Chapter 16
Aerobic and Anaerobic Biotreatment of Olive Oil Mill Wastewater in Lebanon

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Abstract Olive oil mill wastewater (OMW), known in Lebanon as Zibar, is one of the two by-products obtained during olive oil extraction. OMW represents a serious environmental pollution problem especially for underground and surface water. Aerobic and anaerobic OMW biotreatment processes were developed and improved and showed promising success. A bacterial mixture of 10 strains (*Aquaspirillum dispar, Bacillus cereus/thuringiensis, Brevibacterium otitidis, Klebsiella pneumoniae, Proteus penneri/vulgaris, Pseudomonas fluorescence* biotype F, *Pseudomonas marginalis, Pseudomonas mendonica, Pseudomonas sp. and Pseudomonas viridilivida*) and five yeast cultures (*Candida boidini, Candida memodendra, Candida mogii, Pichia haplophia* and *Sacharomyces ludwigii*) were isolated from OMW, purified and reused in OMW aerobic biotreatment. Pilot- (5,000 L) and industrial-scale (25,000 L) biotreatments were performed. After 14 days of pilot-scale biotreatment, a 69.6% in biochemical oxygen demand (BOD) and a 68.3% reductions in chemical oxygen demand (COD) values were achieved, while a 71.0% BOD and a 66.9% COD reduction were scored after 31 days of

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industrial-scale biotreatment. Anaerobic OMW biotreatment done at the experimental laboratory scale using omasomal juice as microbial starter achieved a reduction of 67.4% BOD and 65.9% COD with 37.2 L of biogas production per liter of Zibar after six weeks of incubation. The employed aerobic and anaerobic OMW biotreatment processes, developed at the LAU Biotechnology Labs, that achieved acceptable BOD and COD reduction rates and produced biogas, are low cost technologies and suitable for possible application in small rural olive mills in Lebanon and in the Middle East.

16.1. Introduction

In the Mediterranean basin and since early civilizations, olive trees were valued as symbols of wisdom, peace, abundance and glory.

The olive tree is most adapted to the Mediterranean climate and is one of its characteristic species (Figure 16.1). The Mediterranean region being poor in water resources, rendered the olive tree to become an economically beneficial target. Olive trees depend only on rainfall and don't need irrigation.

On the other hand, olive oil extraction is one of the most polluting agro-industrial sectors worldwide due to the formation of the olive oil mill wastewater (OMW). It is one of the two by-products obtained from olive oil extraction (Papadimitriou et al., 1997), known in Lebanon as Zibar.

16.2. Objectives of Study

The aim of this study is to improve Zibar treatment and bioremediation employing low cost technologies at laboratory and semi-industrial scales, the objectives are:

- Isolation, purification, identification and development of microbial cultures used in OMW biotreatment.

Figure 16.1 Hundreds of years old olive trees in Hasbaya, South Lebanon.

- Reduction of OMW organic load and toxic effects, thus minimizing its environmental threats.
- Development and operation of pilot aerobic and anaerobic treatment facilities at LAU Byblos campus and an aerobic industrial low-cost facility in Hasbaya, South Lebanon.
- Production of non-harmful products from the OMW biotreatment such as biogas and agricultural fertilizer.

16.3. OMW

OMW accounts for up to 50% (v/v) of the total olive oil mill output, while the olive oil accounts for 20% (v/v) and the remaining 30% (v/v) are the solid residue (Baccari et al., 1996; Hamdi et al., 1991; Pérez et al., 1998) known in Lebanon as Jift. More than 7.3 million tons per year (t /y) of OMW is generated worldwide, coming from the extraction of around 1.8 million tons per year of olive oil from 9.7 million tons of olives (Feria, 2000). Many studies report that OMW is a major pollutant to surface and ground water resources in the Mediterranean basin. It is one of the least biodegradable natural compounds due to its high phytotoxic phenols content, colored organic substances and high organic matter concentration (Al Khudari, et al, 2004; Paredes et al., 1986; Saez et al., 1992). OMW is a strongly smelling phytotoxic waste known for its antimicrobial activity (Pérez et al., 1992; Tuncel and Nergiz, 1993). General approximated constituents of the OMW could be established (Table 16.1) where each constituent alone has a negative environmental impact if disposed without treatment with the phenolic OMW being the most polluting constituents (Ragazzi and Veronese, 1989). OMW biological oxygen demand (BOD) could reach values as high as 100 g/L while the chemical oxygen demand

