Bioremediation of Olive Oil Mill Wastewater: Chemical Alterations Induced by *Azotobacter vinelandii*†

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An environmentally friendly bioremediation system of olive oil mill wastewater (OMWW) is studied with respect to its physicochemical characteristics and degradation efficiency on major characteristic constituents. The method exploits the biochemical versatility of the dinitrogen fixing bacterium *Azotobacter vinelandii* (strain A) to grow in OMWW at the expense of its constituents and to transform it into an organic liquid fertilizer. The system eliminates the phytotoxic principles from OMWW and concomitantly enriches it with an agriculturally beneficial microbial consortium along with useful metabolites of the latter. The end product, branded "biofertilizer", is used as soil conditioner and liquid organic fertilizer. Growth of *A. vinelandii* in OMWW results in the decline of content of most of the compounds associated with phytotoxicity, and this is confirmed by the assessment of degradation yields. In parallel, during the process several other compounds noncommittally undergo degradation and biotransformation. More specifically, the biofertilization system is capable of achieving removal yields as high as 90 and 96% after 3 and 7 days of treatment, respectively. Statistical analysis of the results showed that between the periods of operation no significant difference occurs with respect to the degradation yield. Moreover, the degradation yield from 3 to 7 days of continuous operation of the system remains almost unaltered during 2 consecutive years.

Keywords: *Olive oil mill wastewater; biowheel type reactor; Azotobacter vinelandii; bioremediation; chemical study*

INTRODUCTION

Olive oil mill wastewater (OMWW) is a notorious pollutant of both terrestrial and aquatic ecosystems in the Mediterranean region (Moreno et al., 1990; Mendia et al., 1986; Servis, 1986). OMWW is a turbid, dark, and acidic in reaction (pH 4.5−5.5) effluent that carries a high organic and polyphenol load and emanates a sharp characteristic odor. It displays antibacterial properties, inhibits seed germination, and is phytotoxic (Perez et al., 1986; Bonari et al., 1993; Capasso et al., 1992, 1995; Paixao et al., 1999).

The OMWW composition may vary according to cultivar, harvesting time, health of the olives, and technology used in the extraction process. In general terms, the organic fraction contains a complex consortium of phenolic substances, some nitrogenous compounds (especially amino acids), organic acids, sugars, tannins, pectins, carotenoids, oil residues, and almost all of the water soluble constituents of the olives (Balice et al., 1984; Ramos-Cormenzana, 1986). The inorganic fraction contains chloride, sulfate, and phosphoric salts of potassium as well as calcium, iron, magnesium, sodium, copper, and other trace elements in various chemical forms. The inorganic constituents at the concentration levels found in OMWW are not toxic; quite the reverse, they may potentially serve as good sources of plant nutrients. The phytotoxicity of OMWW is due to the phenolic substances and some organic acids such as acetic and formic acid, which are often produced along with other microbial metabolites during storage. Due to its phenolic constituents, OMWW inhibits several groups of bacteria and fungal species (Whitehead, 1964; Ramos-Cormenzana, 1986; Fleming et al., 1986; Gonzalez et al., 1990; Juven et al., 1970; Paredes et al., 1986; Moreno et al., 1987; Perez et al., 1992; Whitehead, 1964; Ramos-Cormenzana et al., 1996). This property affects both the aerobic and anaerobic methods of treatment (Maestro Duran et al., 1991).

An aerobic method of treatment has been developed and has been in use in Greece during the past few years (Balis, 1995; Balis et al., 1987, 1993, 1996; Chatjipavlidis et al., 1996; Flouri et al., 1990). The method exploits the capacity of an isolate of *Azotobacter vinelandii* (strain A) to grow in OMWW and its versatility in transforming the OMWW into an organic liquid fertilizer and soil conditioner. *A. vinelandii* is a free-living N_2-fixing bacterium that uses numerous compounds, including phenolics, as energy and carbon sources (Rubenchik, 1963; Chen et al., 1993; Wu et al., 1987). The particular strain was isolated from a calcareous soil repeatedly treated with OMWW (Balis, 1995) and was found to be particularly efficient in fixing molecular nitrogen when grown in OMWW (Balis, 1995; Balis et al., 1996; Papadelli et al., 1994, 1996; Echaliotis et al., 1999). The strain was therefore used for the bioremediation of OMWW, through a method developed

† This paper is dedicated to our coauthor Constantine Balis, who passed away unexpectedly April 7, 2000.

