Bioprocess Engineering 8 (1992) 79–84

Bioprocess Engineering

Integrated biological process for olive mill wastewater treatment

M. Hamdi, J. L. Garcia and **R. Ellouz,** Sfax, Tunisia

Abstract. The biological process for OMW treatment is based on an aerobic detoxification step followed by methanization step and aerobic post-treatment.

The first aerobic detoxification step of OMW supplemented with sulfate and ammonium was carried out by the growth of Aspergillus niger in a bubble column. This step decreased OMW toxicity and increased its biodegradability because of phenolic compounds degradation. Growth of A. niger resulted in 58% COD removal, with production of biomass containing 30% proteins (w/w). Filtration of OMW was enhanced by this fermentation because the suspended solids were trapped in the mycelium. The filtrate liquid was then methanized using an anaerobic filter packed with flocoor. This reactor showed a short start up and a good stability. COD removal was around 60% and the methane yield (l CH_4/g COD removed) was close to the theoretical yield.

The anaerobic filter effluent was treated in an activated sludge fluidized reactor containing olive husk as a packing material. Husks were maintained in fluidization state by the aeration. This step induces COD removal at 45% and sludge (up to 2 g/dm^3).

The entire process allowed a global COD reduction up to 90%; however, the black colour due to polyphenolic compounds with high molecular weight persisted.

1 Introduction

In the olive growing mediterranean countries, the olive mill wastewater (OMW) production passes beyond 30 millions m^3 per year [1]. The volume of olive black water produced by the traditional press process is 0.5–0.8 m^3/ton of olive [2]. Generally, more diluted wastewaters are produced by continuous process, but the polluting organic load expressed in weight of processed olives, is practically independant of the processing method at a level of 45–55 kg BOD_5 per ton olives. The BOD_5 and COD maximum concentration reach 100 and 220 kg/m^3 respectively [3]. In olive wastewater produced by the traditional mill and press process, the average concentration of volatile solids (vs) and inorganic matter were 15% and 2% respectively. The organic fraction includes sugars, tannins, polyphenols, polyalcohols, pectins and lipids [1]. Anaerobic digestion of OMW can be carried out only on a highly diluted substrate because aromatic compounds are toxic for methanogenic bacteria [2, 4]. Pretreatment of OMW by fermentation with Aspergillus niger which removes phenolic compounds [5] decreases the toxicity for methanogenic bacteria and facilitates anaerobic digestion [4].

Literature analysis of present situation of biological treatment of OMW shows that activated sludge and methanization are efficient only when OMW is diluted and/or pretreated (Fig. 1). Since the cost of activated sludge is five times higher than that of anaerobic digestion [2], the methanization is used as main step of treatment of OMW in this proposed process. When the dilution of OMW reduces the volumetric capacity of digestors and the distillation is very expensive, a biological pretreatment replacing dilution and distillation was chosen. Toxicity and biodegradability studies of OMW showed that polyphenolic compounds responsible for wastewater black colour, present little toxicity and are not easily biodegradable. On the contrary, tannins are highly toxic but biodegradable [6]. Since toxic compounds as tannins and simple phenolic are better biodegradable than darkly colored polyphenols, the role of the biological pretreatment will be the detoxification but not the decolorization. The microorganism which is the most efficient to remove tannins is A. niger [7]. Moreover A. niger metabolise many phenolic compounds identified in OMW as shown in Table 1. Indeed the pretreatment of OMW by A. niger reduces the toxicity of methanogenic bacteria because of elimination of phenolic compounds and oil [4].