Table 16.1 Characteristics and composition of olive oil mill wastewater (Lopez, 1992; Skerratt and Ammar, 1999)

Olive oil mill wastewater characteristic	Value
Color	Intensive violet–dark brown up to black
Odor	Strong specific olive oil smell, foetid smell
pH	4.5–6.0
Water content	83–92%
Organic and volatile material	7–15%
Mineral solids	1–2%
Residual oil	0.3–10.0%
Total sugars	2–8%
Reducing sugars	1–8%
Polyalcohols	1.0–1.5%
Protein	0.5–7.5%
Pectins and tannins	1.0–1.5%
Phenols	17%
Suspended solids	5–35 g/L
BOD_5	65–70 g/L
COD	40–200 g/L

(COD) around 200 g/L (Ubay and Ozturk, 1997). The environmental problems linked to OMW are not confined to water, but some phytotoxic effects were also observed especially on plants germination and premature fall of the fruit and vegetables senescence (Feria, 2000).

New measures were implemented with many chemical, biological and physical methods being suggested due to the great failure of the land spreading and lagooning methods (Hoyos et al., 2002). With yearly increases in olive oil production and thus more OMW release into the environment no practical solution to the Zibar problem exists yet. Each olive mill may adopt a different system of waste treatment. The most adopted solutions include: adsorption, aerobic treatment, anaerobic treatment, composting, decolorization, drying or evaporation, electrolysis, filtration, membrane filtration, ultrafiltration, precipitation or flocculation, lagooning, thermal treatment, treatment by fungi and wet oxidation; and these solutions could be used separately or applied in combination (Improlive project A2, 2000).

16.3.1. Distribution of Olive Oil Production

Olive oil production is almost concentrated in the Mediterranean basin (Ettayebi et al., 2003). Regions dominated by a Mediterranean climate such as California, South Africa (Cape) and some regions in Mexico, Argentina and Australia are also involved in olive oil production. According to the United Nations Convention on Trade and Development records based on data from Food and Agriculture Organization Statistics (FAOSTAT), eight countries were found to produce 96% of the world olive oil in 2003 with the rest of the world contributing to the remaining 4% as shown in Figure 16.2 (FAOSTAT, 2003).

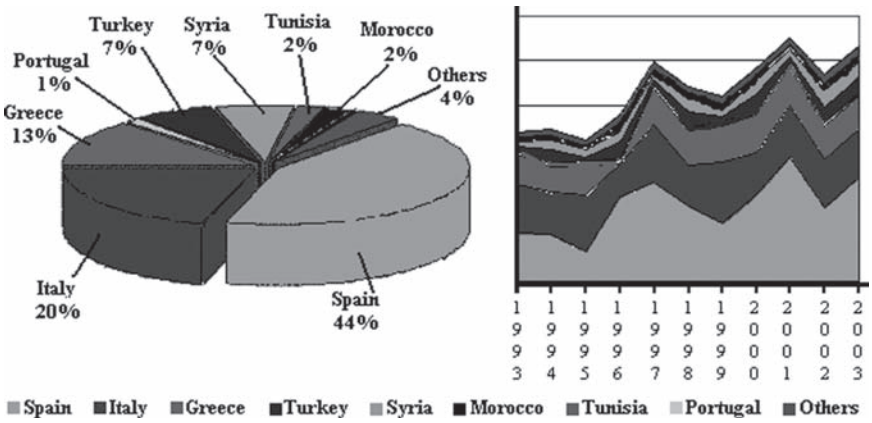

Figure 16.2 Main olive oil-producing countries in 2003 and the evolution of world production during the last 10 years (Food and Agriculture Organization Statistics, 2003)

Figure 16.3 Crude Zibar discharge into the Hasbani River by a local olive mill (Hasbaya, Zibar project, LAU)

Lebanon is one of the small olive oil producers. According to the FAOSTAT database, Lebanon produces around 6,000 tons per year of olive oil (Improlive project A2, 2000). The sector is mostly family owned and inherited from generation to generation as is the case with other Mediterranean countries; many Lebanese depend fully or partially on the income they generate from the olive oil industry; however, what distinguishes Lebanon from other countries is the absence of governmental supervision. Strict laws are not available to protect the environment from the OMW damages and if they exist are not implemented (Hashwa, 2003). It is estimated that around 1.6 million L of Zibar (BOD about 50,000 mg/L) is produced in Lebanon per year and disposed directly in the areas surrounding the olive mills in digs, wells, rivers, lakes and valleys (Al-Khudary et al., 2004). However, all alternatives used will lead to the seepage of OMW into the underground water table or other water sources (Hashwa, 2003).