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10.1021/jf991060v CCC: $19.00
Published on Web 04/26/2000

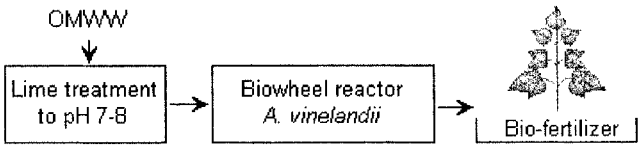


Figure 1. Schematic representation of the bioremediation process of OMWW.

by Balis and his associates (Balis et al., 1993). During the process, after an initial phase of adaptation, the bacterium grows rapidly, fixing molecular nitrogen and concomitantly detoxifying the OMWW (Balis, 1995; Balis et al., 1996; Echaliotis et al., 1999). Moreover, the bacterium produces copious amounts of extracellular polysaccharides similar to alginates (Zafeiropoulou 1997; C. Balis, unpublished data) and excretes plant growth promoting factors.

The purpose of this work was to study the physicochemical status of OMWW before, during, and after the bioremediation process and to assess the degradation efficiency on major characteristic constituents. The bioremediation process was carried out in a pilot plant that was constructed in an olive mill operating at its maximum capacity. Thus, the results are considered quite representative and highlight the operational features of the system.

EXPERIMENTAL PROCEDURES

Biofertilization Plant. The bioremediation process of OMWW is depicted schematically in Figure 1. The plant is a modification of the one constructed in Messinia (Chatjipavlidis et al., 1996). It consists of a pretreatment tank for liming and a bioreactor of 25 m^3 capacity, both made of concrete. The latter is equipped with a rotating biowheel type air conductor. This is a perforated stainless steel cylinder 2 m in diameter by 2 m in length, filled with tubular pieces of PVC (5 cm in diameter by 5 cm in length) that offer quite an extensive surface area. The air conductor is immersed to its two-thirds level into the liquid and revolves on its axis at a rate of 8 rpm. The unit is installed and operates in Peta's Olive Oil Co-operative in Epirus, northwestern Greece. During the process the OMWW goes through two distinct stages. In the first, the effluent is treated with calcium hydroxide at a rate sufficient to bring the pH to ~8−10 and then is transferred into the bioreactor (stage II), where it is mixed with a population of *A. vinelandii* (strain A). The process is carried out in a repeated fed-batch culture system with a cycle time 3 or 7 days, according to the experimental design, with a residual volume one-third of the total.

Sampling. Three samples from different stages of the remediation system were taken and analyzed during the olive oil campaigns of 1998 and 1999. More specifically, samples were collected from (a) the raw OMWW that was coming out directly from the decanter of the olive oil mill, (b) the liming tank, and (c) the bioreactor after 3 and 7 days of residence time. The plan of sampling was based on the experience obtained from the operation of a similar plant (Chatjipavlidis et al., 1996). Amounts of 2.5 L were collected in dark bottles, and the samples were brought to the laboratory to be analyzed immediately.

Apparatus. Conductivity was measured using a conductivity/TDS meter from the Hach Co. (Loveland, CO). A Varian (Mulgrave, VIC, Australia) AA-300 atomic absorption spectrometer in the flame mode was used for metal analyses. A single-beam Hach spectrophotometer DR3000 was used throughout the study for the analysis of inorganic species. HPLC measurements were performed with Shimadzu LC-10AD liquid chromatographs (Shimadzu Corp., Kyoto, Japan). Each instrument was a completely integrated system equipped with a ternary gradient solvent delivery and six port injection valves (Rheodyne, Cotati, CA). Gas chromatographic analyses with flame ionization detector were performed using a Carlo Erba (Milan, Italy) chromatograph (HRGC 5300 Mega series) equipped with a capillary column SE-54, 25 m × 0.32 mm, thickness of 0.45 μm, operated in the split/splitless injection mode. A Hewlett-Packard (Geneva, Switzerland) 5890 series II gas chromatograph coupled with a 5971A mass selective detector operating in the electron impact mode was used for peak identification. Injections of 1 μL were made by using a 7673 autoinjector (Hewlett-Packard).

Analytical Procedures. Chemical oxygen demand (COD) was measured spectrophotometrically after the samples had been treated with potassium dichromate and sulfuric acid at 150 °C for 2 h. Total phosphorus was measured spectrophotometrically with the ascorbic acid method after the organic phosphorus had been oxidized with sodium persulfate and sulfuric acid (Clesceri et al., 1989). Chloride was measured by ion chromatography/conductivity detection (Shimadzu CDD-6A) after the sample had been treated with 0.5 N nitric acid.

Metals were analyzed as follows: An aliquot of homogenized sample was transferred to a glass beaker, and 3 mL of concentrated nitric acid was added. The mixture was placed on a thermostating plate and evaporated to near dryness. Five milliliters of concentrated nitric acid was added to the residue, the beaker was covered with a watch glass, and heating was maintained until brown fumes stopped evolving. Concentrated hydrochloric acid was added until complete dissolution of the matter, and the solution was made up to the desired volume with double-distilled water.

