International Biodeterioration & Biodegradation 88 (2014) 162–168

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Short communication

Integrated biological approaches for olive mill wastewater treatment and agricultural exploitation

Lucia Aquilanti [a], Manuela Taccari [a,*], Donatella Bruglieri [a], Andrea Osimani [a], Francesca Clementi [a], Francesca Comitini [b], Maurizio Ciani [b]

[a] Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy
[b] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy

ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form
18 December 2013
Accepted 18 December 2013
Available online 19 January 2014

Keywords:
Free-living nitrogen-fixing bacteria
Azotobacter
Bioferment
OMW aerobic treatment
Composting

ABSTRACT

In this study, an integrated biological approach, consisting of the aerobic treatment of olive mill wastewater (OMW) for the production of a N_2-fixing culture followed by laboratory-scale composting, was investigated for the agricultural exploitation of this agro-industrial waste. In the first stage, presumptive azotobacteria isolated from soils annually treated with OMW and three reference cultures were screened for their capacity to abate water-soluble polyphenols from sterile lime-treated OMW. Two soil isolates, molecularly identified as *Azotobacter chroococcum* and the reference strain *Azotobacter* spp. GP1 were selected for the formulation of a bacterial consortium to be exploited for the production of a N_2-fixing culture in a 2-L laboratory fermenter by using sterile neutralized OMW. Once assayed for its residual antimicrobial activity, the resulting bioferment was applied to wheat straw piles at the start of the laboratory-scale composting process, and the properties of the final compost were assayed. The effect of regular application of OMW during prolonged composting (136 days) was also evaluated and total abated polyphenols were quantified.

The preliminary results of this first study were an aerobic OMW bioferment enriched with N_2-fixing bacteria and characterized by a significantly reduced antimicrobial activity, and a significant improvement in the compost stability through the inoculation of straw piles with this N_2-fixing bacterial culture.

1. Introduction

Italy is among the major producer countries in the world of olive oil, together with Spain and Greece. The extraction of olive oil is principally performed by discontinuous (press-based) or continuous (centrifugation-based) technologies, which lead to the production of both solid (olive husk or olive pomace) and liquid (olive mill wastewater, OMW) waste products (except for two-phase centrifugation systems which produce only solid residues) (Otles and Selek, 2012). OMW is characterized by a high content of inorganic salts, principally phosphates, and organic matter (COD, 50–150 g L^{-1}) consisting of nitrogenous compounds, organic acids, sugars, tannins, pectins, carotenoids, oil residues, and phenolic substances (Piperidou et al., 2000; Mekki et al., 2006; Piotrowska et al., 2006; Anastasiou et al., 2011).

In the last decades, the remarkable increase in olive oil production has rendered the disposal and treatment of OMW a serious problem for the olive oil industry (Rozzi and Malpei, 1996). Controlled land spreading of untreated OMW undoubtedly represents the simplest and most economical strategy for the management and disposal of this by-product (Otles and Selek, 2012). However, the direct application of OMW on agricultural soils results in either positive or negative effects, the latter being mainly ascribable to the high mineral salt content, low pH, and great abundance of compounds with phytotoxic and antimicrobial activities (Otles and Selek, 2012). Accordingly, a number of physical, physicochemical and even biological strategies have been proposed for the treatment of OMW (Otles and Selek, 2012); among these, biological processes including aerobic treatments with selected microorganisms and composting, are undoubtedly the most environmentally compatible and the least expensive (Mantzavinos and Kalogerakis, 2005).

Aerobic treatments are primarily aimed at degrading a fraction of the OMW pollutants through an oxidative process. To date, a number of bacteria have been assayed, either alone or in consortia, for the aerobic treatment of OMW; these include members of the genera *Arthrobacter*, *Sphingomonas*, *Ralstonia*, *Bacillus*, *Pseudomonas*, and *Azotobacter* (Knupp et al., 1996; Ramos-Cormenzana

* Corresponding author. Tel.: +39 071 2204987.
E-mail address: taccari.manuela@libero.it (M. Taccari).

0964-8305/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2013.12.010

et al., 1996; Ehaliotis et al., 1999; Di Gioia et al., 2001; Niaounakis and Halvadakis, 2006).

The latter are free-living nitrogen-fixing bacteria, which are broadly dispersed in neutral to alkaline soils, water and sediments. Due to the low content in nitrogenous organic compounds and richness in carbon sources, OMW is an ideal substrate for the growth of azotobacteria. Isolates of either *Azotobacter chroococcum* or *Azotobacter vinelandii* proved to actively grow (García-Barrionuevo et al., 1992) and fix atmospheric nitrogen (Balis et al., 1996; Papadelli et al., 1996) in sterile undiluted OMW, and both species were found to use aromatic compounds as a carbon and energy source (Anastasiou et al., 2011). For *A. vinelandii,* the capacity to produce fertility-promoting metabolites (Fiorelli et al., 1996) and exopolysaccharides (Niaounakis and Halvadakis, 2006) in diluted OMW has also been demonstrated. In Greece, an isolate of *A. vinelandii* (strain A) has been exploited in a pilot plant for the aerobic treatment of OMW, with very promising results in terms of removal of phytotoxic compounds (Piperidou et al., 2000).

