removal of phenols by microalgae is attributed to biodegradation processes rather than bioadsorption processes. The bio-treatment of wastewaters depends on the limit of normally occurring microorganisms to degrade pollutants exhibited in the aquatic environment. Some Microalgae have been effectively utilized in the treatment of wastewaters containing pesticide, phenols, hydrocarbons and detergents. This is essentially because of their ability to metabolize nitrogen, phosphorus, carbon and sulfur sources. They have additionally been utilized to diminish nitrogen, ammonia and phosphorous in sewage and different farming wastes (Al-Dahhan *et al*., 2018).Microalgae are capable of biotransforming phenolic compounds, by utilizing phenol as a carbon source to development (Baldiris-Navarro *et al*., 2018).

CONCLUSION

The present study indicates that microalgae *Nostoc muscorum*, *Anabaena oryzae* and *Spirulina platensis* are able to grow in wastewater from olive oil production. The algae during their growth decreased the total phenolic content of wastewater by 50-80%. Growing microalgae in wastewater has economic as well as environmental importance in reducing the production cost of algae and decreasing the amounts of untreated wastewater.

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