Table 1. Phenolic compounds contained in OMW and references of their degradation by Aspergillus niger [5]

Compounds	References content	References degradation
p-Hydroxyphenylacetate	[8]	[12]
Protocatechuate	[8]	[12]
Cinnamate	[9]	[12]
Quercitin	[10]	[12]
Oleuropein	[10]	[12]
Tannins	[3]	[7]
Polyphenols	[1]	[13]
Anthocianins	[11]	[14]

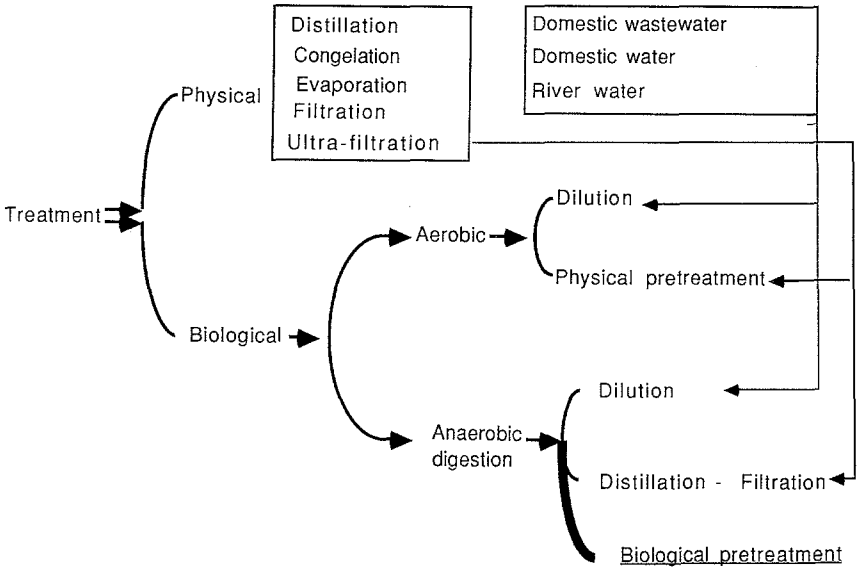

Fig. 1. Biological process used for OMW treatment; (—) cited in literature; (—) proposed in this work

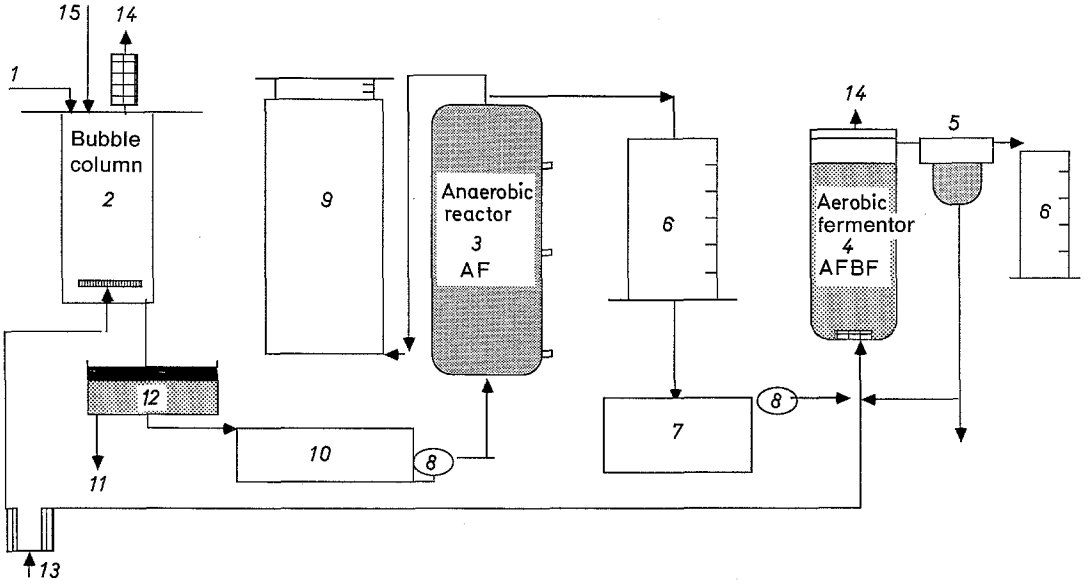

Fig. 2. Schematic diagram of experimental apparatus: *1* OMW input, *2* bubble column, *3* anaerobic filter reactor, *4* aerobic fluidized bed fermentor, *5* settler, *6* effluent, *7* feed reservoir of AFBF, *8* pump, *9* gazometer, *10* feed reservoir of anaerobic filter, *11* biomass, *12* filtration, *13* air-meter, *14* gas-liquid separator, *15* salts

In this paper, a three step biological process is proposed for the treatment of undiluted OMW: Pretreatment by Aspergillus niger in bubble column, biomethanization in anaerobic filter and pretreatment in aerobic fluidized bed fermentor.

