BIOLOGICAL INFLUENCE OF SOME MICROORGANISMS ON OLIVE MILL WASTEWATER

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Abstract

A cyanobacterial strain, *Spirulina platensis* besides two strains of green algae, *Dunaliella saline* and *Chlorella vulgaris* and the fungus *Pleurotus columbinus* were propagated on 10 % concentration of olive mill wastewater at a steady state. For *Pleurotus columbinus* , it was also propagated on the previous concentration at an agitated state. The most efficient strain for decreasing chemical oxygen demand and phenolic compounds was *Pleurotus columbinus* propagated at 150 rpm in shaker which diminishes them by laccase enzyme. The second efficient strain in decreasing, chemical oxygen demand and phenolic compounds was *Chlorella vulgaris* followed by *Spirulina platensis* while *Dunaliella saline* which was the least efficient one. *Pleurotus columbinus* was propagated either in a steady state or an agitated at 150 rpm in shaker donate high amount release of total carbohydrates . The main observation is the small value of growth which may be due to absence of nutrients in the medium. The use of such effective strains will help in minimizing the hazard impact of olive mill wastewater. Accordingly, the waste water produced should be primary treated before being dumped in water bodies.

INTRODUCTION

Olive oil industry is one of the most important industries in the world especially in the Mediterranean countries. Many environmental problems take place from that industry due to the production of huge amounts of wastewater which contain heavy burden of organic matter and poly phenolic compounds. Olive mill wastewater (OMWW) when discharged into surface water which used for irrigating soils affects physical and chemical properties of soils such as porosity and pH . In addition , the high concentration of reducing sugars stimulate microbial respiration and lower dissolving oxygen concentrations (Niaounakis and Halvadakis, 2006) . In olive mill wastewater, chemical oxygen demand (COD) could reach up to 220 g/l as was mentioned by Papanikolaou *et al*. 2008. Several studies were carried out to reduce the phenolic compounds and COD content of this wastewater by using different treatment methods. Green algae and cyanobacteria play an important role in bioremediation of polluted wastewater with heavy metals and organic polycyclic hydrocarbons pollutants. (Torres *et al*., 2008). Semple *et al*. (1999) showed that

eukaryotic algae like *Chlamydomonas*, *Chlorella*, *Dunaliella* and *Scenedesmus* are capable of biotransforming and biodegrading aromatic pollutants commonly in nature and wastewater, enhancing the degradation potential of the micro-biota and eliminating pollutants from the respective ecosystem. The most promising microorganisms of white rot fungi such as *Coriolus*, *Phanerochaete*, *Lentinula, Ganoderma* and *Pleurotus* have the ability to degrade poly phenolic compounds, lignin, removal of COD and decolonization (Anastasiou *et al*., 2011).

This work aims at reducing the phenolic compounds and COD from OMWW using different algal strains propagated in a steady state for 30 days compared to *Pleurotus colombinus* propagated in both a steady state and an agitated state at the same period.

MATERIALS AND METHODS

Materials

Microorganisms used

One strain of cyanobacteria (*Spirulina platensis*) and two strains of green algae (*Dunaliella saline* and *Chlorella vulgaris*) were obtained from Agricultural Microbiology Research Department, Soils, Water and Environment Res. Inst. (SWERI), Agric. Res., Center (ARC) . One strain of white rot fungi (*Pleurotus columbinus*) was obtained from Unit of Mushroom Production , Faculty of Agriculture , Ain Shams University .

Spirulina platensis was grown on Zarrouk medium (Zarrouk, 1966). *Dunaliella saline* was grown on Johnsons medium (Johnson *et al*., 1968). Bold medium (Nichols and Bold, 1965) was used for the green alga *Chlorella vulgaris*. *Pleurotus columbinus* was grown on Potato Dextrose Agar medium , PDA (Martin, 1950) .

Biological Treatments

All microbial strains were cultured on 10 % concentration of olive. Mill wastewater (OMWW) at steady state and at ambient temperature. *Pleurotus columbinus* was also grown in shaker at 150 rpm and at ambient temperature. *Pleurotus columbinus* which was grown in shaker has a symbol * (*Pleurotus columbinus* *)

Methods

Determination of chemical constituents of OMWW

Chemical oxygen demand (COD) was determined according to the method described by APHA *et al*. (1992). Total phenols were determined spectrophotometrically as described by Swain and Hillis (1959). Total sugars were determined by the method of Dubois *et al*. (1956). Dry weight was determined by drying the biomass in the oven at 70^{o} C.

RESULTS AND DISCUSSION

The microorganisms used varied in their effects on pH changes (Table 1). It is clearly noticed that cultivation of cyanobacteria represented by *Spirulina platensis* elevated pH of the medium than the other microorganisms during propagation periods. This might be due to the formation of $CO_2/H_2CO_3/HCO_3^{-1}/CO_3^{-2}$ system which is a very useful buffer system for maintaining the alkaline pH which helps to prevent carbon depletion (Keskinkan *et al*., 2012).

