Journal of Industrial Microbiology (1996) 16, 155–162

Microbial consortia for the aerobic degradation of aromatic compounds in olive oil mill effluent

N Zouari and R Ellouz

Centre de Biotechnologie de Sfax, BP 'W' 3038, Sfax, Tunisia

Aerobic consortia that grow on olive oil mill effluent (OOME) were obtained by enrichment. Several cultures were capable of metabolizing monoaromatic compounds, supplied as the sole carbon source at 2 g L^{-1}. Some consortia degraded mixtures of seven aromatics (4 g L^{-1}) after 1 week of incubation at 32°C. The consortia were also active against monoaromatics of the undiluted OOME. This reduced the inhibitory effect of phenolic compounds prior to the anaerobic digestion of OOME at batch scale. No inhibition of the anaerobic microbial populations was noticed with treated OOME. From the most active consortium, nine different bacterial strains were isolated and shown to grow on simple aromatic compounds. Removal of 50% of the initial chemical oxygen demand and degradation of almost all of the simple aromatics in undiluted OOME was obtained with reconstituted bacterial mixtures. A slight reduction in colouration was due to adsorption of coloured compounds to bacterial cells. Presumably, the consortia could not reduce and degrade the coloured compounds in OOME.

Keywords: olive oil mill effluent; aerobic consortia; inhibitory effect; aromatic compounds; culture enrichment; reconstituted consortia

Introduction

Many industrial wastewaters are difficult to treat biologically because of their inherent recalcitrance to degradation and high pollution potential. Olive oil mill effluent (OOME) is of interest in Tunisia and represents a serious source of pollution in the Mediterranean area. In the olive-growing countries of this region, olive mill wastewater production is estimated to reach more than 30 million cubic meters every year [7]. The maximum BOD_5 (Biological Oxygen Demand) and COD concentrations are as high as 100 and 220 kg m^{-3}, respectively [2].

Several biological processes used for industrial and domestic wastewaters were tested for the treatment of olive oil mill effluent [4]. These processes, however, could not be applied to OOME because of the microbial inhibition caused by toxic components, eg tannin-like compounds or by products of the acidification and biodegradation of polymeric substances [9,10,16]. We showed that when treating OOME at a batch scale, reuse of the anaerobic sludges caused inhibition of the anaerobic bacteria even at low COD concentrations (1 g L^{-1}) of OOME [16]. This inhibition was due to aromatics and in part to volatile fatty acid (VFA) accumulating in the sludge. We have also found that the anaerobic sludge digestion of OOME in UASB-like reactors was instable. This instability was clear even at low loading rate (2.5 g COD L^{-1} day^{-1}) using continuously fed reactors, constant loading rates or intermittent feedings. COD was removed not only by utilization for growth and maintenance of active biomass but also by adsorption of aromatic compounds to bacterial cells.

While the aerobic degradation of simple phenolics has been widely investigated, the anaerobic digestion of tannins has been almost overlooked. Hydrolysable tannins can be rapidly degraded anaerobically. The same conclusion could not be drawn about the degradability of condensed tannins [6]. For OOME, tannin-like compounds include condensed and hydrolysable tannins, monomeric flavoids and simple phenolics [3]. The continuous anaerobic or aerobic degradation of total phenolic compounds in OOME has not been investigated so far.

Wastewaters containing phenols have traditionally been treated using physical and chemical processes. Recently, anaerobic biological processes were used with moderate success for the treatment of specific phenolics in wastewaters [5,15]. We have found, however, that for complex phenolic mixtures, such as OOME, the aerobic treatment of aromatics is a key step in effluent treatment. Indeed, attention has been paid to aerobic processes responsible for degradation of individual aromatics [8]. Recently, some bacteria capable of degrading phenolic acids have been isolated, but only a few strains, identified as *Pseudomonas* spp, utilized phenolics such as gallic acid [13]. Hinteregger *et al* [11] isolated a phenol-degrading *Pseudomonas putida* by enrichment culture from soil. This bacterium metabolized phenol, cresols, chlorophenols and other monophenolic compounds as its sole source of carbon and energy.

The aim of the current work was to identify aerobic consortia able to degrade the main phenolic compounds of OOME. Presumably, in each consortium, different bacterial species would mediate a separate segment of the coupled reaction sequence of degradation [8].

Materials and methods

OOME composition

The OOME was obtained from a local olive oil mill and stored at −20°C until required for experimental use. The

Correspondence: N Zouari, Centre de Biotechnologie de Sfax, BP 'W' 3038, Sfax, Tunisia
Received 27 February 1995; accepted 29 November 1995

average composition of the crude OOME was determined. It contained high concentrations of COD (220 g L^{-1}), BOD (88 g L^{-1}), organic suspended solids (190 g L^{-1}), total Kjeldahl nitrogen (TKN: 1.2 g L^{-1}), condensed tannins (2.3 g L^{-1}), hydrolysed tannins (7 g L^{-1}), monomeric flavoids (1.5 g L^{-1}) and simple phenolics (6.4 g L^{-1}). The measurements of COD, BOD, TKN, suspended solids and aromatics were carried out according to standard methods [1].