Despite the existing environmental laws and regulations, disposal of Zibar into the nearest lands, streams and rivers by many olive oil mills, is still being practiced to avoid bearing additional economic costs of olive oil production in Lebanon (Figure 16.3).

16.3.2. Components of OMW

OMW constituents are similar in all olive oil mill production, but they differ in concentrations. Approximated constituents of the OMW and their relative concentrations are given in Table 16.1.

Each constituent of the OMW alone presents a threat to the environment and when combined together the threat is amplified. Tannins for example, that come from the olive skin, are not harmful for humans, animals or plants; however, they add a dark black–brown coloration to the water affecting light penetration. As a consequence, photosynthesis of many aquatic plants is impaired, respiration by aquatic organisms is diminished and the vision of many aquatic animals is weakened, leading to inability to locate their food sources. In the soil, OMW, containing acids, minerals and organics, could destroy the cationic exchange capacity of the soil. This in turn may inhibit growth of microorganisms, the soil–air and the air–water balance and consequently reduces the soil fertility (Improlive project A2, 2000).

16.4. Methodologies, Applications and Results

Several trials of Zibar biodegradation were performed at different scales. We report here on the results obtained with the pilot- and industrial-scale trials while the Lab and intermediate scale trials were reported elsewhere (Mhanna, 2006).

BOD, COD and pH values were measured and monitored through all trials to evaluate OMW properties changes before, during and after the treatment processes. Temperature and dissolved oxygen (DO) were additionally monitored to ensure optimal growth conditions for the microbial cultures.

16.4.1. Microbial Cultures

In most trial treatments, mixed bacterial and yeast cultures were used as inocula for starting and expediting the biodegradation process. The individual bacterial and yeast species were isolated in our laboratory from fresh crude Zibar.

The following microorganisms constituted the aerobic microbial culture according to the results obtained using the Biolog Identification System (Biolog Inc., USA): 10 bacterial strains: *Aquaspirillum dispar, Bacillus cereus / thuringiensis, Brevibacterium otitidis, Klebsiella pneumoniae, Proteus penneri/vulgaris, Pseudomonas fluorescence* biotype F, *Pseudomonas marginalis, Pseudomonas mendonica, Pseudomonas sp*. and *Pseudomonas viridilivida* and five yeast strains: *Candida boidini, Candida mogii, Candida memodendra, Pichia haplophia* and *Saccharomyces ludwigii*.

16.4.2. Pilot-Scale Aerobic Treatment

The pilot plant was a 5,000-L tank, with a working volume of around 4,000 L. This type of tank supplied and manufactured locally by Nassar Technology Group (NTG), Lebanon is used normally for domestic package wastewater treatment and was adapted for Zibar biotreatment (Figure 16.4).

Figure 16.4 Aerobic OMW pilot (5,000 L) treatment plant (LAU, Byblos Campus, Lebanon)

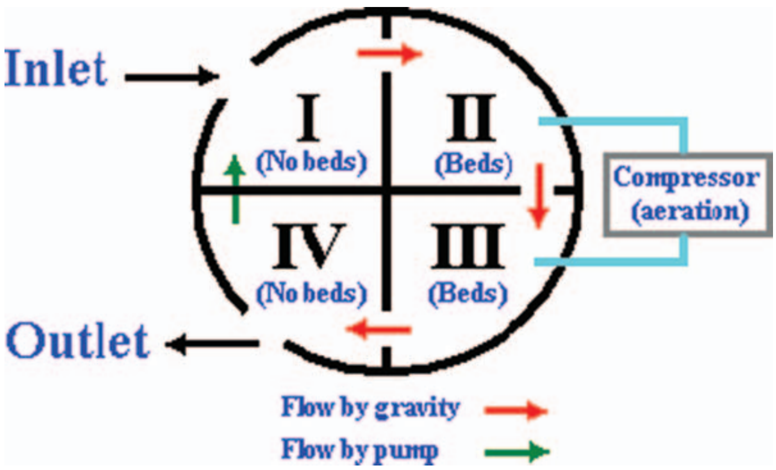

Figure 16.5 OMW cycle inside the pilot treatment plant at LAU, Byblos campus

The pilot tank (Figure 16.4), located at the LAU campus, comprised four interconnected compartments that allow continuous flow, aeration and/or sedimentation of circulating fluid (Figure 16.5).

Compartment II and III contained bed columns and were aerated with an air compressor (Rietschele, Germany; 1 bar, 43.5 m^3/h) housed on the top of the tank.

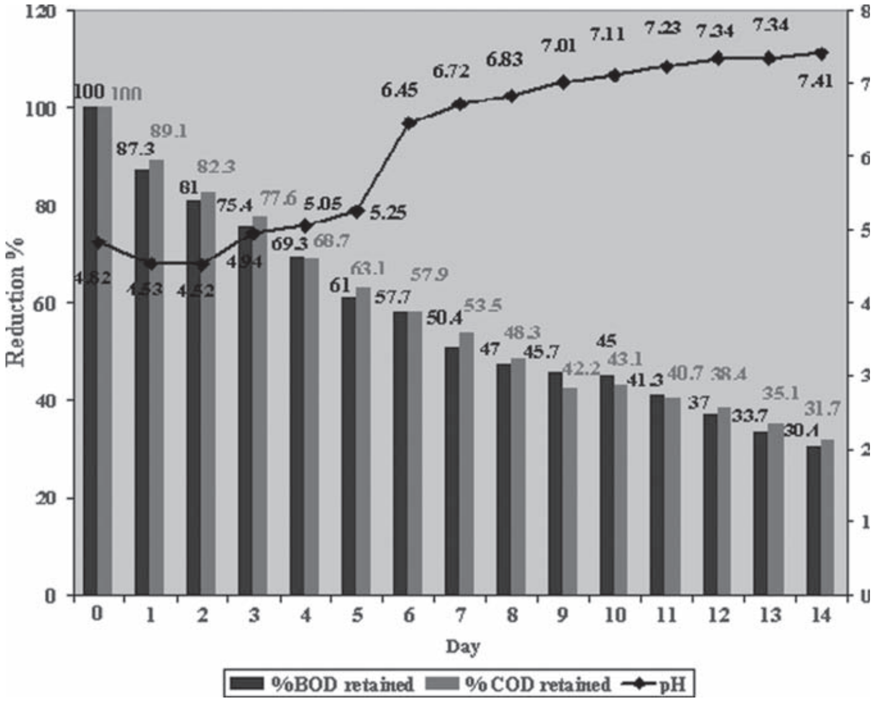

Figure 16.6 BOD, COD and pH changes in the pilot plant scale (5,0001) aerobic treatment of crude Zibar

Compartment IV contained a submersible pump, while compartment I was simply a Zibar holding container. Zibar was filled from the inlet of compartment I, overflowed from compartment I to II, III and then to IV. The cycling was sustained by a submersible pump located in compartment IV, a conical shaped chamber designed for biomass sedimentation (Figure 16.5).

In this series of trials a maximum of 69.6% reduction in BOD and 68.3% in COD values were achieved after 14 days of continuous treatment while an increase of 2.59 in the pH was detected (Figure 16.6).

16.4.3. Industrial-Scale Aerobic Treatment

The industrial-scale Zibar treatment assembly, located at Hasbaya, southern Lebanon, that was installed at the Ziad Abou Ghyda's olive oil mill comprised five large connected tanks (5,000 L each) filled with approximately 25,000 L of freshly pressed crude Zibar (Figure 16.7).

Zibar was pumped from the nearby olive oil mill by a polyethylene pipe (P1) to an intermediate holding tank (T0). Zibar then flowed by gravity from T0 to the

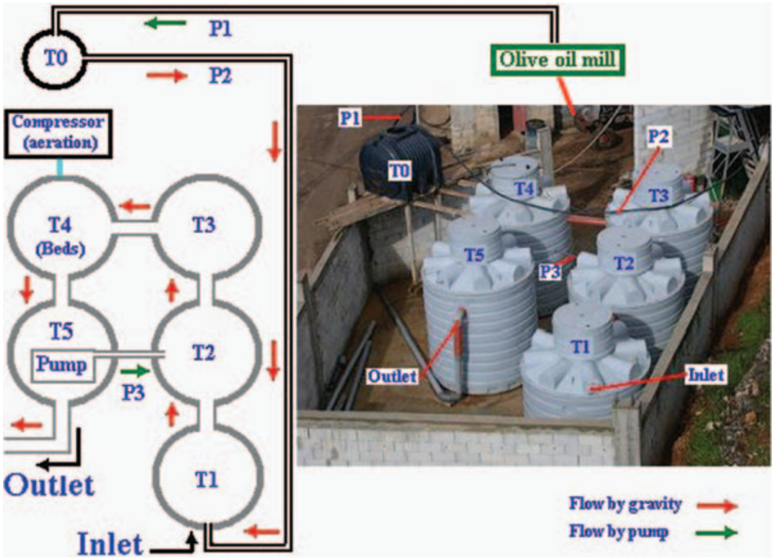

Figure 16.7 Industrial-scale (25,000 L) treatment plant at Hasbaya (P1–3, pipes; T0 holding tank; T1–5 treatment tanks connected in series as described in sketch)