The method used for the extraction of organic components is outlined in the schematic diagram of Figure 2 and constitutes a modification of the analytical scheme proposed elsewhere (Belitz et al., 1994). Homogenization and centrifugation of an aliquot of sample to separate the liquid from the solid phase preceded the implementation of the analytical method. The phases were acidified to pH 2, a saturated solution of sodium chloride was added, and subsequently both were extracted with diethyl ether. Two distinct fractions, the aqueous and the organic, resulted from this preliminary treatment.

Chromatography. The operating parameters of the gas chromatographs were as follows: detector temperature, 280 °C; injector temperature, 240 °C; oven temperature, 50 °C (hold 2 min), 7 °C/min to 280 °C (hold 15 min). Helium was used as carrier gas regulated at 1.0 mL/min.

Analyses were performed in triplicate, and the results are given as mean values. Relative standard deviation for inorganic analyses does not exceed 2.5%, and for organic analyses it lies around 5%.

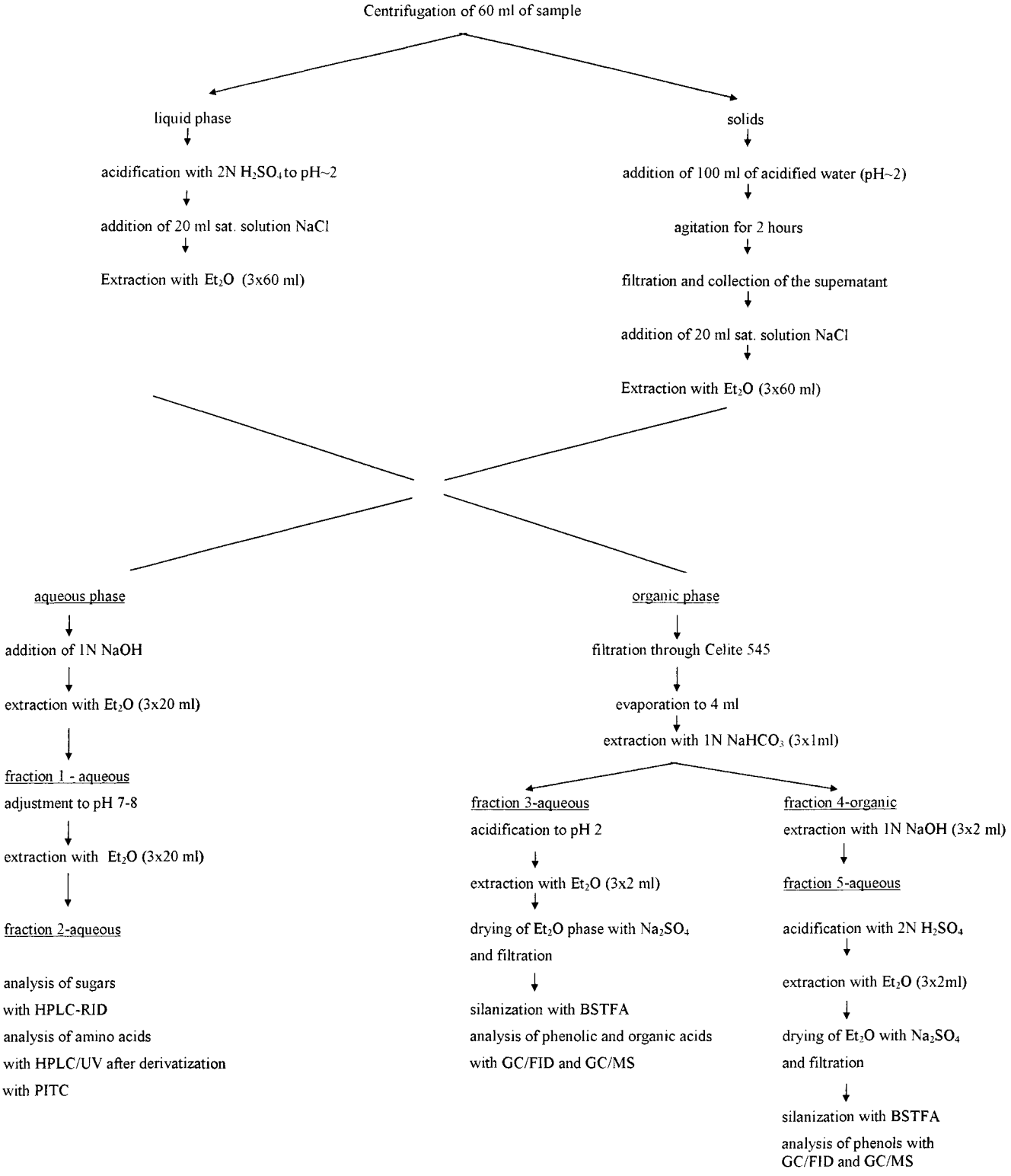
Sugars were analyzed by HPLC/refractive index detection (Shimadzu RID-10A) with a Supelcogel column C-611, 30 cm × 7.8 mm, 5 μm, thermostated at 60 °C. The mobile phase was double-distilled water at a flow rate of 0.5 mL/min.