Selection of consortia

The aerobic environments in which aromatic-degrading bacteria were potentially present were selected from muds and wastewaters from a local tannery factory, muds from an aerobic domestic wastewater treatment plant and soil samples from a local eucalyptus field. Each was used as an inoculum. The culture medium was based on OOME pretreated for 2 months, using combined aerobic and anaerobic processes (data not shown). All readily assimilable organic matter was degraded leaving complex organic materials as the sole carbon source. The pretreated OOME (p-OOME) contained 98 g L^{-1} COD, 107 g L^{-1} organic suspended solids and 0.85 g L^{-1} TKN. All cultures were carried out in 250-ml flasks containing 50 ml of medium and shaken at 250 rpm at 32°C. The pH was adjusted to 7.2. The steps of the enrichment are shown in Figure 1.

Since p-OOME contained a low concentration of nitrogen (0.85 g L^{-1}), some growth factors and a nitrogen source necessary for bacterial growth were added. The following media were used for enrichment cultures at 32°C and pH 7.2: p-OOME, p-OOME + 0.25 g L^{-1} yeast extract, p-OOME + 0.25 g L^{-1} yeast extract + 1 g L^{-1} ammonium chloride. After 2 weeks of culture, samples were harvested and plated on cetrimide agar (Merck; cat. 5284), a *Pseudomonas*-selective agar and on nutrient agar. Colonies from each plate were harvested and used as inocula without mixing the different cultures. These are defined as reconstituted consortia.

The enrichment culture of the crude and reconstituted consortia was continued on standard medium consisting of p-OOME, 0.25 g L^{-1} yeast extract and 1 g L^{-1} ammonium chloride.

Test of the consortia activities

After five successive cultures of 2 weeks, the consortia were tested for their ability to grow at 32°C on simple phenolic compounds in the following medium (g L^{-1}): $(NH_4)_2HPO_4$ 1.0; KH_2PO_4 2.0; K_2HPO_4 3.0; $MgSO_4$ 0.5; $MnSO_4$ 0.05; $CaCl_2$ 0.001; yeast extract 1.0; peptone 2.0 and phenolic compound 2.0, pH 7.2. Five millilitres of each consortium preparation served to inoculate 50 ml of culture medium contained in 250-ml flasks. The cultures were shaken at 250 rpm for 1 week. The cell-free culture medium was used as control. Aerobic degradation of the aromatic compounds was followed spectrophotometrically. The results are expressed as a percentage decrease in the optical density (OD) at the indicated wavelength corresponding to the maximal absorbance of the compound. The results were calculated using the following formula:

$$\frac{(E_{t0} - E_{tx})}{E_{t0}} \times 100 = \%\ \text{OD decrease (tx = 1 week)}$$

Aerobic degradation of a mixture of seven phenolics by the consortia was also studied. The phenolic mixture contained (g L^{-1}): gallic acid 1.0; *p*-coumaric acid 0.5; 3-hydroxybenzoic acid 0.5; cinnamic acid 0.5; protocatechuic acid 0.5; syringic acid 0.5 and *p*-hydroxyphenylacetic acid 0.5, in the medium described above. The control consisted of uninoculated medium. The changes in composition of the phenolics after 1 week's incubation were determined by gas chromatography (GC). The results are expressed as a percentage of the residual concentration of each compound after 1 week of incubation. The concentrations were determined by GC analysis. The results were calculated using the following formula:

$$100 - \frac{(C_{t0} - C_{tx})}{C_{t0}} \times 100 = \%\ \text{residual concentration (tx = 1 week)}$$

The final processing step consisted of aerobic incubation of the consortia with undiluted centrifuged OOME which was adjusted to pH 7.2 using 2 N NaOH. The changes in the monophenols in the culture medium were monitored at 2-day intervals by GC analysis after extraction. The cell-free culture medium served as a control.

Isolation and characterization of bacteria from consortia

Aerobic growth in liquid culture was carried out on the selective medium (p-OOME supplemented with 0.25 g L^{-1} yeast extract and 1 g L^{-1} ammonium chloride). It was followed by plating appropriate dilutions on solidified medium composed of (g L^{-1}): trypticase, 15; soya bean peptone, 5; sodium chloride, 5; agar, 15; pH 7.2. The purity of each isolated strain was confirmed by streaking it up to five times. Morphological, biochemical and physical properties of the isolated strains were determined according to Marchal *et al* [12].