2 Material and methods

2.1 Reactors

A schematic diagram of the experimental setup is given in Fig. 2. The 2.5 dm^3 plexiglass bubble column (2) was 8 cm in diameter and 150 cm in height. Air from an oil-free compressor was metered with a calibrated orifice (13), sterilized with an air filter, and sparged into the medium through perforations on the sparger plate at the bottom of the column. The gas emerging from the column top was water-cooled in a condenser (14).

The bubble column (2) cooperates with an anaerobic reactor (AF) (3) and aerobic fluidized bed fermentor (AFBF) (4). The 3.5 dm^3 anaerobic reactor consists of a 50 cm long, 12 cm internal diameter glass tube. The AF was packed with 750 g of PVC rings with a volume of 600 cm^3. This support medium had a porosity of 83%. The AFBF is a 30 cm long,

6 cm internal diameter glass tube. The volume of this reactor was 0.5 dm^3. It was filled with 50 g of husk provided from an olive oil extraction process. The aeration was 0.8 VVM. The volume of the settler (5) coupled with this fermentor to recycle sludge was 0.5 dm^3. The three reactors (2), (3), (4) are enclosed in a non-reactive jacket through which warm water circulated to maintain a temperature of 35 °C.

2.2 Olive mill wastewater composition and reactor operation

The strain of Aspergillus niger used for pretreatment of OMW was isolated from OMW at the Biotechnology Center in Sfax, Tunisia. Spores of the organism were produced on a medium containing 50% (V/V) OMW; NO_3NH_4 (5 g dm^{-3}); $SO_4(NH_4)_2$ (5 g dm^{-3}); KH_2PO_4 (1 g dm^{-3}); and agar (Difco Lab., Detroit, MI, USA) (18 g dm^{-3}) for one week at 30 °C.

OMW was supplemented with ammonium nitrate (6 g dm^{-3}) and ammonium sulfate (3 g dm^{-3}). After inoculation, 10^7 spores of A. niger/dm^3, the OMW was prefermented in the bubble column (2) during 72 h. The pH of OMW was 5.6 and was not controlled during fermentation.

The inoculation and adaptation of the anaerobic reactor to prefermented OMW treatment has been described elsewhere by Hamdi and Garcia [15]. The AFBF was inoculated by a mixture of bacteria isolated from prefermented OMW.

The prefermented OMW was filtered (12) diluted twice by water to obtain 25.86 g COD/dm^3, adjusted to pH 7.2 with $Ca(OH)_2$ and pumped into the AF (3) with a loading rate of 2.12 g COD/dm^3 d. The effluent of AF with 9.83 g COD dm^{-3} was pumped into AFBF (4) with a loading rate of 1.4 g COD/dm^3 d. Both reactors (3) and (4) were fed using pumps (8) operated sequentially by programmer. The hydraulic retention time were 12 and 7 days in AF and AFBF respectively.

2.3 Analytical methods

Total solids (TS) were obtained by drying the sample overnight at 105 °C. Total suspended solid (TSS) were obtained by centrifugation at 4000 g for 15 min, the settled solids were then dried overnight at 105 °C. The ash content was determined after calcination of the dry sludge at 600 °C for 1 h. The difference between total solids and ash content was defined as volatile solids (VS).

Gas flow rates were measured by liquid displacement (9) in Fig. 2. Methane and volatile fatty acids (VFA) were analysed by gas chromatograph as described by Hamdi [4]. The chemical oxygen demand (COD) was determined by the method described by Knechtel [16].

Gel filtration on sephadex G-50 was used to analyse the polyphenolic compounds present in raw and treated OMW. Three cm^3 of product were filtered and placed on a sephadex coarse G-50 column (3 × 50 cm) previously equilibrated with distilled water. This column was washed with 400 ml of distilled water at a flow rate of 0.33 cm^3/min. The effluent was collected with a 3 cm^3 tube. These fractions were measured spectrophotometrically at 280 nm.

For phenolic acid analysis, 2 cm^3 of centrifuged and acidified samples of OMW and treated OMW were mixed with 4 cm^3 or organic solvent (2 v/1 v: ethylacetate-acetone). The phenolic fraction consisting mainly of monomeric compounds was analyzed with a Shimadzu gas chromatograph GC-9A with a flame ionization detector, fitted with a capillary column packed with SE 30 (SCOT). N_2 was used as carrier gas at a flow rate of 60 cm^3/min with H_2 and air flows of 35 and 500 cm^3/min, respectively. The temperature of the oven, the injector and the detector was 300 °C.

3 Results and discussion

The OMW detoxification by A. niger was carried out in the bubble column batch culture. In the anaerobic reactor and aerobic fluidized bed fermentor the anaerobic digestion and aerobic post-treatment took place respectively. These reactors were fed sequentially in order to reduce the bacteria inhibition by phenolic compounds.

3.1 Aerobic pretreatment of OMW by A. niger in bubble column

Contrary to the growth of this fungus on OMW in a stirred fermentor, which had to be stopped because the mycelium obtained was adhering to the agitation paddles and inner surface of the fermentor [5], the bubble column prooved to be adequate for OMW pretreatment with A. niger [6].

The biomass grows metabolizing glucose, reducing sugars, pectins, oil and phenolic compounds [5]. This biomass formed contains olive pulp particules (50–90% cellulose and lignin) [17]. The protein content was 30%. Growth was limited when oxygen uptake rate (OUR) was higher than 90 mmol/dm^3 · h and the pO_2 was lower than 2% [6]. The limitation was due not only to the reactor mass transfer performances but also to the characteristics of OMW, which are not favourable to oxygen dissolution. During the fermentation, particles of substrate adhered to the mycelium or were trapped, especially when pellets were formed. The pellet formation depended on the COD and TSS concentrations in the OMW and on the amount of the inoculum (spores/dm^3 of OMW) [18]. Contrary to the Erlenmeyer flask cultures, where the pellet size was up to 5 mm [5], in bubble column, the mycelium growth gave rise to suspension of filamentous hyphae and smooth pellets. This suspension is less favourable to oxygen transfer with filamentous suspensions [19]. At the end of fermentation and after elimination of the biomass by filtration, the characteristics of prefermented OMW are reported in Table 2. Although the OMW was sterilized, the bacteria concentration was increased during fermentation especially after mycelium growth. The concentration of methanol at the end of fermentation was in the average of 0.5 g/dm^3. The carboxylic acids and polyols issued from

Table 2. Composition of OMW and prefermented OMW

Characteristics (g/dm^3)	OMW	Prefermented OMW
COD	136.4	56.2
TS	134	61.3
TSS	19.5	4.6
Reducing sugars	15.2	5.4
Glucose	24	trace
Number of bacteria per cm^3	$8 \cdot 10^3$	$5.4 \cdot 10^6$
pH	5.54	5.6

COD: Chemical oxygen demand, TS: total solids, TSS: total suspended solids

Table 3. Performances of different fermentors

Fermenters	ΔCOD (%)	ΔCOD* (%)	ΔTSS (%)
Bubble column	58.7	64.8	76.4
Anaerobic reactor	60.4	63.4	74.4
Fluidized bed	33	46	–

* After elimination of residual TSS by centrifugation

metabolism of A. niger were not quantified. In addition of protein production, the pretreatment removes 58.7% and 76.4% of COD and TSS respectively (Table 3). Moreover, the bioconversion of OMW by A. niger improves highly its filtration kinetic because the TSS was trapped by the fungal pellets [20].

Gas chromatographic analysis of OMW during the fermentation (Fig. 3) showed that some of the simple phenolic compounds had disappeared, whereas others had accumulated, due to tannin and polyphenols degradation [6]. Analysis of raw OMW by gel filtration chromatography showed that it contained two main groups of polyphenols (Fig. 4A). The phenolic compounds of the first group eluated in 30–80 fractions corresponding to simple phenolic compounds and polyphenols like tannin and anthocyanins. The polyphenols of the second group, 8–30 fractions, give dark brown to black colour to the water. Sephadex G 50 chromatography of prefermented OMW shows that A. niger is especially efficient in the reduction of simple phenolic compounds and polyphenols like tannin and anthocianins. The polyphenols that resisted fermentation seem to be partially polymerized by aeration as described for the aqueous spruce burk [21] which increase the absorbance of 8–10 fractions.