Amino acids were determined after derivatization with Edman reagent by HPLC-UV at 254 nm using an Accubond cyano column thermostated at 30 °C (Edman et al., 1967; Murphy et al., 1987). The mobile phase was maintained at a nominal flow rate of 1 mL/min and consisted of 0.2 M ammonium acetate (pH 5.4)/acetonitrile/methanol at a ratio of 70:12:18.

Statistical Analysis. Analysis of variance (ANOVA) of data was conducted using the Statistica software package (version 5.1, StatSoft Inc., Tulsa, OK) with the following variables: VAR 1, period of operation (two levels, first year, second year); VAR 2, samplings within the period of operation (three levels, i.e., the three samplings performed); VAR 3, bioremediation time (two levels, 3 days and 7 days); VAR 4, compounds (43 levels, as many as the compounds studied); and VAR 5, degradation yields (independent variable).

RESULTS AND DISCUSSION

The chemical composition of OMWW by virtue of its natural origin and other uncontrollable variables that are involved in its production is not constant. Despite



BSTFA: N,O-bis (trimethylsilyl)trifluoroacetamide, PITC: phenylisothiocyanate, Et_2O: diethyl ether

Figure 2. Outline of the employed analytical method.

this difficulty, we attempted to identify the nature of the main compounds present in raw OMWW (Balice et al., 1984; Gonzalez-Vila et al., 1992) and to follow their fate during the bioremediation process.

All of the experimental results with respect to identification of compounds have been drawn on the basis of comparison with standards or by interpretation of mass spectra.

Table 1 presents the mean values of the concentrations of metals as well as some important parameters to assess the fluctuation during bioremediation of OMWW. The high conductivity value is due to the presence of increased concentrations of major metal ions in the OMWW. This parameter along with calcium content increased considerably in the first stage (i.e., liming). Chloride and sodium levels are influenced by

Table 1. Mean Values of Physicochemical Parameters and Metallic Ions in the OMWW after 3 and 7 Days of Treatment

sample[a]	conductivity, ms/cm	COD, g/L		total P, mg/L		N/NH_3, mg/L		Cl, mg/L		Cu, mg/L		Zn, mg/L		Mn, mg/L		Fe, mg/L		Na, mg/L		Ca, mg/L		K, mg/L		Mg, mg/L		B, mg/L	
	B[b]	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
infl	10.0	161	178	197	81	170	164	406	533	1.7	2.1	2.0	2.4	1.2	1.5	9.0	18.3	23.4	199	187	195	3650	2380	119	92	13.4	14.7
3d	15.0	124	129	205	81	317	276	344	487	2.0	2.7	7.1	5.3	1.5	1.9	15.5	25.4	51.0	269	2770	2710	4100	3050	233	175	25.1	16.3
7d	21.5	179	188	210	85	265	287	355	472	1.9	3.1	6.4	7.6	1.5	1.7	14.3	26.4	44.7	255	2700	2600	4960	3150	225	185	25.0	18.2

[a] infl, raw OMWW; 3d, after 3 days of incubation; 7d, after 7 days of incubation. [b] A and B represent the first and second period (year) of operation of the system.

the quality of the water that is used in the olive oil extraction process. The elevated concentrations of the other metals that were observed on the last day of sampling are due partly to the concentration of the material because of water evaporation. In the case of iron and zinc their increase is due partly to the lime additions and partly to corrosion of some metallic parts of the bioreactor. After 3 days of operation, the COD diminished to ~70%; on the seventh day of batch operation it rose again and recovered a large share of the original value. Probably, the initial decline of COD emanates form the rapid consumption of sugars and other easily consumable substrates by the *Azotobacter* population. Subsequently, it rises again because of the biosynthesis and accumulation of new microbial products such as microbial biomass and other *Azotobacter* metabolites that are formed as a result of nitrogen fixation (Brown et al., 1968; Garcia-Barrionuevo et al., 1992; Martinez-Toledo et al., 1985, 1988). Nitrogen in its ammonium form follows a similar trend of increase over the bioremediation period; this is in concurrence with the assumed action of *Azotobacter* toward nitrogen fixation. Elements such as potassium, magnesium, and boron are present in soil in chemical forms not always directly available to the plants, but they may become so through the action of soil microbes. These metallic species stand at sufficiently high concentrations in the untreated OMWW and at slightly elevated levels in the final product. Consequently, these elements along with the wealth of the other trace elements present in the final product constitute an important source of nutrients that may become available to plants through the action of the *Azotobacter* population.

The great diversity of organic acids and phenolic compounds in OMWW merit special consideration. The analytical characteristics of the processed OMWW (biofertilizer) are presented in Table 2 and offer good evidence for the bioremediation efficacy of the biorector with respect to the foregoing compounds.