Preparation of bacterial inocula

To prepare an inoculum, each strain was cultured for 15 h in liquid medium containing (g L^{-1}): trypticase, 15; soya bean peptone, 5; and sodium chloride, 5, pH 7.2. The OD at 650 nm of each culture was then almost 4, (average CFU: 10^8 cells ml^{-1}). Bacterial biomass was harvested by centrifuging the culture media at 8000 *g* for 15 min and washing the pellet twice with physiological saline. The consortium was reconstituted by mixing the nine components. The resulting OD was almost 40. The inoculum was 10% (v/v). Cell numbers in cultures were determined microscopically using a Thoma cell.

Activity of isolated bacteria on aromatic compounds and on OOME

The isolated bacteria were tested for their activity towards simple phenolic compounds using the same medium, aromatic mixtures and culture parameters described above for the test of consortia activities. Centrifuged OOME was used undiluted to test the activity of the isolated bacteria. Five millilitres of each bacterial inoculum or the bacterial mixture served to inoculate 50-ml portions of culture medium

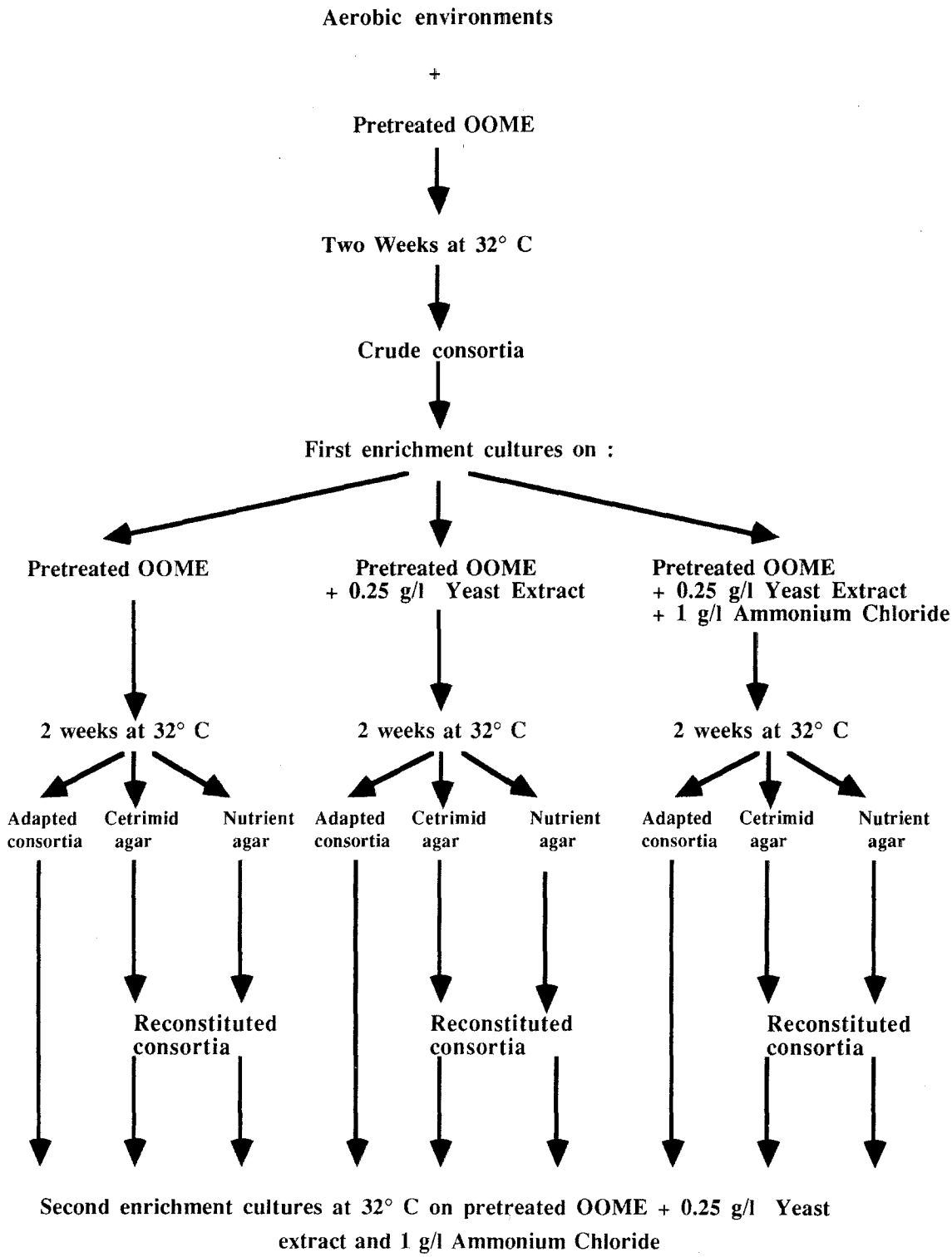

Figure 1 Steps in the acclimatization of the consortia.

contained in 250-ml flasks. The cultures were shaken at 250 rpm for 1 week at 32°C. The cell-free culture medium was used as control. The COD and the OD at 280 nm and 390 nm were determined in the centrifuged culture media after appropriate dilution.

