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Structural characterization of olive mill waster-water after aerobic digestion using elemental analysis, FTIR and ^{13}C NMR

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Abstract

Aerobic digestion of olive mill waster-water (OMW) has been conducted under various medium conditions to determine the best treatment involving good stabilisation and maturity of these residues. Analysis by various chemical methods of elemental analysis, FTIR and ^{13}C NMR spectroscopies show the high content of raw olive mill waster-water of the less condensed structures of phenols, organic acids, alcohol, fatty acids and simple sugars. Aerobic digestion involved an humification process through two mechanisms—biodegradation and polycondensation. These mechanisms were highly influenced by medium conditions. In the cases of natural acid pH, either in the presence of soil micro-flora or the yeast *Saccharomyces cerevisiae*, aerobic digestion has been less developed due to the antimicrobial effect of free phenol. Neutralization of pH enhances the development of microbial activity and the humification process seems greatly influenced by means of neutralization. In the case of neutralization by lime, the intense oxidation of organic compounds occurred and humification involved polyphenol condensation. While, in the case of neutralization by phosphate, more oxidation of sugars has been observed, and polycondensation in contrast developed through N-linkage. Accordingly, treatment of olive mill waste-waters by soil micro-flora with neutralization of pH by phosphate could be considered the best treatment that allows good stabilisation of organic matter and high preservation of nitrogen in humic form. This treatment corrects further the deficiency of the two elements phosphorus and nitrogen.

Keywords: Olive mill waste-water; Aerobic digestion; Humification; Neutralization; Lime; Phosphate; Soil micro-flora; Yeasts

1. Introduction

Morocco is a Mediterranean country in which a part of the economy is based on olive culture and the production of olive oil. It has been subjected to numerous difficulties in the disposal of olive mill waster-water OMW produced after trituration [1–3]. A ton of triturated olives produces about 500 to 1200 kg of effluents [4]. This waste causes large-scale environmental problems because of its high polluting power due to a high organic load and a high antimicrobial activity exerted mainly by various phenolic compounds [5,6]. The organic fraction includes sugars, tannins, polyphenols, polyalcohols, pectins and lipids. Some of these substrate including sugars and polyalcohols can serve as carbon and energy sources for growth of microorganisms. However, its high content of monomeric and polymeric phenols involves high chemical and biochemical oxygen demands (COD and BOD) [7]. Monomeric phenols exhibit phytotoxic effects [8] and antimicrobial activity [9]. Polymeric phenols have a lignin-like structure as their most recalcitrant fractions and are mainly responsible for the typical colour of OMW [9].

The main difficulties recorded of treatment of olive mill effluents are related to: (a) seasonal operation; (b) high territorial scattering and high organic loading composed mainly of long-chain fatty acids and phenolic compounds which are difficult to biodegrade [10].

A preliminary treatment of these effluents and their valuation is recommended. A number of OMW treatment methods have been employed in recent years and these can be divided

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into physico–chemical methods or biological methods. Physico–chemical methods including flocculation, ultrafiltration and thermal concentration, are generally very expensive and/or unable to solve the problem completely of the need to dispose of a sludge derived from the process. Biological methods are based on production of proteins, activated carbons, poly-hydroxy-β-butyrates, exopolysaccharides, composting, anaerobic and aerobic digestion [7,9]. The latter methods have certain clear benefits due to the potential utilisation of their bioproducts. Most studies have been focused on bioremediation as a means of reducing the polluting effect of OMW. Removal of the monocyclic aromatic compounds from OMWs greatly reduces the toxicity of this waster-water. Aerobic treatment has a great efficiency in degrading polyphenolic compounds [11,12]. This great decrease has been explained either by microbial metabolism of phenols as an energetic source or their incorporation during humic substance neoformation [2,7,9,13,14]. Humification is widely recognised as a process allowing a turn-over of organic matter and has been suggested in numerous studies as a good index of stability and for assessing of the agronomic value of organic residues intended for agricultural manuring [15–17].