The raw OMWW is markedly rich in some phenolic compounds and organic acids. L-Lactic acid, citric acid, acetic acid, oleic acid, caffeic acid, palmitic acid, and 4-hydroxyphenylethyl alcohol are predominant, although differences in their concentrations are noticed between samples taken within the same period (year) of operation. For these constituents the bioremediation process resulted in a decrease ranging from 66 to 99% after 3 days of incubation depending on the initial concentration. After 7 days of incubation, some of them (e.g., caffeic acid, palmitic acid, and 4-hydroxyphenethyl alcohol) exhibited degradation yields at levels very close to 100%. The rest of the studied organic acids and phenolic compounds are reduced to the bare minimum within the treatment period of time. The reported compounds account for a small fraction of the vast number of the phenolic compounds that occur in the complex matrix of OMWW. Nonetheless, the degradation system is capable of abating most of the studied compounds, especially those that are present in the initial waste at low concentration levels (Lopez-Aparicio et al., 1977).

Amino acids are at appreciable levels in the raw waste as well as in the final (biofertilizer) product. With some minor exceptions, after 3 days of incubation, they are degraded to ~70%; after 7 days, the yield is not significantly improved. It should be pointed out that the amino acid concentrations in "biofertilizer" refer to the total amount of both plant and microbial origin.

From a quantitative point of view, sugars constitute the most important organic fraction of OMWW. Although some of them were not detected in the studied OMWW (mannose, arabinose, etc.), there were a few present at considerably high levels. All sugars were degraded to a great extent after 3 days of treatment. However, almost complete degradation was attained after 7 days of incubation.

Analytical results between raw and limed OMWW are almost the same (data not shown) within experimental error. In this regard, the initial 6-h pretreatment with lime does not bring about any changes to the composition of the organic fraction of raw OMWW.

At first glance, no major differences were observed on the degradation efficiency of the biological treatment system after 3 and 7 days of incubation, yet differences among sets of data based merely on visual inspection do not offer unequivocal conclusions on yields of degradation because of the small differences between the respective values and the large number of the compounds involved. To detect whether there is any significant variation on the degradation, an ANOVA was performed. Considering that the local heterogeneity in the bioreactor is inconsequential due to the continuous mixing of the substrate, a multiple ANOVA was chosen to test whether the variations in the yields of bioremediation are significantly greater than the variation due to random error of measurements.

The ANOVA analysis gave a new insight into the interpretation of the results. More explicitly, it was found that the differences within the first variable and between the first and third variables are insignificant (level of significance = 80%). In other words, no significant differences exist in degradation yields between the periods of operation. Also, the degradation yield from 3 to 7 days remains almost unaltered from year to year. Figure 3 delineates some of the interactions among the factors considered. From the first to the second sampling for both 3 and 7 days of treatment, the degradation yield increased and almost leveled off. The differences among the samplings within the same period of operation could be ascribed to differences between different batches of OMWW and the involvement of uncontrolled factors such as fluctuation of temperature, sunlight, and rainfall during the operation of the system. Differences are also noticeable between the degradation yields of the

Table 2. Results of the Chemical Analysis of Raw and Bioremediated OMWW after 3 and 7 Days of Treatment[a]