first tank T1 through P2 (Figure 16.6). T1 was used for storage and settlement. Tanks 2, 3, 4 and 5 formed a closed treatment assembly (Figure 16.6). The biological reactor (T4) with compressor, plastic beds and diffusers was inoculated with bacterial and yeast mixed cultures and was sampled at fixed time intervals. The Zibar flowed from T4 into T5, an after-clarification step tank; where a submersible pump removed treated Zibar intermittently back to T2. This closed system was kept running for about four weeks. The treated Zibar was discharged out of T5 subsurface outlet into the neighboring Hasbani River (Figure 16.3). Zibar sludge sedimentation occurred in Tanks T2 and T3 served as sedimentation tanks allowing the partially clarified Zibar to reach the aerated Bioreactor tank (T4). T4 was inoculated with a freshly prepared mixed yeast and bacterial culture transported upon need from the 80 km distant LAU campus. The starter culture (inoculum) reached about 5% (v/v) of the total treated volume. Zibar samples were taken from T4 and occasionally from all other tanks on a weekly basis between October to March and were analyzed at LAU labs for Zibar biodegradation as indicated by changes in BOD_5, COD, as well as other parameters such as DO, pH and temperature.

Unlike the pilot plant scale, trials on the industrial scale stretched over a prolonged period of time reaching 31 days, where the freshly pressed crude Zibar was used directly from the olive mill. The microbial cultures were supplemented on a weekly basis. After 31 days of fermentation, a maximum decrease of 71%

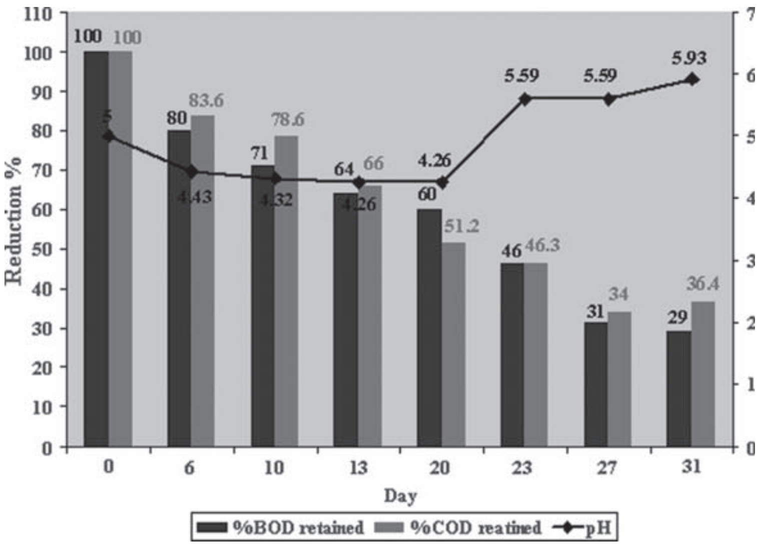

Figure 16.8 Biochemical oxygen demand, chemical oxygen demand and pH changes during industrial-scale (25,000 L) aerobic treatment of olive oil mill wastewater

in the BOD and 63.6% in the COD values and 0.93 increases in the pH were detected as illustrated in Figure 16.8.

16.4.4. Laboratory-Scale Anaerobic OMW Treatment

Erlenmeyer anaerobic flasks (Figure 16.9) were filled with 200 mL of a mixture of basal medium (Demirer and Speece, 1998), Zibar and omasomal juice (cows' third stomach). A control solution was also included to monitor the BOD base value and to determine the possible omasomal juice activity on the total BOD of the mixture.

The flasks were incubated in a water bath at 37 °C and vented to collect the biogas in inverted graduated burettes as shown in Figure 16.9. Changes in BOD, COD and pH values as well as biogas production were measured on a weekly basis for a period of six weeks.