Table 1. Periodical fluctuations in medium pH values due to growth of the various microorganisms. *

Period per day / Microbe strain	Zero time	10 days	20 days	30 days
Spirulina platensis	4.6	8.4	8.3	8.2
Dunaliella saline	4.6	4.7	4.6	4.8
Chlorella vulgaris	4.6	5.5	5.5	6.2
Pleurotus columbinus	4.6	5.4	5.2	5.0
*Pleurotus columbinus**	4.6	5.5	5.6	5.7

*Propagation of *Pleurotus columbinus* on 10 % concentration of olive mill wastewater in an agitated state at 150 rpm for 30 days.

Table (2) shows that *Dunaliella saline* exhibited the least effect on COD in diluted OMWW during treatment periods (40.5 % removal after 30 days of propagation), while *Pleurotus columbinus** was the highest (81.0 % removal after the same elaborated period). *Chlorella vulgaris* and *Pleurotus columbinus* propagated in steady state were mostly in the same effect throughout treatment periods. For algal strains, *Chlorella vulgaris* was the most affecting COD followed by *Spirulina platensis* then *Dunaliella saline*. These results agree with Markoua *et al*. (2012) who stated that *Spirulina platensis* can remove 73.18 of COD presented in OMWW. Morever, Valderrama *et al*. (2002) reported that *Chlorella vulgaris* propagated on 10 % concentration of OMWW can diminish COD to 61 %. OMWW inoculated by *Pleurotus columbinus* at steady state and agitated at 150 rpm removed 66.7 and 81.0 % of COD after 30 days of propagation, respectively.

Table 2. COD values of microbial strains propagated on 10 % concentration of OMWW through 30 days. *

Period per day / Microbial Strains	Zero time	10 days	20 days	30 days
	Content g/l	Content g/l	Content g/l	Content g/l
Spirulina platensis	4.2	3.3	2.2	1.7
Dunaliella saline	4.2	3.3	3.0	2.5
Chlorella vulgaris	4.2	2.7	2.0	1.6
Pleurotus columbinus	4.2	2.7	2.0	1.4
*Pleurotus columbinus**	4.2	1.8	1.3	0.8
LSD 0.05	0	0.92	1.03	0.33

*Propagation of *Pleurotus columbinus* on 10 % concentration of olive mill wastewater in an agitated state at 150 rpm for 30 days.

The results in Table (3), reveal obvious phenol removal by *Pleurotus columbinus* either in steady state or agitation than any algal strain used. On the other side, *Spirulina platensis* shows high efficiency in phenol removal followed by Chlorella vulgaris then *Dunaliella saline*. Elimination or decreasing phenolic compounds is related to phenol oxidase enzyme presented in some genera of algae such as *Spirulina* and *Chlorella*, or laccase enzyme which is considered one of the family of phenol oxidase enzymes presented in some genera of white rot fungi like *Pleurotus*. Sherman *et al*. (1991) stated that a lot of green algae like *Chlorella*, *Stigeoclonium*, *Microspora*, *Ulva* and *Spirogyra* have polyphenol oxidase enzyme. Kirkwood *et al*. (2003) ascribed the capability of *Spirulina* to deal with pollutants and degrade the phenolic compounds to photosynthesis phenomena which make cyanobacteria do not need any external carbon source because of CO_2 fixation. Kunamneni *et al*. (2007) mentioned that *Pleurotus* produces a group of enzymes such as laccase, manganese peroxidase and veratryl alcohol oxidase that have the ability to degrade poly phenolic compounds.

Table 3. Effect of microbial strains on decreasing of phenolic compounds during 30 days on 10 % concentration of OMWW*

Period per day	Zero time	10 days	20 days	30 days
Microbial strains	Content g/l	Content g/l	Content g/l	Content g/l
Spirulina platensis	0.215	0.082	0.078	0.046
Dunaliella saline	0.215	0.158	0.133	0.114
Chlorella vulgaris	0.215	0.115	0.103	0.063
Pleurotus columbinus	0.215	0.058	0.037	0.021
*Pleurotus columbinus**	0.215	0.021	0.014	0.012
LSD 0.05	0	0.02	0.01	0.02

*Propagation of *Pleurotus columbinus* on 10 % concentration of olive mill wastewater in an agitated state at 150 rpm for 30 days.

Distinct differences in carbohydrate behavior between *Spirulina platensis* and *Dunaliella saline*., *Chlorella vulgaris* that propagation in steady state and *Pleurotus columbinus* which was propagated in both steady and agitated state towards carbohydrate behavior through propagation on diluted OMWW are observed . Table (4) reveals that algal strains are consumer to carbohydrate, while fungal strain is producer. *Chlorella vulgaris* is the highest consumers of carbohydrate at 30 days of propagation on 10 % concentration of OMWW, followed by *Spirulina platensis* then *Dunaliella saline* at the same condition, while *Pleurotus columbinus* in steady state is the highest producer than in agitated state. Hodaifa *et al*. (2009) reported that microalgae consumed total carbohydrate by 65 % and assimilated it to form biomass. The percentage of confirmed carbohydrate is determined by nearly 65 %. On the other hand, mushroom production is manipulated to produce exo--polysaccharides used in some potential pharmaceutical application such as lintanine ,schyzophyline and grifron D from *Lentinula*, *Schizophyllum* and *Grifola* , respectively. Food manufactures have directly employed exo-polysaccharides of mushrooms by fermentation to prepare drinks or capsules for sales (Wu *et al*. , 2008).