	1st period (year of operation)									2nd period (year of operation)								
	1st sampling			2nd sampling			3rd sampling			1st sampling			2nd sampling			3rd sampling		
	infl	3d	7d	infl	3d	7d	infl	3d	7d	infl	3d	7d	infl	3d	7d	infl	3d	7d
carbonic and phenolic acids/alcohols (mg/L)																		
citric acid	2460	756	593	842	167	98	1050	149	105	350	140	101	440	165	101	400	148	88
succinic acid	23.4	8.5		16.6			20.5			28.8			23.5			38.6		
L-lactic acid	3140	950	740	8010	1620	1310	6700	1350	940	9600	2400	2100	7400	1920	1460	6750	1510	1200
fumaric acid	1.2	0.4	0.1	0.9			1.6			1.6			0.9			1.4		
sebacic acid				7.1			5.3			8.1			4.3					
azelaic acid	11	2.4		3.9			7.5			9.2			8.4			3.5		
caffeic acid	147	21.6	7.5	12.4			122	4.6		253	21.7	12.6	81	7.2		155	8.8	
trans-cinnamic acid	0.2			0.1			1.0			2.1			1.6			0.2		
3,4-dihydroxybenzoic acid	8.2	2.4					6.5			10.7			6.8			7.6		
p-hydroxybenzoic acid	1.7	0.2		2.6	0.4		3.4	0.5	0.2	4.2	0.9		1.8	0.3		3.1	0.7	
3,4,5-trimethoxybenzoic acid	6.6	1.8	0.5	8.2			7.3			8.5	1.9	0.6	7.6	1.7		4.5	0.6	
3,4-dimethoxybenzoic acid	1.3			1.3			1.5			1.2			0.9			1.3		
vanillic acid	6.0	0.9	0.4	15.1			21.2	0.5	0.2	17.8	2.9	1.1	23.8	3.8	0.5	21.5	3.8	1.3
p-coumaric acid + 4-hydroxycinnamic acid	10.9	2.0	0.8	5.6	0.8	0.2	6.4	0.7	0.2	6.5	0.8	0.6	9.8	1.4	0.6	5.5	0.6	
syringic acid	11.0	3.4	2.0	14.2			12.7			12.9	2.0	1.1	11.6			10.9		
ferulic acid	6.1	0.2		0.8			5.8			6.2			5.7			0.9		
phenolacetic acid				0.1			0.7			1.1								
benzoic acid	0.4			0.3			0.9			0.6			0.4			0.3		
cyclohexanecarboxylic acid	0.4			0.1			0.2			0.6			0.3			0.1		
2-phenoxyethanol + pelargonic acid	2.1	0.8	0.6	3.8	0.4	0.2	4.5	0.4	0.2	3.2	1.1	0.8	3.6	0.4		1.7		
acetic acid	530	212	175	2550	430	275	1920	286	195	1660	330	308	3150	320	272	2250	360	305
isovaleric acid	17.7	2.9		27.2	3.2	0.2	31.5	4.4	0.2	32	4.9	1.3	15	2.3		27.0	4.1	
n-valeric acid	5.0	1.9	0.8	4.9	0.9	–	6.9	0.9		5.4	1.6	0.6	4.9	0.7		5.2	0.5	
n-caproic acid	1.5			22.9	2.7	0.5	13.7	1.8	0.2	22.8	3.3	1.2	2.5			26.7	2.6	
2-hydroxymethylbutyric acid	6.3			12.5			23.5			7.8			6.5			5.1		
2-methyl-2,4-pentanediol																		
2-hydroxyisocaproic acid	0.6			0.8			0.8			0.9			0.5			0.7		
2-hydroxycaproic acid	0.2			0.1			0.1			0.4			0.1			0.1		
2-methyladipic acid																		
4-hydroxyphenylethyl alcohol	63.5	2.3	0.5	108	1.3	0.2	217	2.3	0.5	75.3	5.1	1.5	99	6.9	2.8	176	12.3	1.7
D-3-phenyllactic acid	1.7			1.8			2.0			1.6			1.7			2.3		
pimelic acid	2.4	0.7	0.4	0.9			3.2			4.2	1.6		0.8			0.6		
suberic acid	5.8	1.8	0.8	8.1	1.8	0.6	18.1	2.1	0.7	8.7	1.5	1.3	19.5	2.9	1.6	16.5	1.8	1.0
palmitic acid	135	13.4		109	22.0	1.1	174	25.7	1.5	135	14.9		166	28.2	5.0	105	12.5	2.1
oleic acid	934	14.5	4.0	533	3.5	0.8	1150	4.8	1.2	1250	48.6	25.1	943	18.5	9.5	725	23.0	3.5
phytol	8.8	2.3	1.9	8.7			7.6			8.5	1.4		10.5	2.0		7.5		
formic acid	133	59.1	27.0	32.2	4.5	3.1	232	6.2	2.9	35.4	9.9	6.4	56.7	9.6	6.4	130	16.2	10.3
amino acids (μg/L)																		
glycine + proline	377	110	55	376	107	50	415	104	55	430	140	63	400	120	56	380	112	55
histidine	4.2	0.7	0.5	3.3	0.7	0.3	5.4	1.1	0.5	6.2	0.9	0.6	4.4	0.7	0.5	4.9	0.9	0.7
arginine	31	8.8	2.0	79	9.5	2.2	61	8.5	2.0	65	19	5.9	52	14	4.4	46	12.4	4.4
tyrosine	490	141	1.0	440	91	1.1	530	89	2.9	515	140	96	500	140	98	450	125	90
sugars (%)																		
raffinose	1.45	0.19	0.05	2.41	0.30	0.15	2.82	0.35	0.15	0.48	0.06		0.42	0.06		0.51	0.07	
lactose	0.11						0.16			0.12			0.07	–		0.13	–	
glucose	0.20	0.01					0.35			0.12	0.03		0.10	0.03		0.14	0.04	
fructose	0.43	0.10		0.22	0.02		0.42	0.02										