Accordingly, for the purpose of investigating the importance of humification as a detoxification process involving a transformation of polyphenols from their free forms to more stable humic structures, aerobic digestion of olive mill waster-water has been conducted. Different preliminary treatments have been performed to determine the best medium conditions for the humification process. Medeci et al. [18] has proposed neutralization of pH as a mean of avoiding the antimicrobial effect of polyphenols following their transformation to phenate form. Therefore, in this study, aerobic digestion has been carried out in the presence of soil micro-flora in natural acid pH or after neutralization by lime or natural phosphate, as well as after inoculation by yeasts (*Saccharomyces cerevisiae*) at natural acid pH. Various chemical techniques to follow humification process (elemental analysis, ^{13}C NMR, FTIR) have been applied to olive mill waster-water at raw state RW and after 10 days of aerobic digestion of various trials.

2. Material and methods

2.1. Olive mill waster-water sampling

The olive mill waster-water studied were taken from a semi-industrial unit of trituration of olive by pressure in Marrakech (Morocco), during the oliveculture season 2000/2001. Table 1 illustrates the various physico–chemical characteristics of these effluents [14].

2.2. Preliminary preparation of olive mill waster-water

Olive mill waster-water cannot be directly treated since it is high in organic matter, mainly in phenols with a high antimicrobial potential. A dilution of the raw olive mill waster-water with distilled water of 1/10 was applied [19]. To avoid any contribution of the natural flora of olive mill waster-water, sterilisation of the solution was carried out in an autoclave at 120 °C during 15 min was carried out.

Table 1
Principal physico–chemical characteristics of the raw olive mill waster-water

Parameters	Values
pH	4.7
Electric conductivity (ms/cm)	16.2
Dry matter (g/L)	85.9
Ash (g/L)	16.6
Chemical oxygen demand (g of O_2/L)	161 ± 5
Organic carbon (% M.S.)	47 ± 0.27
Total phenols (g/L)[a]	4 ± 0.01
Total Kjeldahl Nitrogen (mg/L)	588 ± 0.04
Ratio C/N	67.4
$N–NH_4^+$ (mg/L)	108
$N–NO_3^-$ (mg/L)	126
Total phosphorus (mg/L)	110

[a] Phenols are expressed as catechin.

Two types of inoculation were applied:

1) Inoculation by soil micro-flora: 2 l of sterilized olive mill waster-water are mixed with 5 g soil, and a trial was conducted at natural acid pH (Trial 1: E1) or at pH neutralized by addition of lime $CaCO_3$ (Trial 2: E2) or by addition of 50 g of natural phosphate taken from Khouribga city (Morocco) (Trial 3: E3).

   Microbiological characterization of soil inocula was achieved and results are expressed as colony forming units CFU/g fresh soil: fungus = 15.10^6 UFC/g fesh soil; yeasts = 11.10^6 UFC/g fesh soil; mesophilic flora = 24.10^6 UFC/g fesh soil.
2) Inoculation by baker's yeasts (*Saccharomyces cerevisiae)*: 1 g of yeast are dissolved in 2 l sterilized olive mill waster-water and the trial was carried out at natural acid pH (Trial 4: E4).

2.3. Aerobic digestion

Attempts were supervised for 10 days in polyethylene mini-digestors with a capacity of 2 l (diameter: 11 cm; height: 24 cm), equipped permanently with air flow, which has been adapted after numerous trials to obtain optimal microbial activity. The experiment was conducted at ambient temperature of about 25 ± 3 °C.

Sampling of olive mill waster-water were carried out before the treatments (diluted and sterilized raw olive mill waster-water = RW) and after various attempts I, II, III, IV (end of treatments = E1; E2; E3; E4).

The lyophylised samples of raw olive mill waster-water RW and of solutions obtained after various treatments E1; E2; E3; E4 were analysed by various chemical techniques: