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Winery wastewater treatment by combination of *Cryptococcus laurentii* and Fenton's reagent

Cátia Santos [a], Marco S. Lucas [b], Albino A. Dias [a], Rui M.F. Bezerra [a], José A. Peres [b], Ana Sampaio [a,*]

[a] Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas (CITAB), Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta dos Prados, 5001-801 Vila Real, Portugal
[b] Centro de Química de Vila Real (CQVR), Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta dos Prados, 5001-801 Vila Real, Portugal

HIGHLIGHTS

- First report on WW treatment by two basidiomycetous yeasts followed by Fenton's reagent.
- Both yeasts had identical pollution abatement performances but *C. laurentii* was faster.
- H_2O_2 concentration was critical in COD and TPP removal.
- WW treated by biological and Fenton reagent is within legal limits for released in natural waters.

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ABSTRACT

Winery wastewaters (WW) have high levels of organic matter, resulting in high COD and BOD and suspended solids. This paper studies the combination of biological and chemical processes in WW treatment. Among 10 yeast isolates, *Filobasidium* sp. (AGG 577) and *Cryptococcus laurentii* (AGG 726) were selected due to their superior performance in COD removal. During WW degradation, COD and total polyphenols (TPP) content removal of 89–90% for *Filobasidium* sp. and 90–93% for *C. laurentii* were obtained. However, despite similar degradation efficiency for both yeasts, COD kinetics and pH evolution during treatment reveals that *C. laurentii* presents a faster response than *Filobasidium* sp. The toxicity (inhibition of *Vibrio fischeri* luminescence) of *C. laurentii* treated WW decreases to an inhibition value below 2.5%. However, treated WW exceeds the legal limits, making necessary an additional treatment. In this case, the selection of Fenton's reagent as a chemical final polish step process is a good compromise between efficiency and lower practical complexity. The best results for both COD and TPP removal were obtained with H_2O_2 initial concentration of 39.2 mM and a H_2O_2:Fe^{2+} molar ratio of 15:1. The combined *C. laurentii* – Fenton's reagent treatment of WW achieved a total reduction of 98% and 96%, for COD and TPP, respectively.

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1. Introduction

Wine production is inevitably accompanied by the generation of solid and liquid wastes. The liquid effluents, usually referred as WW are mainly originated from various unit operations such as washing of the presses used to crush the grapes, rinsing of fermentation tanks, barrels, bottles and other equipments or surfaces. A winery produces around 1.3–1.5 kg of effluent per liter of wine produced (Lucas et al., 2010).

Winery wastewater is characterized by a high organic load and acidic pH. The high COD and BOD, mainly due to the concentrations of soluble sugars, organic acids, alcohols, polyphenols, tannins and structural polymers (Malandra et al., 2003; Jin and Kelly, 2009; Souza et al., 2013) turn these effluents into an environmental problem. Hence, a responsible management of these effluents requires that their potential environmental impacts be minimal and within an acceptable range.

Current methods for winery wastewater treatment include physical, chemical and biological processes. Physical processes only do a phase transfer of the pollutants and chemical processes are normally more expensive (Beltran-Heredia et al., 2005). The conventional biological treatments are not a complete solution to the problem due to the high bio-recalcitrance and toxicity of phenolic contaminants present in WW (Benitez et al., 1999). Thus, it is important to find alternative solutions.

Microorganisms have a key role in biological WW treatment, and are involved either in aerobic or anaerobic treatments, being

* Corresponding author. Address: Universidade de Trás-os-Montes e Alto Douro, Departamento de Biologia e Ambiente, Quinta dos Prados, 5001-801 Vila Real, Portugal. Tel.: +351 259350106; fax: +351 259350480.
E-mail address: asampaio@utad.pt (A. Sampaio).

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responsible for significant COD reductions (Petruccioli et al., 2002; Eusébio et al., 2004; Ganesh et al., 2010; Souza et al., 2013). Studies on the microbial community in WW aerated bioreactors indicated that in number bacteria dominated over fungi (Malandra et al., 2003; Eusébio et al., 2004; McIlroy et al., 2011). Within fungi, yeasts are more representative than filamentous fungi, ranging from 10^2 to 10^5 CFU g^{-1} of sludge (Yang et al., 2011) and are specific to wastewater treatment plants, varying with wastewater type (Yang et al., 2011; Evans and Seviour, 2012). However, for WW mostly of the yeast species found by culture-independent methods (Evans and Seviour, 2012) as reported by others (Sabate et al., 2002; Malandra et al., 2003; Eusébio et al., 2004) and many are associated with the vineyard environment.

The combination of biological and chemical processes, allows a further reduction of organic content, particularly of non-biodegradable compounds. AOP allow the destruction of complex organic constituents. Fenton's reagent is a homogeneous catalytic oxidation process resulting from the combination H_2O_2 and Fe^{2+} in acid conditions. This AOP has the ability to act as an oxidant of a wide variety of organic compounds, both aromatic (phenols, polyphenols) and aliphatic compounds (alcohols, ketones, aldehydes) (Bigda, 1995; Neyens and Baeyens, 2003). In Fenton's reagent, hydroxyl radicals are generally referred as the primary oxidizing chemical species generated in accordance with fundamental equation (Peres et al., 2004).