Optimization of the nitrogen source for bacterial growth on OOME

Optimization of the nitrogen source was carried out using a reconstituted consortium. The medium consisted of undiluted OOME + 1 g L^{-1} yeast extract and variable concen-

trations of ammonium chloride or ammonium sulfate. Five millilitres of the bacterial mixture served to inoculate 50-ml portions of culture medium in 250-ml flasks. The cultures were shaken at 250 rpm for 1 week at 32°C. The culture medium without nitrogen source was used as control. Cell numbers in cultures were determined microscopically using a Thoma cell.

Gas phase chromatography (GC)

Simple aromatics were analyzed by GC in a gas chromatograph (Shimadzu GC-9A). The phenols were extracted using ethylacetate/acetone (2:1) at pH 2.0. Two millilitres of the solvent were added to 1 ml of culture medium, centrifuged and the organic phase was harvested. GC conditions were: column SE 30 (30 m length, 0.5 mm ID, methyl silicone in the solid phase, injection temperature of 280°C and detector temperature of 300°C), carrier gas N_2 (60 ml min^{-1}), split at 100 ml min^{-1} corresponding to a pressure of 1.2 kg cm^{-2}, detector FID (H_2 at 0.5 kg cm^{-2}, air 0.5 kg cm^{-2}), temperature gradient: 100 to 280°C (4°C min^{-1}), injection of 1 μl of silylated sample. Silylation of the dried samples was carried out by incubation with 100 μl pyridine and 50 μl trimethyl silane at 60°C for 15 min.

Test of the methanogenic activity

The test adopted to evaluate anaerobic digestion of the crude and the pretreated OOME consisted of a semi-stirred system; serum bottles (500 ml) were stirred manually twice a day. The bottles were connected to a serum bottle liquid displacement system containing 1.5% NaOH (CO_2 absorption) for methane measurement. The digesters contained 200 ml autodigested seed sludge (sewage sludge and cow slurry) and 300 ml diluted OOME (3.5 g COD L^{-1}). All experiments were carried out at 32°C and at an initial pH of 7.2.

Results and discussion

Culture enrichment

The first step was the selection of aerobic consortia that were able to grow on pretreated OOME. The problem with conventional selection and isolation procedures is that these are designed to select single organisms capable of degrading a particular compound and, frequently, only prototrophic strains are isolated [14]. For this reason, the enrichments were carried out using p-OOME supplemented with yeast extract and ammonium chloride.

Growth in closed liquid culture, followed by plating aliquots from appropriate dilutions on solid media, resulted in the physical separation of microorganisms [8]. The reconstituted and the adapted consortia showed different activities. The results obtained with 14 consortia, numbered 1 to 14, are shown in Table 1. Four of the most interesting consortia were reconstituted (consortia 4, 7, 9 and 13) and offered additional possibilities of microbial degradation of aromatic compounds in OOME.

Biodegradation of aromatic compounds

The results presented in Table 2 show that the adapted crude consortia were able to catabolise each aromatic compound found in p-OOME aerobically. All aromatic compounds were susceptible to degradation by the selected consortia, suggesting the presence of natural aerobic microorganisms that could degrade monoaromatics. The decrease of the OD of the culture medium at 200–220 nm indicated the loss of phenolics. Microbial growth and decrease of total COD in the culture media indicated the consumption of aromatics by consortia (data not shown). We cannot however, exclude the possibility that the decrease of OD and COD were associated to some extent with adsorption of such material to cell surfaces.

All the consortia were tested on a mixture of phenolic compounds in order to evaluate their total biodegradative potential. The GC analyses of aliquots determined after 1 week of incubation showed the changes in the phenolic composition deduced from the GC spectra obtained with the most interesting cultures (Table 1). Only consortia 5, 11, 13 and 14 achieved almost complete degradation of all components of the phenolic mixture. With other consortia, some peaks disappeared completely and smaller unidentified peaks appeared. Furthermore, certain microbial consortia were unable to degrade all substrates completely. The inability to attack all aromatics could be due to the absence of the appropriate microbial strain(s), or it could be caused by inhibition due to the phenolic concentration (4 g L^{-1}) which is very high, or even to more sophisticated aspects such as the lack of syntrophic strains.

Biodegradation of OOME

All consortia were active on aromatic compounds of OOME, giving rise to either partial or complete biodegradation. The results reported in Figure 2 show a sequential degradation of phenolics by consortium 5 grown on undiluted fresh OOME. The responses varied, but it is clear that some compounds disappeared more rapidly than others. Due to the complex composition of OOME, the disappearance of the most important peaks shown with untreated medium could be obtained only after 2 weeks of incubation. Each consortium was unique in this respect.

