Bioremediation of Olive Mill Wastewater By Fungi

Thesis
Submitted in partial Fulfillment of the requirements for M.Sc. degree in Microbiology

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2013

Introduction

Water scarcity in the Mediterranean countries of the arid zone resulted from high rates of population growth, urbanization and industrialization. Large quantities of fresh water supplies will be diverted to meet the growing water demand in municipal and industrial sectors in the region. In order to overcome water shortages, there is an alternative plan for saving water which is in the use of non-conventional water resources such as the treated waste water.

The olive oil consumed in the world is mainly produced in the Mediterranean countries. Olive agriculture and oil extraction is growing and becoming of great importance in Egypt, north Sinai. The olive oil industry with an estimated production of 40 thousand tones per year and 10–30 million m^3 of olive mill wastewater (OMW) generated every year from the production of olive oil (Niaounakis and Halvadakis, 2006).

These effluents are composed of vegetation water of olives plus washing and proceeding waters in addition to soft pulp tune and oil in the form of a very stable emulsion.

The organic fraction of OMW is composed of sugars, N-compounds, organic acids, fats as well as polyphenols, pectin, tannin, residual oil polyalcohol, polysaccharides (Greco *et al*., 1999).

High value of chemical oxygen demand (COD) of OMW coupled with its phenol content inhibit the natural organic load degrading capability of the microflora in water bodies (Lanciotti *et al.*, 2005).

Phenolic compounds, which are usually present in OMW consist of monocyclic aromatic compounds, such as hydroxytyrosol, tyrosol, catechol, methylcatechol, caffeic acid. Due to the instability of OMW phenols, they tend to polymerize during storage into condensed high-molecular weight polymers (Crognale *et al*., 2006). The last mentioned are recalcitrant to biodegradation and toxic to most bacteria and fungi. Therefore the incorrect disposal of OMW may cause serious and large scale environmental problems and it is urgent to develop a suitable and environmental friendly treatment. Instead of OMW disposal, an approach of using this waste as a resource to be valorized is of greater interest.

The general treatment alternatives available for the treatment of the agro-industrial wastewater can be divided into two major treatment categories: (1) physical/chemical treatment systems and (2) biological treatment systems. All physical/chemical treatments have serious limitation. On the other hand, biological treatment (aerobic/anaerobic) is an alternative for its safety, efficiency, low cost and environmental friendly treatment. Biological treatment refers to the utilization of living organisms of contaminants for the purpose of reducing the concentration of contaminants in an environment. Even simple biological systems are complex mixture of thousands of biochemical reactions being conducted by a variety of biological organisms. This complexity often produces an amazing ability by the biological system to adapt to the treatment of a wide variety of pollutants.

Microorganisms capable of degrading chemicals in industrial wastes were isolated and used in designated commercial preparations for pollution control. They consume the organic material and at the same time produce biomass and other valuable products, thereby, they are considered suitable in biological treatments (Horan, 1990).

Great interest has been focused on biological treatment using aerobic bacteria and fungi for bioremediation of olive-mill wastewater. Aerobic biological methods seems to be especially suitable since the treatment leaves a residue of lower toxicity and diminished phenol content (Balis *et al*., 1996 & Sbai *et al*., 2000).

Fungi are important in the biological treatment of some industrial wastes and in composting of organic solid waste, because they tolerate low pH levels, nitrogen-limiting conditions. Fungal genera utilize a wide range of simple aromatic compounds and have high activities of the several catabolic enzymes (Bitton, 1994).

Fungi use OMW as media, reducing COD, remove simple phenolics, and effective at reducing coloration of OMW.

The effect of supplement addition to OMW, alone or in different combinations stimulate the enzymatic activity of microorganism (Odokuma and Dickson, 2003).

The use of single or combined method of treatment may aid in increasing the efficiency, the resulting treated water may be employed as a growth media or as a biofertilizer.

AIM OF WORK

In biological treatment of wastewater, different types of wastewater such as OMW can be employed as substrate to study the ability of microorganism to biodegradate polyphenols, decolourisation and decrease of COD.