The biogas (mostly methane) production under anaerobic conditions was followed over a period of six weeks under constant culture and incubation conditions. The formed gas mixture (CO, H_2, N_2, CO2, CH_4 and H_2S), which was bubbled first through a 2-L Erlenmeyer flask containing a solution of 20 g/L of KOH to remove CO_2 and other trace gases, was then released inside the inverted burettes (Figure 16.9). This

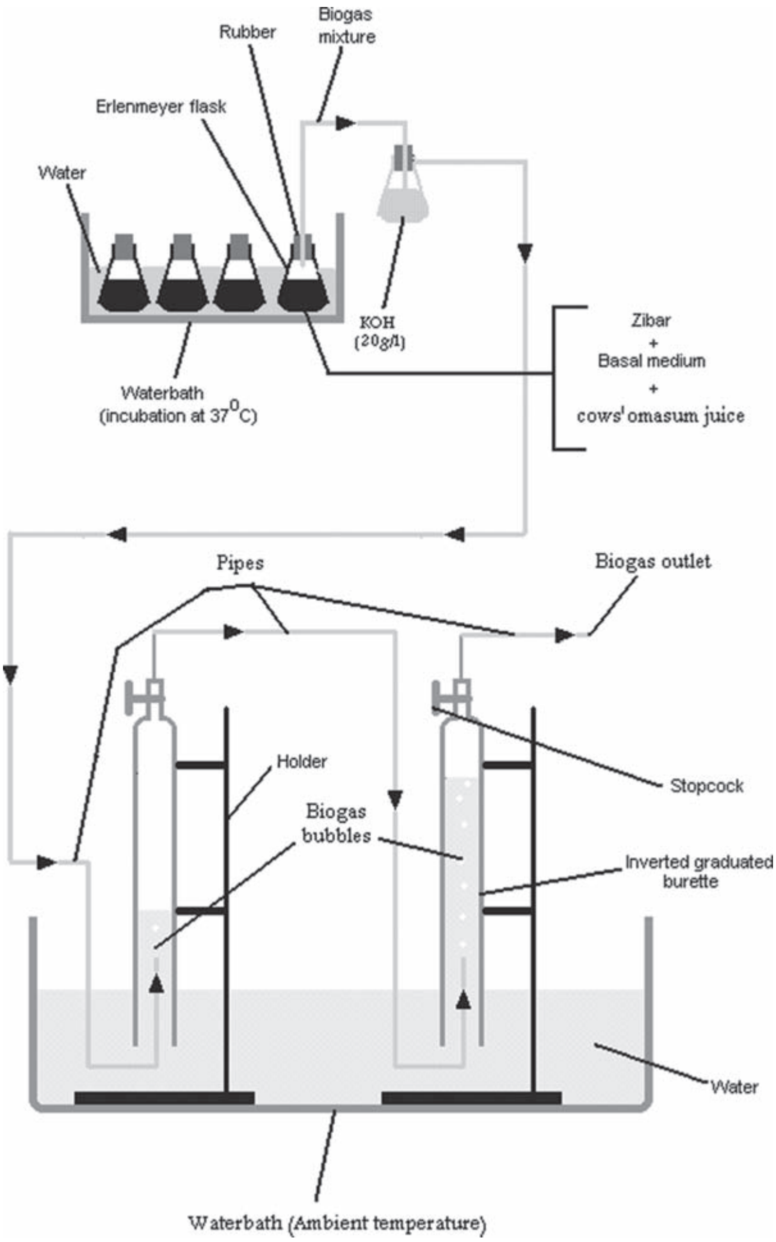

Figure 16.9 Sketch of the anaerobic system used for biogas collection

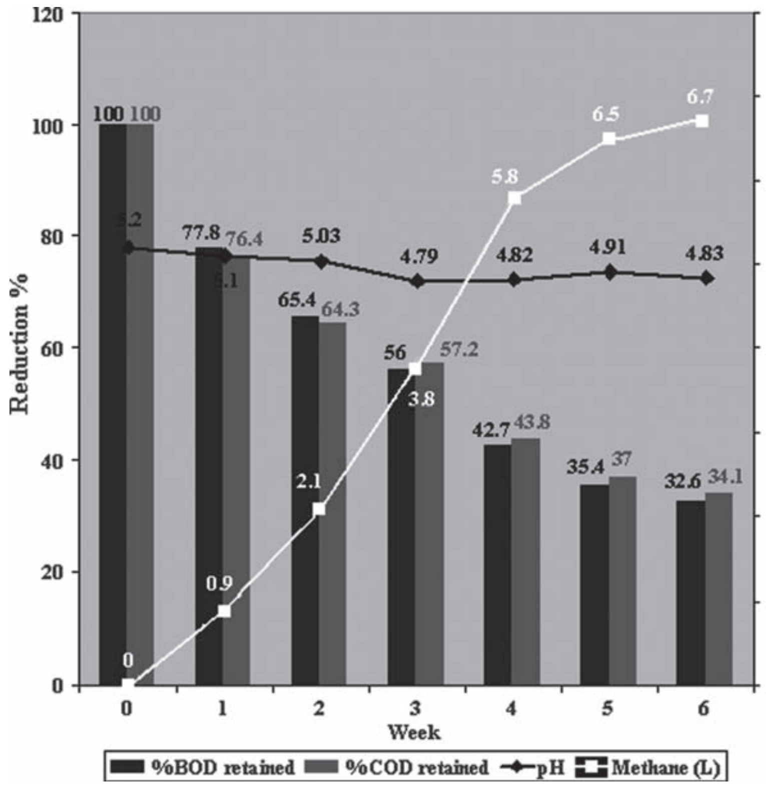

Figure 16.10 Anaerobic Zibar treatment of a crude Zibar using omasomal juice as inoculum showing changes in biochemical oxygen demand, chemical oxygen demand, pH values and Biogas production

released gas mixture was not qualitatively determined and was assumed to be 99% methane according to Demirer and Speece (1998) and Ergüder et al. (1999).

A maximum reduction of 67.4% in BOD and 65.9% in COD values and a decrease of 0.37 in the pH were established after six weeks of incubation of crude Zibar with anaerobic bacterial cultures (omasomal juice). During this period 37.2 L of biogas were produced per liter of Zibar as shown in Figure 16.10.

16.5. Discussion

The uncontrolled disposals of OMW in the environment represent a serious environmental problem. The antimicrobial activity (Capasso et al., 1995; Paixao et al., 1999), the inhibition of seed germination (Bonari et al., 1993; Perez et al., 1986), the phytotoxicity to herbage crops (Capasso et al., 1992; Tomati and Galli, 1992) and the production of stale odor by OMW have been demonstrated. OMW pollution is not limited to lands, but also could reach aquatic environments, surface and underground waters (Moreno et al., 1990; Mendia et al., 1986). Accordingly, high OMWs organic concentration and content of antimicrobial compounds, such as phenols, should be subjected to pretreatment before being discharged in the environment (Ehaliotis et al., 1999). OMW treatment can be achieved satisfactorily