This system can be considered eco-friendly since the H_2O_2 not consumed in the reaction is harmless and quickly decomposed to water and oxygen, and also because Fe^{2+} is a cation normally present in natural aquatic systems particularly in reducing conditions such as groundwater.

The main aim of this work is to remove the pollution of a WW using an eco-friendly technology in order to not exceed legal limits for discharge into surface water bodies. In order to meet this criterion a combination of bioremediation (using yeast species) followed by an AOP (Fenton's reagent) was implemented. As far as we know, our work is the first report on WW biological treatment using wild basidiomycetous yeast isolates (*Filobasidium* sp. and *C. laurentii*) followed by Fenton's reagent.

2. Experimental

2.1. Winery wastewater

The original WW used in this study was obtained in a Portuguese winery ("Adega Cooperativa de Vila Real") located in the Douro region (northeast of Portugal).

Table 1 summarizes the physical–chemical characteristics of the WW which corresponds to the average values of two collected samples. Before performing biological and chemical tests the WW was coarsely filtered through a filter of 2 mm mesh and stored at −20 °C in plastic cans. For each experiment a new frozen sample was used in order to minimize discrepancies among experiments.

Table 1
WW characterization and discharge limits determined by Portuguese legislation.

Parameter	WW	Discharge limits
COD (mg L^{-1})	3820 ± 115	150
TPP (mg GAE L^{-1})	190 ± 10	–
pH	4.9 ± 0.2	6.0–9.0
TOC (mg L^{-1})	1628 ± 17	–
TSS (mg L^{-1})	1215 ± 120	60
VSS (mg L^{-1})	960 ± 95	35
BOD (mg L^{-1})	2110 ± 105	40
TN (mg L^{-1})	198 ± 6	15
TP (mg L^{-1})	28 ± 2	10
K (mg L^{-1})	987 ± 30	–
BOD/COD	0.55 ± 0.01	–

2.2. Reagents

For yeast maintenance, yeast malt agar (YM) was purchased from BD Difco and the analytical grade reagents used in medium base (MB) composition were from Sigma–Aldrich.

Fenton experiments were performed using $FeSO_4.7H_2O$, H_2O_2 (30% w/w), Na_2SO_3, H_2SO_4 and NaOH. All analytical grade reagents were provided by Panreac Química.

2.3. Analytical procedures

Total suspended solids (TSS), volatile suspended solids (VSS), total-N (TN), total-P (TP) and K were determined according to Standard Methods (APHA, 1992). COD analyses were performed in a COD reactor from HACH and a HACH DR2010 spectrophotometer was used for colorimetric measurement. BOD was evaluated by the respirometric method, using OxiTop. In Fenton reagent experiments before the COD analysis, the samples were conditioned to remove excess H_2O_2 by adding sodium sulfite. Hydrogen peroxide concentration was controlled during the treatments using Merck Peroxide Test (0–25 mg H_2O_2 L^{-1} and 0–100 mg H_2O_2 L^{-1}). The evolution of pH was determined by a pH-meter (CRISON 507). The TPP content was measured in filtered samples by spectrophotometry at 765 nm using the Folin–Ciocalteau reagent (Merck) (Singleton and Rossi, 1965) and the results expressed as mg gallic acid equivalent (GAE) L^{-1}. A Jasco V-530 UV/VIS (Tokyo, Japan) double-beam spectrophotometer was used whenever necessary.

Total organic carbon (TOC) was monitored by measuring the direct injection of filtered samples into a Shimadzu TOC-V CSN analyzer, equipped with an ASI-V autosampler, provided with an NDIR detector and calibrated with standard solutions of potassium phthalate.

Toxicity assessment was performed using a commercial toxicity screening kit (BioTox). The biological agent is a freeze-dried preparation of the marine bacterium *Vibrio fischeri* (NRRL number B-11177). To provide optimal living conditions for this test microorganism, different samples of WW and controls were prepared with non-toxic 2% sodium chloride solution adjusted to pH 7. The light emission was measured with a luminometer (Aboatox 1253) at 0 min and after a contact period of 30 min at 15 °C. The inhibition effect of the samples was compared with a control to obtain the percent of inhibition (% INH) determined as described in the following formula:

$$\%\mathrm{INH} = 1 - \frac{T_{30}/T_0}{C_{30}/C_0} \times 100 \quad (1)$$

where T_0 = light in sample vial at 0 min; T_{30} = light in sample vial at 30 min; C_0 = light in control vial at 0 min and C_{30} = light in control vial at 30 min. All the experiments were carried out in duplicate and average values are presented.

2.4. Yeast isolates origin and maintenance

In the present work were used ten yeast isolates: (i) nine obtained from decomposed leaves in a freshwater wetland located in Santarém, Central Portugal, with the culture references AGG 577, AGG 585, AGG 598, AGG 667, AGG 691, AGG 711, AGG 721, AGG 726, AGG 729, and (ii) one, M33, from a olive mill wastewater, previously identified as *Candida oleophila* (Lucas et al., 2006). Yeast isolates identification was achieved throughout sequencing the D1/D2 domain of 26S rDNA, using the forward and reverse primers NL1 and NL4 by Biopremier, Portugal. The obtained sequences

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