[a] infl, raw OMWW; 3d, after 3 days of incubation; 7d, after 7 days of incubation. L-Malic acid, malonic acid, oxalic acid, mannose, arabinose, and ramnose were not detected in any samples. Detection limits: succinic acid, 8.3 mg/L; fumaric acid, 0.1 mg/L; sebacic acid, 3.3 mg/L; azelaic acid, 1.5 mg/L; L-malic acid, 5.0 mg/L; malonic acid, 8.0 mg/L; oxalic acid, 4.0 mg/L; caffeic acid, 7.0 mg/L; *trans*-cinnamic acid, 0.1 mg/L; 3,4-dihyrdoxybenzoic acid, 2.1 mg/L; *p*-hydroxybenzoic acid, 0.1 mg/L; 3,4,5-trimethoxybenzoic acid, 0.5 mg/L; 3,4-dimethoxybenzoic acid, 0.7 mg/L; vanillic acid, 0.4 mg/L; *p*-coumaric acid and 4-hydroxycinnamic acid, 0.6 mg/L; syringic acid, 2.0 mg/L; ferulic acid, 0.1 mg/L; phenylacetic acid, 0.1 mg/L; benzoic acid, 0.3 mg/L; cyclohexanecarboxylic acid, 0.1 mg/L; 2-phenoxyethanol and pelargonic acid, 0.4 mg/L; acetic acid, 7.0 mg/L; isovaleric acid, 0.5 mg/L; valeric acid, 0.5 mg/L; *n*-caproic acid, 0.1 mg/L; 2-hydroxymethylbutyric acid, 2.0 mg/L; 2-methyl-2,4-pentanediol, 1.5 mg/L; 2-hydroxyisocaproic acid, 0.2 mg/L; 2-hydroxycaproic acid, 0.1 mg/L; 2-methyladipic acid, 0.8 mg/L; 4-hydroxyphenylethyl alcohol, 0.3 mg/L; D-3-phenyllactic acid, 0.9 mg/L; pimelic acid, 0.2 mg/L; suberic acid, 0.8 mg/L; palmitic acid, 2.0 mg/l: oleic acid, 0.6 mg/L; phytol, 1.0 mg/L; formic acid, 2.0 mg/L; raffinose, 0.01%; lactose, 0.01%; glucose, 0.01%; fructose, 0.01%; mannose, 0.01%; arabinose, 0.01%; rhamnose, 0.01%.

first sampling of each period of operation and the other two samplings. Conceivably, when the pure culture is introduced into the limed OMWW, the adaptation of the inoculum is poor. With the passage of time, the improved adaptation of *Azotobacter* population to OMWW leads to better efficiency of treatment. This corroborates well with evidence reported elsewhere (Ehaliotis et al., 1999) using a laboratory-scale reactor.

The mean degradation yield values of the studied compounds are presented in Figure 4. The fluctuations observed are attributed to predictable factors such as the incubation time, different degradation yields of the system to the studied compounds, and their varying concentrations. In general, the mean degradation yields are ideally high at levels approaching 100%.

CONCLUSION

Given time and OMWW composition, the *Azotobacter*-based bioremediation system is in all respects beneficial and environmentally friendly. The phytotoxicity is eliminated, and the processed end product can be used