Even after 2 weeks of incubation, however, degradation of all the phenolic compounds was incomplete (as shown in GC spectra). Such materials may be inherently recalcitrant, but there is also the possibility that the existing environmental conditions were not sufficiently favourable. In the latter case, optmization of the culture parameters would be useful in order to improve the rate of phenolic degradation.

Degradation of some aromatics in OOME by the aerobic consortia may remove the inhibitory effect of OOME on methanogenesis. Consortium 5 was particularly effective in this regard. The anaerobic treatment of OOME is the final objective of our interest.

Anaerobic digestion in batch reactors was carried out using OOME treated with consortium 5. The reuse of the same sludge every 2 weeks to treat crude and pretreated OOME was studied. The results illustrated in Figure 3 show no inhibitory effect after up to three cycles with OOME treated with consortium 5, whereas the inhibition was clear in the untreated materials. This finding confirms the implication of simple aromatics in the inhibitory effect of OOME and is in agreement with our previous results relative to the anaerobic digestion of OOME in UASB-like reactors. Our

Table 1 Aerobic degradation of monoaromatic compounds mixed in the mineral medium using the most interesting consortia

Consortium number	New peak	Per cent remaining after 1 week of:						
		p-Hydroxyphenyl acetic acid	Protocatechuic acid	Syringic acid	*p*-Coumaric acid	Gallic acid	Cinnamic acid	*p*-Hydroxybenzoic acid
(Control)	0.0	100.00	100.0	100.0	100.0	100.0	100.0	100.0
1	+	43.7	27.3	10.0	100.0	100.0	73.7	66.4
2	0.0	0.9	1.9	13.5	11.0	8.1	6.8	9.6
3	0.0	24.3	16.1	100.0	100.0	100.0	10.3	8.1
4	0.0	0.0	0.0	100.0	9.3	6.3	5.7	0.0
5	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
6	+	24.9	6.8	5.2	30.2	7.9	0.0	4.7
7	+	10.3	0.0	3.0	100.0	22.9	11.0	6.8
8	+	0.0	0.0	100.0	0.0	19.6	0.0	11.7
9	+	0.0	0.0	100.0	21.0	0.0	0.0	0.9
10	+	0.0	0.0	100.0	100.0	0.0	0.0	4.1
11	0.0	0.0	0.0	9.7	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	17.3	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	4.3	0.0	0.0	0.0	3.5
14	0.0	0.0	0.0	1.0	0.0	0.0	0.0	8.1

Consortia 4, 7, 9 and 13 were reconstituted. The results are expressed as a percentage of the residual concentration of each compound after 1 week of incubation. The symbol + indicates the presence of an unidentified new peak that appeared in several cases.

Table 2 Aerobic degradation of monoaromatic compounds by crude consortia after 1 week of incubation

Inoculum sample	Per cent decrease in OD (at wavelength) for:					
	p-Hydroxyphenyl acetic acid (276 nm)	Gallic acid (258 nm)	Syringic acid (262 nm)	Protocatechuic acid (250/287 nm)	*p*-Hydroxybenzoic acid (270 nm)	Cinnamic acid (270 nm)
Soil I-1	84.6	31.9	22.6	74.4/46.6	60.3	92.3
Soil I-2	87.1	59.0	50.7	75.6/57.3	67.7	94.2
Soil I-3	86.3	62.1	51.9	78.0/60.8	81.3	95.3
Soil II-1	86.6	32.7	39.4	70.1/41.3	66.0	95.3
Soil II-2	87.0	54.1	45.1	70.8/44.6	74.3	95.8
Soil II-3	86.9	61.7	56.5	69.3/44.1	88.6	97.1
Tan 1	68.2	39.8	94.3	70.3/33.8	61.4	94.7
Tan 2	84.7	64.1	36.7	74.6/40.0	68.3	96.9
Tan 3	85.0	62.8	96.1	76.3/39.9	87.1	97.0
Mud 1	79.0	41.3	95.9	68.9/39.9	70.1	92.9
Mud 2	88.4	58.6	91.7	64.6/38.3	84.4	96.3
Mud 3	83.6	54.1	94.3	72.1/43.3	85.6	97.1

The results are expressed as a percentage of the decrease in OD at the indicated wavelength, corresponding to the maximal absorbance of the compound. Soil I-1, Soil I-2, Soil I-3, Soil II-1, Soil II-2 and Soil II-3 indicate six different soil samples from a local eucalyptus field. Tan 1, Tan 2 and Tan 3 indicate three different wastewaters from a local tannery factory, Mud 1, Mud 2 and Mud 3 are three different muds used for the selection of aromatic-degrading bacteria.

results were in accordance with those of Boari *et al* [4]. These authors improved the anaerobic treatment of OOME in UASB reactor by the use of acclimatized sludge inoculum, based on building up of anaerobic microbial populations able to detoxify the wastewater by metabolizing the inhibitory compounds.