by various techniques. As these techniques have a high fixed and operational cost, the majority of olive mills cannot afford to adopt them leading to the spread of the pollution problems. The technical–economic problems such as the requirement of a large installation spaces, professional manpower and high economic costs of the proposed treatment techniques are all limiting factors in the OMW treatment (Ettayebi et al., 2003).

The main objective behind this research study was to develop a low capital cost operational OMW treatment techniques that can reduce the high organic load of OMW, minimize its toxic impacts on the environment and are suitable to be adopted by the Lebanese olive mills in order to minimize the OMW negative environmental impacts. The biological treatments or biotreatments of OMW are seen as treatments of choice, since they are low cost and are capable of converting toxic compounds to useful commercially valuable such as single-cell proteins, agricultural fertilizers, phenolic compounds and biogas (Ettayebi et al., 2003; MINOS Project 2004). This research study was conducted on two treatment strategies to look into effective means for the alleviation of the negative environmental impact associated with the discharge of OMW. These included: an aerobic biotreatment and an anaerobic biotreatment. The aerobic technique was carried at two different fermentation levels the pilot (5,000 L) scaled up to the industrial scale of 25,000 L. These aerobic treatments require relatively simple installation and maintenance; with both scales being low cost and affordable by small- to medium-sized Lebanese olive mills. In contrast the anaerobic biotreatment process, being more complex, was only experimented at the small laboratory scale. In the proposed aerobic treatment, the OMW was assessed in terms of the biodegradation parameters, BOD and COD reduction, while the released biogas under anaerobic conditions was collected and assessed to evaluate the efficiency of the small scale anaerobic fermentation.