This study closely examines the possibility of using both biostimulation of existing microflora and bioaugmentation of microorganisms capable of producing biodegrading enzymes and effective in the treatment of OMW. The research is also concerned with the study of several physical and biological modes of treatment to decrease some environmental parameters in order to meet permissible levels of treatment.

To achieve the aim of this work, the following steps were carried out:

1) Analysis of some parameters of OMW samples were obtained from the industrial effluent.

2) Screening of potent microflora isolates on selective medium.

3) Bioaugmentation through inoculating OMW by specific microorganisms.

4) Biostimulation of existing microflora by addition of different nutrients in different concentrations.

5) Studying of γ- irradiation effect on OMW biostimulation.

6) Using consecutive filtration and biostimulation treatments of the OMW for further treatment of OMW and analysis of some parameters of treated OMW.

8) Studying the applicability of the treated OMW as a biofertilizer and growth media.

Literature Review

Production of olive oil

Demand for olive oil is rapidly expanding world wide due to its healthy image. Olive oil has gained an increasing popularity due to its oleic acid content, which may have different medical effect such as; affecting the plasma lipid/lipoprotein profiles and antioxidative effects due to it's richness in antioxidants. These antioxidants may prevent some human diseases. Olive oil production is an important economic activity of Middle East countries (Delplanque *et al*.,1999; Fito *et al*.,2000).

The anti-oxidant effect of olive oil is well known. This is mainly because of the quantity of phenolic compounds. In addition to this effect, olive oil possesses beneficial properties for the human circulatory system (Visioli and Galli 1998). In particular, olive oil phenols have been beneficially linked to processes that contribute to the prevention of heart disease (in particular hydroxytyrosol), atheroscelosis and cancer (Rafael and Olha, 2010).

The olive oil consumed in the world is mainly produced in the Mediterranean basin countries. Olives grown in the Mediterranean countries constitute about 98% of the global production (Niaounakis and Halvadakis, 2006).

Olive oil extraction Methods:

Olive agriculture and oil extraction is of great importance in Egypt especially in North Sinai. The quantity of Olive mill waste water (OMW) estimated around 20-50 tons/day in the period from September to December (Hegazi *et al*., 2009).

An estimated 10–30 million m^3 of OMW is generated every year as a result of olive oil production (Niaounakis and Halvadakis, 2006).

Olive oil extraction produces vast amounts of residues, in the form of both liquid and solid wastes. OMW and crude olive cake are produced as waste-products in olive oil factories. Olive mill waste water, commonly called alpechin, is the main waste produced by three-phase extraction of olive oil (Paredes *et al*., 1999 and Aktas *et al*., 2001).

Olive oil is a typical valuable agro-industrial product. Extraction of the oil from olive fruit generates large amounts of organic wastes. Olive oil mill wastewater as the liquid by-product was obtained from mechanical olive processing. The volume of this liquid waste (VW) depends on the method used for the oil extraction and varies from 40-60 L for pressing method to 80-100 L for three-phase centrifugation technique (per 100 Kg of olives) (Harwood and Aparicio, 2000).

Pressing (traditional system) and continuous (three or two phase) are the most important extraction processes, applied for olive oil production. Three and two-phase extraction technology differ in the water supplies (Lanciotti *et al.*, 2005). Large amounts of water are required for an extraction process with a three-phase decanter and large amount of liquid by-products is generated (OMW), this by-product is a stable emulsion composed of water, olive pulp and oil.

Olive oil factories changed their production system to a two-phase continuous centrifugation system, which greatly reduces the volume of polluting VW (Alb, 2001), However, the amounts of solid olive mill waste was increased. This OMW has a higher water content (up to 70%) than olive press-cake (OPC) (27-50%) (Tsioulpas *et al*., 2002).

Olive mill Wastewater composition:

The weight composition of OMW is 83-96% water, 3.5-15% organics and 0.5-2% mineral salts (Ehaliotis *et al*., 1999). The organic fraction is composed of sugars (1-8%), nitrogenous-compounds (0.5-2.4%), organic acids (0.5-1.5%), fats (0.02-1%) as well as polyphenols and pectins (1-1.5%), polyalcohols and other tannins (Greco *et al*., 1999). The OMW constituents, quality and quantity, depend on many factors, such as type of olive and its maturity, climatic conditions, region of origin, cultivation methods, harvesting period and specially the technology used for oil extraction (Roig *et al*., 2005).