Of greater significance in the current work, as a result of the separation of the two steps of OOME treatment, was the use of undiluted OOME for aerobic digestion which is important for the anaerobic treatment step.

Bacterial isolates

As described above, consortia showed high activities towards fresh undiluted OOME. The reproducibility of growth may be problematic due to the variable composition of OOME but also the variable nature of the inocula. We therefore decided to isolate microorganisms from one of the most active consortia (5 in Table 1), which originated from a soil sample. Table 3 shows the main properties of nine bacteria isolated from this consortium. Six strains were obligate aerobes, but all were catalase-negative and oxidase-negative, indicating that none could be a *Pseudomonas* sp.

Enrichment using a restrictive medium based on biodegraded OOME is appropriate for isolating interacting microbial communities able to degrade recalcitrant compounds in such a complex medium. Furthermore, there is the question of what effect other organisms, selected also with this enrichment procedure, may have on the activity and function of the microbial community. Also, what

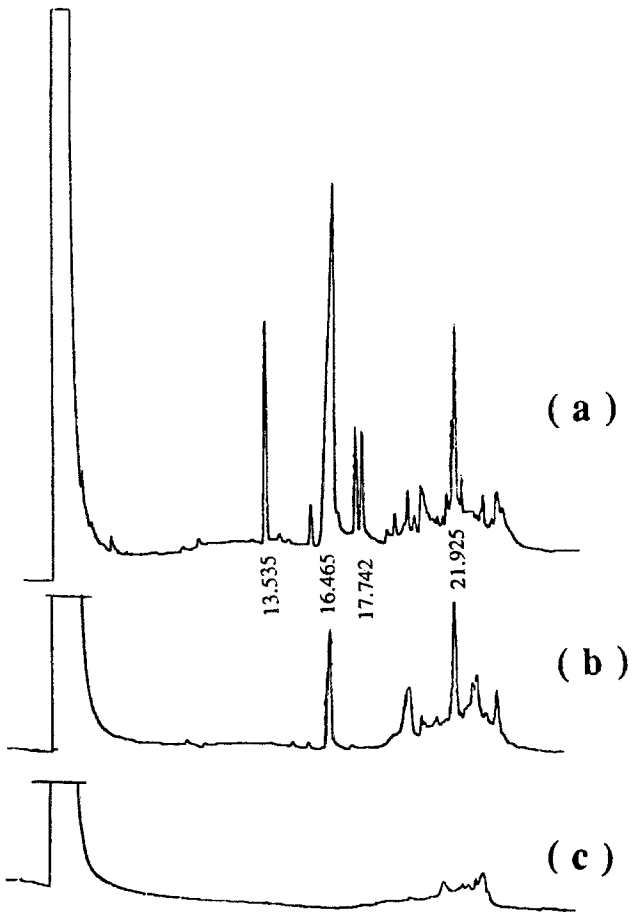

Figure 2 Changes in phenolic substrates with time. Consortium 5 on OOME at days (a) zero, (b) 6 and (c) 15. No changes were observed in a control experiment carried out under the same conditions without microbial inoculum. Numbers indicate retention times (min).

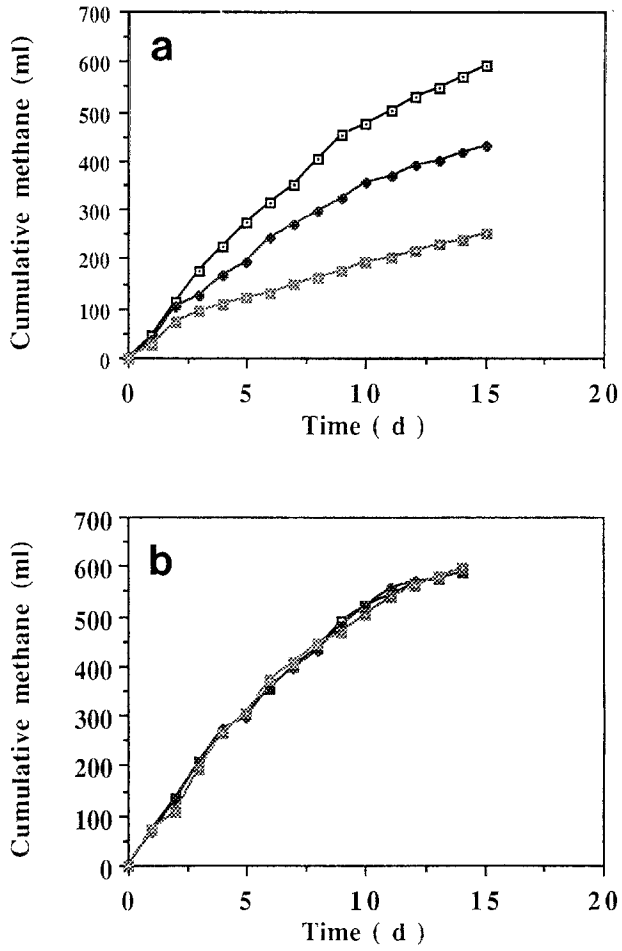

Figure 3 Methanogenic activity of the sludge in batch culture using (a) crude OOME and (b) pretreated OOME by consortium 5. Test of reuse of the same sludge in batch: (—□—) first utilization, (—●—) second utilization and (—■—) third utilization.