3.2 Methanization of prefermented OMW in AF

Calcium hydroxide which precipitates phenolic compounds and long chain fatty acids toxic to methanogenic bacteria, and improves the total alkalinity is beneficient for adjustment of the pH of OMW [22]. In deed, the adjustment of pH of prefermented OMW at 7.2 by $Ca(OH)_2$ enhanced the elimination of the residual tannic compounds and darkly colored polyphenols (Fig. 4B). It was demonstrated that

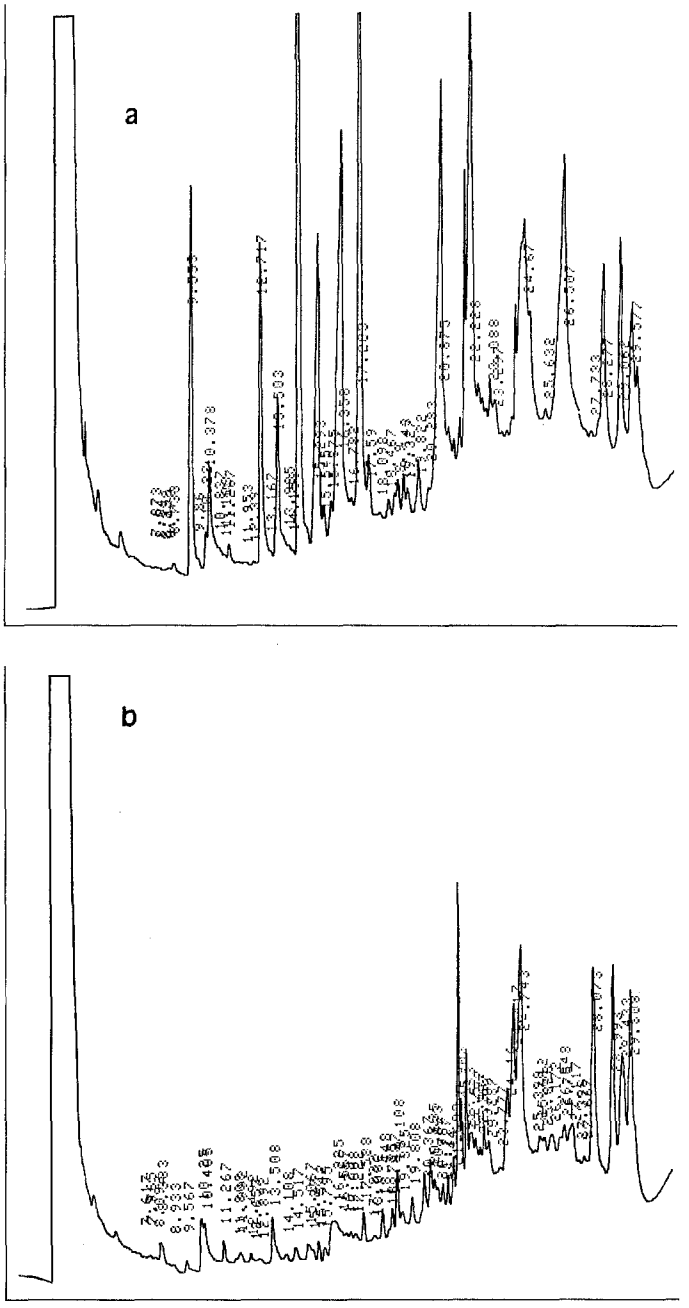

Fig. 3a, b. Gas chromatogram of OMW (**a**) and prefermented OMW by A. niger in bubble column (**b**)

extensive polymerisation of tannins by calcium and/or aeration at pH 6 enhances their elimination and gives a complete detoxification of aqueous spruce burk [21]. Moreover, the A. niger growth produced methanol from pectin which stimulated the methane production by acetoclastic methanogenic bacteria [4]. Previous study showed that start-up of anaerobic filter fed with the OMW was much shorer than of suspended growth reactors such as the anaerobic contact process and the UASB [23]. In deed, the anaerobic filter reactor is very suitable for anaerobic digestion of prefermented OMW because of its stability and high performance [15].