Composting is a further biological technology for the disposal of OMW; it enables the recycling of organic matter through the activity of the endogenous microbiota of the composted solid matrix (Hachicha et al., 2008). Thanks to its high content in carbon and humic substances, compost has a high fertilizing and soil-conditioning value (Hachicha et al., 2008). A considerable bibliography exists on the composting of OMW (Hachicha et al., 2008; Aviani et al., 2010; Cayuela et al., 2010; Altieri et al., 2011; Federici et al., 2011) and sound evidence is available on the fertilizing capacity of compost obtained from this agro-industrial waste (Mantzavinos and Kalogerakis, 2005). However, relatively few data are available on the application of OMW during prolonged composting (exceeding 4 months) (Hachicha et al., 2008) and, to the best of our knowledge, no feasibility studies on the combination of composting with a biological pre-treatment of OMW have yet been carried out.

In this study, an integrated approach, relying on the aerobic pre-treatment of OMW for the production of a N_2-fixing culture followed by laboratory-scale composting was preliminary investigated in laboratory-scale trials for the agricultural exploitation of this agro-industrial waste. Aerobic pre-treatment, performed in a 2-L laboratory fermenter with a consortium of N_2-fixing bacteria opportunely selected for their capacity to abate (and hence indirectly metabolize) polyphenols, yielded a liquid bioferment with a reduced antimicrobial activity, which was further applied to wheat straw piles at the start of the composting process. The effect of the regular application of OMW to composted piles during prolonged composting (136 days) was also evaluated, and the total abated polyphenols were quantified.

2. Materials and methods

2.1. OMW sampling and composition

OMWs were sampled from olive processing plants located in the district of Ancona (Marche Region, Italy) using either the press system (sample 1 and sample 2) or the three-phase centrifugation process (sample 3). For each sample, an aliquot was collected and analysed for: moisture, pH, reducing sugars, and phenols (such as gallic acid), as previously described (Taccari and Ciani, 2011).

The analytical composition of the OMW samples under study was reported as follows: sample 1: moisture, 93.0%; pH 5.16; reducing sugars, 0.5%; phenols, 550.7 mg L^{-1} (as gallic acid); sample 2: moisture, 94.7%; pH 4.9; reducing sugars, 0.3%; phenols (as gallic acid) 1785.6 mg L^{-1}; sample 3 three-phase OMW: moisture, 97.5%; pH 4.73; reducing sugars, 0.25%; phenols (as gallic acid), 189.8 mg L^{-1}.

Samples 1 and 3 were used for the screening of the soil isolates and reference cultures in 250-mL Erlenmeyer's flasks, whereas sample 2 was used for OMW pre-treatment in a 2-L laboratory fermenter and for repeated watering of composted piles during composting.

2.2. Reference cultures

Three reference strains were purchased as lyophilized cultures from the Deutsche Sammlung von Mikrorganismen und Zellkulturen (DSMZ, Braunschweig, Germany): *A. vinelandii* DSM389, *Azotobacter* spp. DSM1721 and *Azotobacter* spp. DSM6428. They were revitalized as indicated by the culture supplier, stored at −80 °C in a mixture of Tryptic Soy Broth (TSB, Oxoid, Milan Italy) and glycerol at a ratio 1:1 (v/w) and screened for their ability to reduce total content in water-soluble polyphenols of sterile CaO-treated OMW. The latter culture, originally isolated from a mixture of soil samples from various countries and deposited as *Azotobacter* spp. strain GP1, was used as a positive control, for its reported ability to use a number of phenolic compounds (including phenol) as carbon and energy sources (Li et al., 1991).

2.3. Isolation of azotobacteria

Soil samples were collected during the spring from uncultivated soils annually treated with OMW. They were withdrawn at a depth of 5–15 cm below the surface, following standard operating procedures (Sheppard and Addison, 2006), transported to the laboratory in sterile glass vials, sieved through a 4-mm-mesh sieve, and stored at field moisture content at 4 °C until use. Prior to the isolation campaign, aliquots of sieved soils were screened for the occurrence of azotobacteria using the soil paste method previously described by Aquilanti et al. (2004a). After five days incubation at 30 °C, *Azotobacter*-like colonies, growing around the soil grains, were sampled and streaked to purity on the same medium. Exopolysaccharides and pigment production was determined on N-free LG medium containing sucrose as a sole carbon source and blue of bromothymol as a pH indicator (Aquilanti et al., 2004a) whereas the Gram staining response and cell morphology were determined by optical microscopy. Long-term storage of Gram-negative, rod-shaped isolates producing exopolysaccharides and organic acids on N-free LG medium was at −80 °C in a mixture of Tryptic Soy Broth (TSB, Oxoid, Milan Italy) and glycerol at a ratio 1:1 (v/w), whereas short-term storage was on Tryptic Soy Agar (TSA, Oxoid) at 4 °C. Cultures selected for their OMW detoxification performance in Erlenmeyer flasks were molecularly identified by Amplified Ribosomal DNA Restriction Analysis (ARDRA) and partial sequencing of the 16S rRNA gene as previously described (Aquilanti et al., 2004b).

2.4. Screening of the bacterial cultures for their ability to abate water-soluble polyphenols in OMW

OMW samples 1 (from a press-based process) and 3 (from a centrifugation-based process) were centrifuged at 3000×*g* for 5 min to remove particulate material and the supernatant was added with lime (CaO) to adjust the pH to 7.5. Previous studies clearly demonstrated that pre-treatment of OMW with lime does not modify the composition of the organic fraction of this by-product (Piperidou et al., 2000).

Aliquots (100 mL) of each CaCO-treated OMW sample were transferred into 250-mL Erlenmeyer flasks, sterilized at 121 °C for 15 min and inoculated to a final bacterial concentration of 10^5–10^6 cells mL^{-1}. Two controls for each OMW sample, consisting of sterile unspiked OMW (negative control) and raw OMW (unspiked unsterilized control) were also assayed. The bacterial load of the