effects do fluctuating environmental conditions have on the composition and structure of microbial communities? These will be quite difficult to answer because of technical difficulties in measuring the activity of one particular microbial community among a background of many organsims.

Activity of isolated bacteria

A mixture of the major monoaromatics of OOME was reconstituted and used for evaluation of the activities of the isolated bacterial strains. Table 4 shows the percentage of each residual aromatic calculated from peak area as obtained by GC analysis. Values less than 10 are from peaks that were considered negligible. Several strains were more efficient towards the total mixture than others, but gallic and caffeic acid were entirely degraded by all strains. The overall effectiveness of the combined strains was complementary since they displayed differential activities to specific aromatic compounds, and when mixed, resulted in almost complete degradation of these phenolics.

As these strains can individually use a range of aromatic compounds, they should be further evaluated for their ability to grow on undiluted OOME. Despite the observed degradative ability on a range of aromatics (Table 4), the isolated strains were unable to grow individually on undiluted OOME. No changes were found in the phenolic compounds via GC analysis. Even when mixed, significant growth was not detected. One possible reason is the lack of some growth factor(s). Since TKN in OOME did not exceed 1.2 g L^{-1}, addition of a nitrogen source in the culture medium was studied. By using an inoculum containing all nine strains, growth on undiluted OOME with two nitrogen sources was evaluated (Table 5). The best bacterial growth was obtained with ammonium sulfate at 2 g L^{-1}. These conditions may, however, be sub-optimal or non-optimal for individual strains.

Reconstitution of the bacterial mixture was a difficult step, because it aimed to reproduce a bacterial population having the same function towards OOME. Thus, in the reconstitution, a dense inoculum of each strain was used. The best results were obtained when the inoculum was prepared as follows: the OD of the culture of each strain was 3.5–4.5 (average CFU: 10^8 cells ml^{-1}); the OD of the reconstituted inoculum was 40; and further experiments used a 10% (v/v) inoculum with incubation at 32°C for 1 week.

Under these conditions, reconstituted consortia were able to give almost 50% reduction of the initial COD. This implies that synergistic interaction may help to overcome the toxicity of OOME.

The optical density (260 nm) of the culture medium was

Table 3 General characteristics of the nine bacterial strains isolated from consortium 5

Strain	Shape	Gram	Aerobe	Mobility on mannitol	Fermentation of mannitol	Nitrate reduction	Oxidase	Catalase
1	Rod	+	Obligate	+	–	+	–	–
2	Coccus	–	Facultative	–	–	–	–	–
3	Coccus	+	Facultative	+	–	–	–	–
4	Rod	–	Obligate	–	+ (late)	–	–	–
5	Rod	+	Obligate	+	–	+ (late)	–	–
6	Rod	+	Obligate	–	+ (late)	–	–	–
7	Rod	+	Obligate	+	–	–	–	–
8	Coccus	–	Obligate	–	+	+	–	–
9	Rod	+	Facultative	+	–	+ (late)	–	–

Table 4 Activity of the isolated strains towards monoaromatic compounds

Strain	Per cent remaining of:						
	Gallic acid	Caffeic acid	Tyrosol	Syringic acid	*p*-Hydroxyphenol acetic acid	*p*-Coumaric acid	Protocatechuic acid
1	0	0	0	0	5.3	0	0
2	0	0	51.9	0	56.1	0	0
3	0	0	92.6	69.2	92.8	67.2	24.6
4	0	0	0	34.3	0	26.8	0
5	0	0	0	35.4	90.9	30.1	0
6	0	0	0	0	6.4	0	0
7	0	0	0	0	0	16.6	0
8	0	0	94.1	0	89.3	79.4	33.9
9	0	0	0	74.8	90.2	22.9	0
Mixture	0	0	0	3.7	2.9	3.8	0
Control	100	100	100	100	100	100	100

The results are expressed as a percentage of the residual compound. (0) indicates complete metabolism; ie the absence of a peak in the GC chromatogram.