Environmental problem:

Unfortunately, the mechanical process of olive oil extraction generates a significant amount of both solid and liquid wastes composed of many complex coloured and recalcitrant compounds with high organic loads (Gonzalez *et al*.,1990 ; Tuncel and Nergiz, 1993). The incorrect disposal of dark coloured OMW effluent cause serious and large-scale environmental problems, particularly in the Mediterranean area (Crognale *et al*., 2006).

The problem of severe limitations, which are introduced by OMW spreading, for example, over agricultural soil or even discharging it in rivers and wastewaters is not only in the Mediterranean regions, but in other countries where olive is produced, similar situations can be found such as in the Middle-East countries (Fadil *et al.,* 2003).

High Chemical oxygen demand (COD) of OMW coupled with its phenol content, that inhibit the natural organic load degrading capability of the microflora in water bodies. In fact the olive pulp is very rich in phenolic compounds (Lanciotti *et al*., 2005) but only 2% of the total phenolic content of the olive fruit remains in the oil phase, while the remaining amount is lost in the OMW (approximately 53%) (Rodis *et al.,* 2002). The phytotoxicity of the olive mill wastewaters can be attributed to the phenolic compounds.

Phenolic compounds are considered to be persistent and recalcitrant in the environment (Duran and Esposito, 2000) , toxic to most bacteria and fungi and are used as a slimicide and disinfectant (EPA/635/R-02/006).

Due to the instability of OMW phenols, they tend to polymerize during storage into condensed high-molecular weight polymers that are difficult to degrade (Crognale *et al.,* 2006). Thereby, uncontrolled OMW disposal can create several risks to the environment rendering it urgent to develop a suitable treatment.

The high toxicity of phenolics, even at low concentrations, has motivated the search and improvement of many treatment techniques such as evaporation (Saez *et al*., 1992), physico-chemical (Rozzi and Malpei,1996; Khoufi *et al*., 2007), filtration (Almirante and Carol,1991) and biological (aerobic/anaerobic) methods (Hartman *et al*.,1984 & Martirani *et al.,* 1996).

Physical and chemical methods for wastewater treatment:

Physical/chemical treatment system utilize physical and/or chemical processes to remove contaminants from wastewater. They can be grouped into the following three categories:

1- Ionizing radiation

2- Sedimentation

3- Filteration

1- Ionizing radition (Gamma radiation):

Radiation in general is the emission of any rays or particles from a source, the energy travels through space (Uvarov and Isaacs, 1986). It is classified to non-ionizing and ionizing radiation such as gamma rays.

Gamma rays emitted from the excited nucleus of Co^{60} have been studied extensively for its effect on microorganisms (Board, 1983). Ionization is not a selective process, any atom or molecule in the path of radiation may be ionized. the predominant constituents of cells stand a better chance of being ionized and therefore affected (Grosch and Hopwood, 1979).

Gamma irradiation of simulated waste water effectively removed several chemical species from the water. The treatment system utilizing a high energy beam also destroyed the organic compound such as, benzene and the probable reaction products such as phenol (William *et al*., 1996).

The death of microorganisms is a consequence of the ionizing action of the lethal damage to microbial DNA. The loss of ability to reproduce is the primary cause of lethality, but damage to other sensitive and critical cell structures e.g. membranes, may also have a similar effect.

Dose levels that are lethal to microorganisms do not necessarily cause inactivation to enzymes, proteins, or any other large molecules. In general, microorganisms differ greatly in their sensitivity to irradiation (Silliker *et al.*, 1980).

2- **Sedimentation:**

Sedimentation process referred to the settling of particulate or coagulated suspended solids from the wastewater. Sedimentation is often used in conjunction with coagulation and flocculation. It is also independently effective for suspended particles, which are unstable in suspension because of their size and density. Sedimentation is an effective treatment for agricultural wastewater containing soil particles which have not formed irreversible colloidal suspensions (Adhoum and Monser, 2004 & Kapellakis *et al*., 2008).