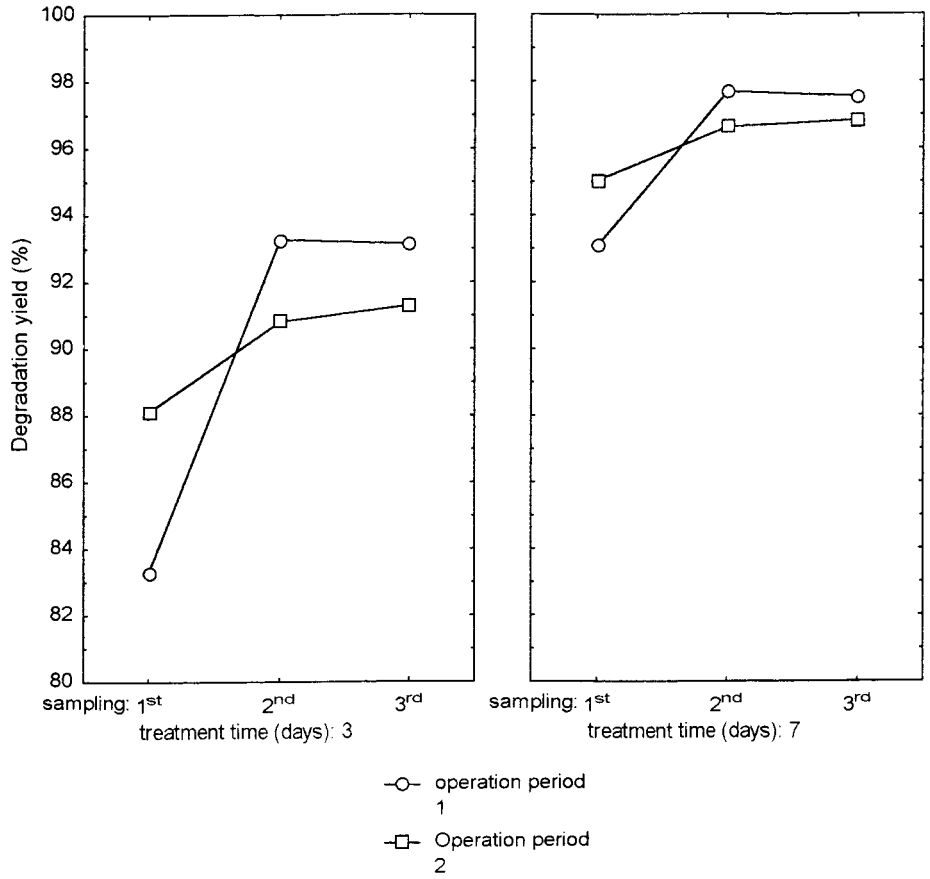


Figure 3. Three-way ANOVA interactions among periods of operation (first year, second year), samplings of different fed-batch within the period of operation (first, second, third), and treatment time (3 days, 7 days) for the bioremediation system.

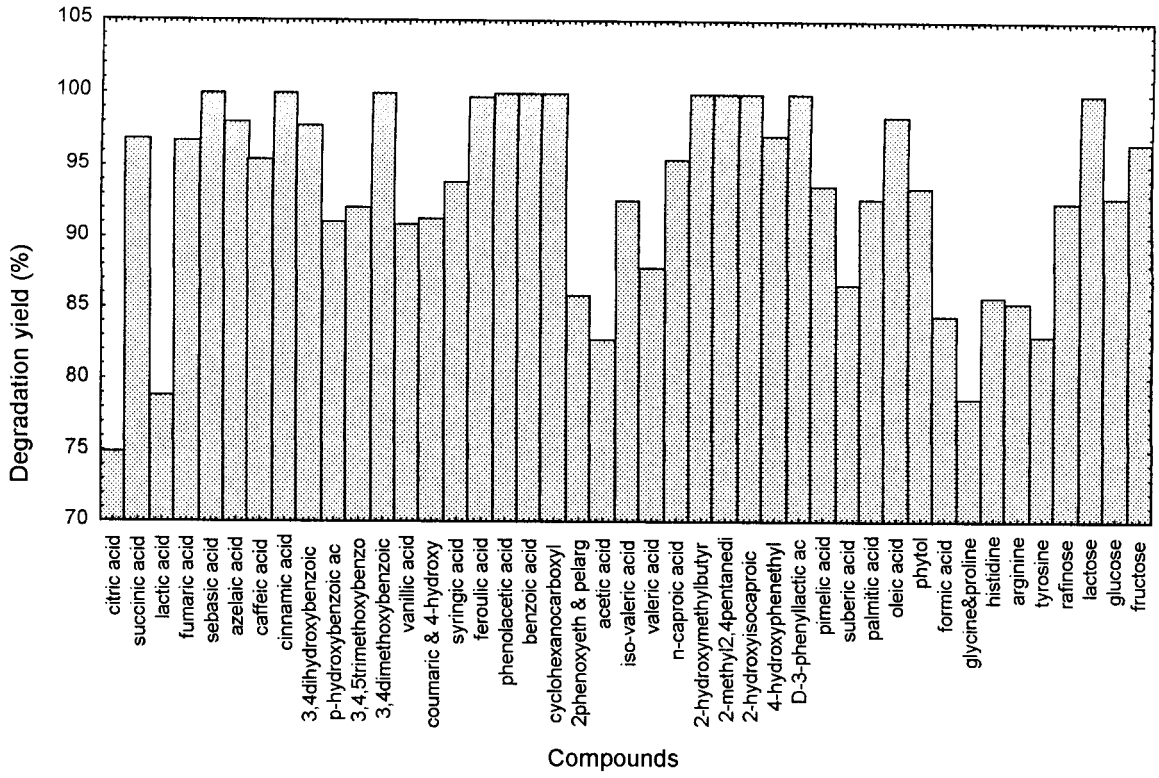


Figure 4. Graphical representation of the mean degradation yields as a function of the compounds studied.

for the fertilization of plants. In fact, the product was used for the fertilization of olive and orange trees. The effects were altogether beneficial and were in accordance with those reported by Chatjipavlidis et al. (1997). The progress or completion of the degradation of most of the compounds that are responsible for its phytotoxicity is confirmed by the obtained degradation yields; several other compounds occurring in OMWW noncommittally

undergo degradation and biotransformation. The technical aspects and the cost analysis of the biofertilization system will be presented elsewhere. At this point it suffices to say that the process is rather simple, cost-effective, and, most importantly, capable of achieving mean removal yields as high as 90 and 96% after 3 and 7 days of treatment, respectively. Finally, statistical analysis of the results showed that between the periods of operation no significant difference occurs with respect to the degradation yield. Likewise, the degradation yield from 3 to 7 days remains almost unaltered during 2 consecutive years.

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Received for review September 27, 1999. Revised manuscript received February 14, 2000. Accepted February 23, 2000. The present work was funded by the EPET II EKVAN P-87 national project. C.B. acknowledges also the support of the EU program IMPROLIVE.

JF991060V