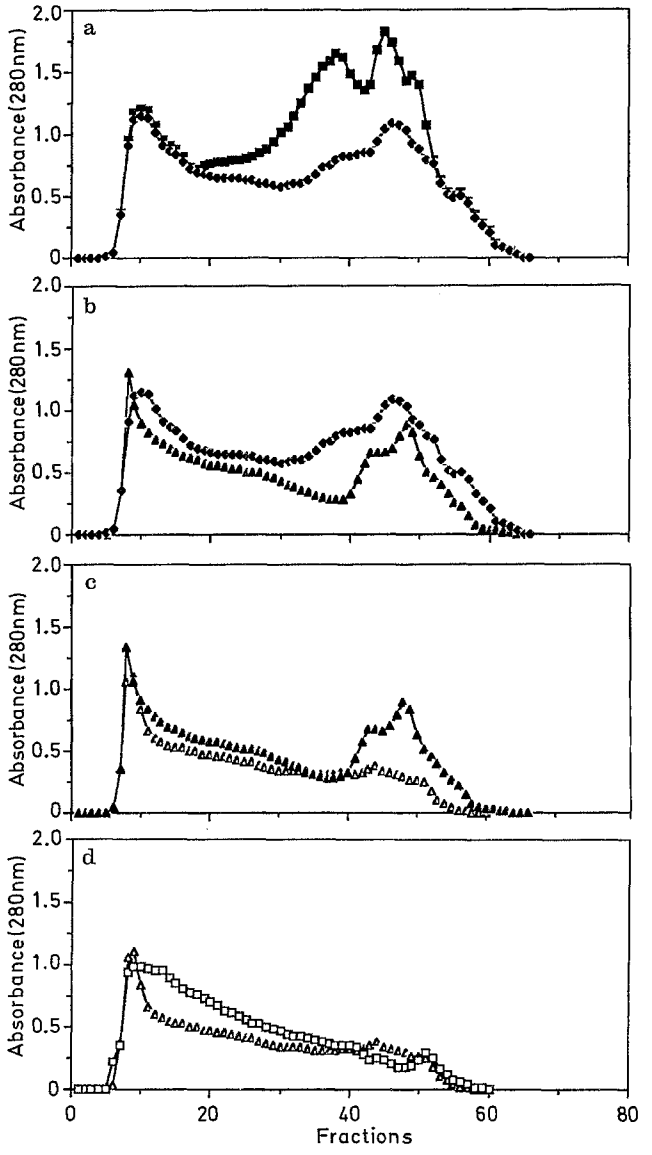

Fig. 4. A: Sephadex G-50 permeation of raw OMW (■), and prefermented OMW by A. niger in bubble column (♦). B: Sephadex G-50 permeation of prefermented OMW by A. niger in bubble column (♦) and neutralized prefermented OMW by $Ca(OH)_2$ at 7.25 (▲). C: Sephadex G-50 permeation of feed (▲), and effluent (△) of anaerobic reactor. D: Sephadex G-50 permeation of feed (△) and effluent (□) of aerobic fluidized bed fermentor

The daily methane production and COD removal obtained in anaerobic filter reactor exceeded those obtained in conventional anaerobic contactors. Moreover, the anaerobic filter reactor gives biogas with a higher percentage of methane and effluent with a lower volatile fatty acids and volatile solids content than anaerobic contactors. The flow of the prefermented OMW through the anaerobic filter reactor changes the characteristics of the substrate as shown in Table 4 and produces daily an average to 2 dm^3 of biogas with 80% of methane. The reduction of COD and TSS were 60.4% and 74.4% respectively (Table 3). The methanization