To perform the different aerobic fermentations, the following microorganisms (bacterial and yeast cultures were isolated and purified from OMW enrichment cultures. The microorganisms included the following bacterial strains: *Aquaspirillum dispar, Bacillus cereus / thuringiensis, Brevibacterium otitidis, Klebsiella pneumoniae, Proteus penneri/vulgaris, Pseudomonas fluorescence* biotype F, *Pseudomonas marginalis, Pseudomonas mendonica, Pseudomonas sp*. and *Pseudomonas viridilivida* and yeast strains: *Candida boidini, Candida mogii, Candida memodendra, Pichia haplophia* and *Saccharomyces ludwigii*. A survey of the literature revealed very little information about OMW microbial aerobic fermentations and the specific species involved. Trials of aerobic fermentation for pilot and industrial scales, to the best of our knowledge, are not reported in the literature and such large-scale fermentation has been done only with anaerobic biotreatment. In the pilot plant

(5000 L) fermentation trials, a 69% reduction in BOD and a 68.0% reduction in COD values were recorded. These values were relatively similar to the ones achieved by Ettayebi et al. (2003; 69.7% COD reduction) while they were better than Mendoça et al. (2004) with 50% COD reduction. But this process required an incubation period of 14 days for the biodegradation crude Zibar inoculated with aerobic microbial mixed cultures.

The NTG pilot reactor in our study was originally designed for aerobic biodegradation of domestic wastewater. The domestic sewage has a pH ranging between 7.5 and 8 and a BOD around 0.35 g/L, while OMW pH varied between 4.5 and 6.0 and a BOD of 50 g/L (Improlive project A1, 2000; Lopez, 1992; Qureshi et al., 2004; Skerratt and Ammar, 1999). During summer time the temperature inside the pilot plant exceeded the optimal temperature of the aerobic microbial culture mixture to reach about 55 °C. Therefore the aeration time was reduced in order to decrease the temperature to cope with optimal needed range. The melting point of fats and long chain fatty acids is often well above ambient temperatures. At such temperatures, these substances become liquid and are more accessible to microorganisms and their lipolytic enzymes. Diffusion coefficients and the solubility of fatty acids in aqueous media increase significantly with rising temperatures allowing for a better mass transfer (Becker et al., 1999). The industrial-scale fermentation runs showed a maximal decrease of 71% of the BOD and 63.6% in the COD values after 31 days of incubation. These are relatively acceptable values. The two levels of aerobic biotreatment achieved acceptable results in BOD and COD reduction rates. The pilot plant and industrial-scale fermentations could be recommended in Lebanon and other Middle East countries as the OMW treatment of choice since they are low cost and can be locally manufactured and maintained.

The anaerobic biotreatment was only conducted on the small laboratory scale. The objective of using the anaerobic technology, inaddition to reducing the organic load, was to produce a valuable energy source, the biogas. OMW anaerobic treatment, inoculated with omasomal juice as the microbial starter culture, resulted in a reduction of 67.4% BOD and 65.9% COD with the concomitant production of 6.7 L of biogas during the six-week fermentation process. To the best of our knowledge the application of bovine omasomal juice in OMW treatment is reported here for the first time in the literature. According to Kamra (2005) the optimal growth for the rumen microorganisms is 39 °C. The experimental incubation temperature was fixed at 37 °C as set by Ergüder et al. (1999) for anaerobic biotreatment using old OMW sludge as inoculum. This could explain the higher values scored by Ergüder et al. (1999), where COD removal efficiencies ranged between 85.4% and 93.4 compared to 65.9 to 67.3% in our hands, and a 11.42 L of biogas during 44 days of incubation compared to 6.7 L during six weeks in our case. Additionally, good results were achieved by Marques (2001) with 70 to 80% reduction in the COD values inside an upflow anaerobic reactor during six to seven days while a removal efficiency of up to 85% in COD values were reported by Sabbah et al. (2003).

16.6. Conclusions

- This study reports that the aerobic microbial culture that was used in OMW biotreatment was constituted of the following microorganisms: ten bacterial strains: *Aquaspirillum dispar, Bacillus cereus / thuringiensis, Brevibacterium otitidis, Klebsiella pneumoniae, Proteus penneri/vulgaris, Pseudomonas fluorescence* biotype F, *Pseudomonas marginalis, Pseudomonas mendonica, Pseudomonas sp*. and *Pseudomonas viridilivida*; and five yeast strains: *Candida boidini, Candida mogii, Candida memodendra, Pichia haplophia* and *Saccharomyces ludwigii*.
- After 14 days of pilot-scale biotreatment, a 69.1% reduction was recorded in BOD values and a 68.2% reduction in COD values.
- A 71.0% BOD and a 63.9% COD reduction was observed after 31 days of industrial-scale biotreatment.
- Anaerobic OMW experimental laboratory-scale biotreatment using omasomal juice as inoculum culture achieved a reduction of 67.8% BOD and 66.6% COD with 6.7 L of biogas production after six weeks of incubation.

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