Table 4. Total carbohydrate consumed or released during propagation of microbial strains on diluted OMWW through 30 days. *

Period per day / Microbial strains	Zero time Content g/l	10 days Content g/l	20 days Content g/l	30 days Content g/l
Spirulina platensis	0.571	0.338	0.345	0.171
Dunaliella saline	0.571	0.463	0.505	0.395
Chlorella vulgaris	0.571	0.377	0.341	0.126
Pleurotus columbinus	0.571	1.096	2.027	2.067
*Pleurotus columbinus**	0.571	1.016	1.081	1.542
LSD 0.05	0	0.16	0.16	0.47

*Propagation of *Pleurotus columbinus* on 10 % concentration of olive mill wastewater in an agitated state at 150 rpm for 30 days.

Table (5) shows small values of growth throughout all propagation periods. This might be due to absence of nutrients during propagation periods. The high values of growth exist at 20 days of propagation for both *Spirulina* and *Dunaliella* while high values of growth are at 10 days propagation for *Chlorella vulgaris* and *Pleurotus* which propagated at either steady or agitated state. Obvious decreases in growth were observed for the three strains of algae. These may be due to depletion of nitrogen presented in the medium.

Table 5. Dry weight of *Spirulina*, *Dunaliella*, *Chlorella* and *Pleurotus* propagated on 10 % of OMWW through 30 days. *

Period per day / Microbial strains	10 days growth mg/l	20 days growth mg/l	30 days growth mg/l
Spirulina platensis	860	1080	50
Dunaliella saline	500	1080	30
Chlorella vulgaris	1200	1080	50
Pleurotus columbinus	1200	1020	1000
*Pleurotus columbinus**	1660	1200	1100
LSD 0.05	122.9	68.04	210.2

*Propagation of *Pleurotus columbinus* on 10 % concentration of olive mill wastewater in an agitated state at 150 rpm for 30 days.

CONCLUSION

The results of this study show clearly that either *Spirulina platensis* , *Dunaliella saline* or *Chlorella vulgaris* has the capability to reduce both COD and phenolic compounds as they have phenol oxidase, *Pleurotus columbinus* is the most efficient microbe to deal with poly phenolic compounds . *Pleurotus columbinus* also secrets exo-polysaccharides which have many beneficial applications in nutritional and pharmaceutical purposes . Further studies will investigate the role of OMWW treated with *Pleurotus columbinus* on the aggregation and amendment of soil properties and enhancement of plant growth. From these results, it is recommended to establish a treatment unit for bioremediation of the olive mill wastewater pollutant before being drained in water bodies.

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التأثير الحيوى لبعض الكائنات الحيه الدقيقه على الماء الناتج من صناعة زيت الزيتون

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تم استخدام احدى سلالات السيانوبكتريا و هى سبيرولينا بلاتينسيس ، و سلالتين من الطحالب الخضراء و هما ديونااليلا سالين ، كلوريللا فولجاريس و أحدى سلالات العفن الابيض الفطريه و هى بليوروتاس كولومبينوس حيث تمت التنميه عند تركيز 10 % من الماء الناتج من صناعة زيت الزيتون فى حالة السكون ما عدا سلالة البليوروتاس التى نمت فى مزرعه ساكنه و مزرعه فى حالة اهتزاز عند 150 اهتزازه فى الدقيقه على درجة حراة الغرفه . سلالة البليوروتاس التى نمت فى حالة الاهتزاز على 150 اهتزازه فى الدقيقه كانت الاكثر كفاءه فى تقليل الاكسجين المحتاج اليه كيميائيا و كذلك المركبات الفينوليه حيث انقصت المواد الفينوليه بواسطة وجود انزيم اللاكيز . و كانت ثانى السلالات فى الكفاءه عند التعامل مع الملوِّثات و هى الاكسجين المحتاج اليه كيميائيا و كذلك المركبات الفينوليه هى الكلوريللا فولجاريس ثم تبعها سلالة السبيرولينا بلاتينسيس ثم اخيرا سلالة الديونااليللا سالين .السلاله الفطريه بليوروتاس كولومبينوس التى نمت سواء فى حالة السكون او الاهتزاز اعطت كميه كبيره من السكريات الكليه المتحرره فى البيئه . و كانت الملحوظة التى اثارت الانتباه هى ضعف النمو و الذى قد يرجع الى عدم اضافة اى عناصر غذائيه الى بيئة النمو . يوصى بإلحاق وحدة معالجه لمصانع إنتاج زيت الزيتون حتى يمكن تلافى الآثار الضاره لمحتواها من الفينولات و المواد الأخرى على خواص الأراضى نتيجة صرفها الى المجارى المائيه بدون معالجه.