Table 4. Composition of feed and effluent of anaerobic filter reactor

Characteristics (g/dm^3)	Feed of anaerobic filter	Effluent of anaerobic filter
COD	25.86	9.43
TS	20.9	9.44
TSS	3.22	0.74
pH	7.2	7.85

Table 5. Composition of feed and effluent of aerobic fluidized bed fermentor

Characteristics (g/dm^3)	Feed of AFBF	Effluent of AFBF
COD	9.83	6.58
TS	10.12	7.3
TSS	0.5	1.87
Number of bacteria per cm^3	–	10^6-10^7
pH	7.8	8.2

step reduces especially the polyphenolic compounds eluated in 40–60 fractions (Fig. 4C). Of the polyphenolic compounds, eluated in 8–30 fractions, and responsible for the black colour of the wastewater only a few were modified. Gas chromatographic analysis of anaerobic filter effluent shows that the simple phenolic compounds were removed as a whole (result unreported). During two years running, the anaerobic filter packed with flocoor has not been optured. The active biomass was retained in AF especially by trapping within and/or between the support elements. The methanogenic bacteria genera observed in sludge fixed in AF were morphologically Methanobacterium, Methanococcus, Methanospirillum, Methanogenium and Methanosarcina.

3.3 Postreatment of methanized OMW in AFBF

The grains of husk used in AFBF were easily suspended by aeration at the bottom of the reactor because of their biological composition (>90% of VS). This support media can be used in fluidization without recirculation of medium. They are constituted especially by lignine and hemicellulose which are difficult biodegradables [24]. The physical parameters as density and sedimentation rate of husk are smaller than those usually observed in fluidized beds. The specific area values of husk are close to those of sand [25]. The population which served to inoculate the AFBF is constituted by bacteria, which belong to genus of Pseudomonas, Achromobacter, Chromobacter, Aeromonas and Serratia. The concentration of these bacteria was in the average 10^6-10^7 bacteria/cm^3. The weak fixation of bacteria on this support may be due to the hydrodynamic conditions in the reactor and to the presence of the phenolic compounds that flocculate the bacteria, giving microgranules, which are easily sedimentable, and which increase the TSS concentration (Table 5). The AFBF removes 46% of COD after elimination

of TSS by sedimentation in settler. However, when the biomass was not eliminated, the COD removal was only 33% (Table 3). The sedimentation of sludge in settler is very easy.

The black colour of OMW, caused by the phenolic compounds having a high molecular weight, was increased by AFBF. The gel filtration chromatography showed that the polyphenols, contained in fractions 6–30, increased because of phenolic compounds partly polymerisation (Fig. 4D). Field and Lettinga [21] noted that tannins, which resist A. niger fermentation, partially autoxided and polymerized by aeration when pH passes 6.

Although the three biological steps remove 90% of COD, the COD concentration of effluent is still high compared to norms, and the black colour of OMW due to the darkly colored polymers persist. The colored effluent may be a hinderance to "cosmetic" discharge norms such as those regulating color. If required, the color norms can potentially be fulfilled by chemical precipitation or by biological mineralization.

Acknowledgement

This research was supported by the UNDP programme DP/TUN/86/010.

