

# Isolation and identification of microorganisms that can biodegrade organic compounds which are present in olive mill waste.

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## Abstract

Isolation of microorganisms that can biodegrade organic compounds which are present in olive mill waste and more specifically microorganisms that can survive at high phenol concentrations was studied in the Environmental Engineering Laboratory for three months in the Department of Environmental Science and Technology in the Cyprus University of Technology. Firstly, liquid crops were created, then the samples were cultivated in solid crops (with phenol as the sole carbon source) and at last isolation and purification of the samples were performed and the single colonies were replicated in liquid crops to check their performance. Eight single microbial culture will be sent to Macrogen The Netherlands Research Center for recognition and identification.

**Keywords:** olive mill wastewater, pollution, cultivation of microorganism, phenol, solid cultures, isolation.

## 1. Introduction

In the olive oil production, the main waste resulting is olive mill wastewater, as a major source of pollution which causes serious problems for olive oil producing countries because of the increased organic pollutant load they contain.

In order to secure the deposition of olive mill wastewater in the environment, two major problems should be solved. First the degradation of the very high organic load they possess and which requires large amounts of oxygen to decompose and secondly the degradation of the water-soluble based phenolic compounds, which are transferred to liquid olive mill wastewater during the processing of olives.

### 1.2 Phenolic content in olive mill wastewater

Olive mill wastewater include a high content of polyphenols. Phenolic compounds imparts olive mill wastewater difficult to degrade by presenting

antimicrobial, antioxidant and phytotoxic properties, giving the waste toxic properties.

The phenolic compounds consist of a benzene ring containing directly one or more hydroxyl groups.

## 2. Materials and Methods

The process of isolating microorganisms is important because it provides microorganism strains for detailed, controlled laboratory studies.

### 2.1 Sampling, enrichment and isolation of microorganisms

To conduct the research needed to follow the process of laboratory culture of microorganisms for their isolation. First, 5 samples (Biocarrier of type K3) from Moving Bed Biofilm Reactor (MBBRs) that had treated olive mill wastewater were the initial inoculum taken from the Water and Air Quality Laboratory of the Department of Environment of the University of Aegean.

The samples were separated into 8 conical flasks which were enriched with Angelidaki Medium substrate with three highly selective nutrient organic substrates such as Phenols, Vanillic acid and Guaiacol. The biomass used (Angelidaki Medium) for the supply of nutrients, trace elements and buffers prepared in accordance with the protocol referred to article Angelidaki et al 2009.

More specifically, for the first enrichment were added to 400 ml of Angelidaki Medium 0.4 g of Phenols, in 200 ml of Angelidaki Medium was added 0.1 g of Vanillic acid and in 200 ml of Angelidaki Medium was added 0.1 ml of Quaiacol.

In each conical flask of the 8 produced with the olive oil samples, was added about 150 ml of each substrate separately.

Conical flask	Liquid oil mill wastewater	Bio - carrier	Organic Phenol substrate	Guaiacol organic substrate	Vanillic acid organic substrate
1 <sup>st</sup>	1 ml	1	150 ml	-	-
2 <sup>nd</sup>	1 ml	1	-	150 ml	-
3 <sup>rd</sup>	1 ml	1	-	-	150 ml
4 <sup>th</sup>	1 ml	1	150 ml	150 ml	150 ml
5 <sup>th</sup>	4 ml	-	150 ml	-	-
6 <sup>th</sup>	4 ml	-	-	150 ml	-
7 <sup>th</sup>	4 ml	-	-	-	150 ml
8 <sup>th</sup>	4 ml	-	150 ml	150 ml	150 ml

By the process of liquid cultures the microorganism is adapted to consume a particular substrate and is thus more easily identified.

Enrichment technique is the most common strategy to be followed. Enrichment cultures were created by direct dilution of the sample in a strongly selective medium and thus microorganisms were isolated in the course of the experiment. The selection of these organic substrates was made as these substances are in the olive mill wastewater. In an enrichment culture, nutrients and incubation conditions are selected to favor the desired organism and restrict the rest. The value of the active acidity (pH) was set to be between 6.20 to 7.40. They were stored in the Incubator.

Then, the samples were cultivated in solid cultures (with phenol as the sole carbon source) with the addition of Agar Bacteriological coagulant thus exposing the microorganisms to more extreme conditions and interrupting the immersion of liquid cultures. Several concentration of phenol were tested 100 mg/L - 2000mg/L). Individual cells plated on the surface of plastic Petri dishes will grow and divide to form colonies. Then, isolation and purification of the single cultures (8 microbes) took place and the singles colonies were re-exposed to liquid media to test their performance. The procedure of enrichment took place by serial dilution over time and phenol as the sole carbon source.

### 3. Conclusion

A total of 14 serum bottles were made of which two were Control Phenols, one Control Quaiacol, six serum bottles Phenols, two Vanillic acid, three Quaiacol. Using inoculation loop, samples were taken from the colonies on the plastic Petri dishes and placed on the corresponding serum bottle by storing them in the Incubator. After two days, three serum bottles which were enriched with Quaiacol were blurred in color while the rest were discarded as no performance was observed. Thus, the same process was reversed. From the new liquid cultures it was observed to impart those that were submerged from biofilm with colonies of Vanillic acid, Mix with biocarrier and Phenols with biocarrier.

Eight single microbial culture will be sent to Macrogen The Netherlands Research Center for recognition and identification.

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