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

Soha S. Mostafa1*, Adel S. El-Hassanin2, Amira Sh. Soliman2, Sayed Rashad3 and Ghadir A. El-Chaghaby3

1- Microbiology Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Egypt
2- Faculty of African Postgraduate Studies, Cairo University, Egypt
3- Regional Center for Food and Feed, Agricultural Research Center, Egypt

*Corresponding author: sohaasayed@yahoo.com

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ABSTRACT

The present study investigates the possibility of growing microalgae in wastewater generated from olive oil 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 (741 ppm), iron (59.78 ppm), magnesium (301 ppm), copper (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 1.87 µg/L, respectively. Dry weights obtained in diluted OWW50% were 1.72, 1.45 and 1.28 g/L for Spirulina, Anabaena and Nostoc, respectively. The values of optical density were 0.38 for Spirulina, 0.29 for Anabaena 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>
<thead>
<tr>
<th>Wastewater</th>
<th>OWW1*</th>
<th>OWW2**</th>
<th>OWW3***</th>
<th>OWW****</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.60</td>
<td>4.53</td>
<td>4.73</td>
<td>4.51</td>
</tr>
<tr>
<td>EC; μs.cm⁻¹</td>
<td>12.7</td>
<td>15.9</td>
<td>30.5</td>
<td>14.85</td>
</tr>
<tr>
<td>Salinity ; ppt</td>
<td>7.2</td>
<td>9.2</td>
<td>18.7</td>
<td>8.41</td>
</tr>
<tr>
<td>TSS;mg/L</td>
<td>3636</td>
<td>99484</td>
<td>613800</td>
<td>44241</td>
</tr>
<tr>
<td>Total phenol ; mg /L</td>
<td>294.5</td>
<td>3797.4</td>
<td>4872.93</td>
<td>2999.4</td>
</tr>
<tr>
<td>NO₃ mg/l</td>
<td>10.97</td>
<td>1.69</td>
<td>4.69</td>
<td>5.12</td>
</tr>
<tr>
<td>NO₂ mg/l</td>
<td>977</td>
<td>8.69</td>
<td>21.46</td>
<td>10.47</td>
</tr>
<tr>
<td>N%</td>
<td>0.30</td>
<td>0.20</td>
<td>0.50</td>
<td>0.31</td>
</tr>
<tr>
<td>Ca ppm</td>
<td>616.7</td>
<td>318.8</td>
<td>1750</td>
<td>741</td>
</tr>
<tr>
<td>Cu ppm</td>
<td>143.7</td>
<td>189.3</td>
<td>541.3</td>
<td>301</td>
</tr>
<tr>
<td>Mg ppm</td>
<td>258</td>
<td>301</td>
<td>386</td>
<td>314</td>
</tr>
</tbody>
</table>

*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 NaHCO₃ 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>
<thead>
<tr>
<th>Treatment</th>
<th>Strain + Culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td><em>Spirulina platensis</em> + Zarrouk medium</td>
</tr>
<tr>
<td>S2</td>
<td><em>Anabaena oryzae</em> + BG11 medium</td>
</tr>
<tr>
<td>S3</td>
<td><em>Nostoc muscorum</em> + BG11 medium</td>
</tr>
<tr>
<td>M1</td>
<td><em>Spirulina platensis</em> + OWW 100%</td>
</tr>
<tr>
<td>M2</td>
<td><em>Anabaena oryzae</em> + OWW 100%</td>
</tr>
<tr>
<td>M3</td>
<td><em>Nostoc muscorum</em> + OWW 100%</td>
</tr>
<tr>
<td>M4</td>
<td><em>Spirulina platensis</em> + OWW 50%</td>
</tr>
<tr>
<td>M5</td>
<td><em>Anabaena oryzae</em> + OWW 50%</td>
</tr>
<tr>
<td>M6</td>
<td><em>Nostoc muscorum</em> + OWW 50%</td>
</tr>
</tbody>
</table>

**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 560 nm (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 (Cᵢ - Cᶠ / Cᵢ), where Cᵢ is the initial concentration of phenols and Cᶠ 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, Anabaena 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, Anabaena 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, Anabaena 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 Anabaena 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](image1.png)

![Fig. 2: Chlorophyll of Spirulina, Anabaena and Nostoc cultivated on different media](image2.png)
Microalgae for decreasing phenolic content of wastewater

Effect of microalgal 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; Bhattacharya et al. 2012).

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, 15th and 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 biosorption processes. The biotreatment 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.

REFERENCES


Microalgae for decreasing phenolic content of wastewater