3-Filtration:

Filtration processes involve a wide variety of gravity and pressure filters, including mixed media and pulsed bed filter, which utilize sand, gravel, diatomaceous earth, cloth, coagulated contaminants by physically straining them from the water. The filtration process mechanisms are complex , but the basic principle is simple. Filtration processes have generally been employed as a polishing step following coagulation and sedimentation rather than directly following coagulation (Pearson, 1989).

The overview of treatment options presented above portrays the myriad of options available to solve a particular wastewater treatment problem. The most common method applied has been the storage of OMW in lagoons, followed by evaporation during summer season (Azbar *et al*., 2004).

This method is not satisfactory since it only reduces the volume of waste, without treating the pollutants, and a black foul-smelling sludge is produced. The physico-chemical methods have the disadvantages of high cost and low efficiency (Borja *et al*.,1998). For example, lime precipitation results in a 40% reduction of the organic matter only along side production of large quantities of sludge which require costly disposal. Reverse osmosis has over 90% efficiency in removing organic matter, but with high operating cost and sludge-disposal problems (Fiestas and Borja, 1992).The tested physico-chemical treatment processes could not be implemented on a large scale because of their high investment and maintenance cost (Kalogerakis *et al*., 2000 & Sbai *et al*., 2000). All physical/chemical treatments have serious limitation.

Bioremediation (Biological treatment):

Microorganisms capable of degrading chemicals in industrial wastes were isolated and used in designated commercial preparations for pollution control. They consume the organic material and at the same time produce biomass and other valuable products, thereby, they could be suitable in biological treatments (Horan, 1990).

Great interest has been focused on biological treatment using aerobic bacteria and fungi for bioremediation of olive-mill wastewater (Balis *et al*., 1996 & Sbai *et al*., 2000).

Biological treatment is a viable treatment alternative for many agricultural wastewater contaminants. Biological treatment in combination with a physical/chemical system is the most feasible alternative (Pearson,1989).

Bioaugmentation:

Bioaugmentation is the use of strains of microbial isolates, which are isolated from the environment to improve some of the processes involved in traditional waste treatment. The early attempts to use microorganisms in the pollution control field have focused on anaerobic digestion. Later on, microorganisms capable of degrading herbicides and other chemicals in industrial wastes were isolated and used in designated commercial preparations for pollution control. In the 1970s, microbial preparations were marketed for enhancing the operational efficiency of waste treatment processes. This approach has been called bioaugmentation (Johnson et al., 1985).

Some of the applications of bioaugmentation are the following (Grubbs, 1984 & Rittmann *et al*., 1990):

Increased BOD removal; Microbial strains may be used to increase BOD removal in wastewater treatment plants (Bitton, 1994).

Reduction of sludge volume; Production of large amounts of sludge is a serious problem associated with aerobic waste treatment, and thus reduction of sludge volumes is highly desirable. The reduction is the result of increased organic removal following addition of a mixed culture of selected microorganisms. Reductions in volumes of generated sludge ranged from 17% to nearly 30% have been documented.

Use of mixed culture in sludge digestion, In aerobic digesters, the use of mixed cultures has led to significant savings in energy requirements. In anaerobic digesters; bioaugmentation has resulted in enhanced methane production.

Biotreatment of hazardous wastes; The use of added microorganisms for treating hazardous wastes (e.g. phenols, ethylene glycol, formaldehyde) has been attempted and has a promising future. Bioaugmentation with *Candida tropicalis* cells have been used to remove high concentrations of phenol in wastewater (Kumaran and Shivaraman, 1988).

Some major drawbacks of bioaugmentation are the need for an acclimation period prior to onset of biodegradation, and short survival or lack of growth of microbial inocula in the seeded bioreactors. Evaluations of some of these commercial products are sometimes negative or inconclusive (Jones and Schroeder, 1989).

Production of microbial seeds:

Microbial strains for enhancing biodegradation of specific chemicals are generally isolated from environmental samples (wastewater, sludge, compost, soil) and are selected by conventional enrichment techniques. They are grown in nutrient media that contain a specific organic chemical as the sole source of carbon and energy or as a sole source of nitrogen. Some of the microbial strains may be subsequently irradiated to obtain desirable mutation (Johnson *et al.*, 1985).

The selected strains are grown in large fermenters and then concentrated by centrifugation or filtration. They are then