References

1. Fiestas Ros de Ursinos, J. A.: Différentes utilisations des margines. In Proc. of Séminaire international sur la valorisation des sous produits de l'olivier. FAO-PNUD. Tunisie. Décembre, pp 93–110, 1981
2. Boari, G.; Brunetti, A.; Passino, R.; Rozzi, A.: Anaerobic digestion of olive oil mill waste-waters. Agricultural Wastes. 10 (1984) 161–175
3. Balice, V.; Boari, G.; Cera, O.; Abbaticchio, P.: Indagine analitica sulle acque di vegetazione. Nota 1. Inquinamento, no. 7–8 (1982) 49–53
4. Hamdi, M.: The effect of pretreatment and agitation on the batch anaerobic digestion of olive mill wastewaters. Bioresource Technol. 36 (1991) 173–178
5. Hamdi, M.; Kadir, A.; Garcia, J. L.: The use of Aspergillus niger for bioconversion of olive mill wastewaters. Appl. Microbiol. Biotechnol. 34 (1991) 829–831
6. Hamdi, M.: A new conception of biological process for olive mill wastewaters treatment. Ph.D. Thesis, (Université de Provence, France) 1991
7. Ikeda, Y.; Takahashi, E.; Yokogama, K.; Yoshimora, Y.: Screening for microorganisms producing gallic acid from chineese and tara tannins. J. Ferment. Technol. 50 (1972) 361–370
8. Cichelli, A.; Solinas, M.: I composti fenolici delle olive e dell'oloi di oliva. Riv. Merceol. 23 (1984) 55–69
9. Balice, V.; Cera, O.: Acidic phenolic fraction of the juice of olives determined by gas chromatographic method. Grasas y Aceites 25 (1984) 178–180
10. Vazequez, R. A.; Maestro, D. R.; Graciani, C. E.: Compentes fenolicos de la aceintuna. Il poliphenoles del alpechin. Grasas y Aceites 25 (1974) 341–345
11. Tanchev, S.; Joncheva, N.; Genov, N.; Codounis, M.: Identification of anthocyanins contained in olives. Georgike Ereuna 4 (1) (1980) 5–13
12. Kieslich, K.: Microbiol transformations of non-steroid cyclic compounds. John Wiley and Sons, Georg Thieme publishers 1976
13. Makasinova, S. Y.; Martakov, A. A.: Assimilation of phenolic compounds by fungi of the genus Aspergillus. Tr In-Ta Mikrobiol Ivirusol AN Kazssr 27 (1982) 30–36
14. Blom, H.; Thomassen, M. S.: Kinetic studies on strawberry anthocyanin hydrolysis by a thermostable anthocyanin-β-glucosidase from Aspergillus niger. Food Chem. 17 (1985) 157–168
15. Hamdi, M.; Garcia, J. L.: Comparison between anaerobic filter and anaerobic contact process for fermented olive mill wastewaters. Biores. Technol. 38 (1991) 23–29
16. Knechtel, R. J.: A more economical method for the determination of chemical oxygen demand. Water Pollut. Control May/June (1978) 25–29
17. Fernandez, M. J.: Olives. In: Rehm, H. J.; Reed, G. (Eds.) Biotechnology, vol. 5. pp 379–397, Verlag Chemie, Weinheim (1983)
18. Hamdi, M.; BouHamed, H.; Ellouz, R.: Optimization of Aspergillus niger growth on olive mill wastewaters. Appl. Microbiol. Biotechnol. 36 (1991) 285–288
19. Smith, E. I.; Greenshields, R. N.: Tower fermentation systems and their applications to aerobic process. Chemical Engineer. (1974) 28–34
20. Hamdi, M.; Ellouz, R.: Use of Aspergillus niger to improve filtration of olive mill wastewaters. J. Chem. Technol. and Biotechnol. (In press 1992)
21. Field, J. A.; Lettinga, G.: Treatment and detoxification of aqueous spruce bark extracts by Aspergillus niger. Wat. Sci. Tech. 24 (1991) 127–137
22. Hamdi, M.; Festino, C.; Aubart, C.: Anaerobic digestion of olive mill wastewaters in fully mixed reactors and in fixed film reactors. Process Biochem. (In press 1991)
23. Rozzi, A.; Passino, R.; Limoni, M.: Anaerobic treatment of olive mill effluents in polyurethane foam bed reactors. Process Biochem. April (1989) 68–74
24. Nefzaoui, A.; Molina, E.; Outmani, A.; Vanbelle, M.: Ensilados de orujo de aceituna tratados con alcalis. Composicion quimica, digestibilidad in sacco y degradabilidae. Archivos de Zootecnia. 33 (1985) 219–236
25. Switzenbaum, M. S.: A comparison of the anaerobic filter and the anaerobic expanded/fluidized bed processes. Wat. Sci. Tech. 15 (1983) 345–358

Received November 8, 1991

M. Hamdi (corresponding author)
R. Ellouz
Centre de Biotechnologie de Sfax
BP-W, 3038 Sfax
Tunisia

J. L. Garcia
Laboratoire de Microbiologie ORSTOM
Université de Provence
3 Place Victor-Hugo
13331 Marseille cédex 3
France