Table 5 Optimization of the nitrogen source using reconstituted consortia

Ammonium sulfate ($g\ L^{-1}$)	Ammonium chloride ($g\ L^{-1}$)	cells ml^{-1} ($\times 10^7$)
0.0	0.0	0.02
0.5	0.0	0.14
1.0	0.0	3.00
2.0	0.0	12.00
3.0	0.0	4.00
4.0	0.0	0.9
0.0	0.5	0.5
0.0	1.0	0.9
0.0	2.0	2.0
0.0	3.0	0.6

reduced to 47% of the initial value, suggesting degradation of some aromatic compounds in OOME, which was confirmed by GC analysis. Only 13% reduction in the optical density at 390 nm was obtained. This means that the colouration of OOME as measured at 390 nm was unaffected. Presumably the consortium could not reduce and degrade the coloured compounds in OOME. The slight reduction of colouration may be simply explained by its adsorption to bacterial cells.

It is noteworthy that this aerobic treatment step in OOME depollution did not need strictly controlled culture conditions, which is an important point in the economic evaluation of the final process. Moreover, the advantage of such a treatment is that OOME could be directly used without any dilution. That is a very important aspect in several Mediterranean regions producing large quantities of OOME as a by-product of the olive oil industry.

Acknowledgements

This work was supported by funds for a research and development project of the UNDP programme DP/TUN/86/010. We are grateful to DE Eveleigh (New Brunswick, NJ, USA) and to MP Nuti (Padova, Italy) for supervising, as UNIDO experts, the advancement of this work and their scientific support. We thank them and Jean-Marc Labat (ORSTOM, Marseilles, France) for critical reading of the manuscript. GC analyses were carried out by Hédi Aouissaoui and Najla Masmoudi (CBS, Tunisia).

References

1 American Public Health Association (APHA, ed). 1980. Standard methods for the examination of water and wastewater. AWWA, WPO, Washington, DC.

2 Balice V, G Boari, O Cera and P Abbaticchio. 1982. Indagine analitica sulle acque di vegetazione. Nota 1. Inguinamento 7–8: 49–53.

3 Balice V, C Carrieri, O Cera and B Rindone. 1988. The fate of tannin-like compounds from olive oil mill effluents in biological treatments. In: Proceedings of the 6th Symposium on Anaerobic Digestion of Wastewater, Bologna, Italy, pp 275–280.
4 Boari G, A Brunetti, R Passino and A Rozzi. 1984. Anaerobic digestion of olive oil mill wastewaters. Agricultural Wastes 10: 161–175.
5 Fedorak PM and SE Hrudey. 1986. Anaerobic treatment of phenolic coal conversion wastewater in semicontinuous cultures. Wat Res 20: 113–132.
6 Field JA and G Lettinga. 1987. The methanogenic toxicity and anaerobic degradability of a hydrolysable tannin. Wat Res 21: 367–374.
7 Fiestas Ros de Ursinos JA. 1981. Différentes utilisations des margines. In: Proc Séminaire International sur la Valorisation des Sous-produits de l'Olivier. Organisation des Nations Unies pour l'Alimentation et l'Agriculture, Tunisie, pp 93–110.
8 Gibson DT and V Subramanian. 1984. Microbial degradation of aromatic hydrocarbons. In: Microbial Degradation of Organic Compounds (Gibson DT, ed), pp 439–485, Marcel Dekker, New York and Basel.
9 Hamdi M. 1991. Effects of agitation and pretreatment on the batch anaerobic digestion of olive oil mill wastewaters. Bioresource Technol 36: 173–178.
10 Hamdi M, A Khadir and JL Garcia. 1991. The use of *Aspergillus niger* for the bioconversion of olive oil mill wastewaters. Appl Microbiol Biotechnol 34: 828–831.
11 Hinteregger C, R Leitner, M Loidl, A Ferschl and F Streichsbier. 1992. Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. Appl Microbiol Biotechnol 37: 252–259.
12 Marchal N, JL Bourdon and CL Richard. 1986. Bacillus. In: Les Milieux de Culture pour l'Isolement et l'Identification Biochimique des Bactéries (Obré A and R Buttiaux, eds), pp 327–330, Doin, Paris.
13 Perez J, A Ramos-Cormenzana and J Martinez. 1990. Bacteria degrading phenolic acids isolated on a polymeric phenolic pigment. J Appl Bacteriol 69: 38–42.
14 Slater JH and D Lovatt. 1984. Biodegradation and the significance of microbial communities. In: Microbial Degradation of Organic Compounds (Gibson DT, ed), pp 181–250, Marcel Dekker, New York and Basel.
15 Wang YT, MT Suidan and BE Rittmann. 1986. Anaerobic treatment of phenol by an expanded-bed reactor. J Wat Pollut Contr Fed 58: 227–233.
16 Zouari N, N Gharsallah, M Hamdi and R Ellouz. 1989. Limitations in the anaerobic digestion of the olive oil mill effluents. In: Biomass for Energy and Industry. Proceedings of the 5th EC Conference (Grassi G, G Gosse and G dos Santos, eds), pp 2.398–2.402, Elsevier Applied Science, London and New York.