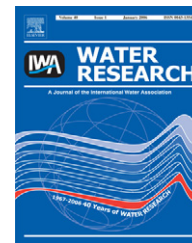


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Prevention of volatile fatty acids production and limitation of odours from winery wastewaters by denitrification

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ABSTRACT

The effect of the addition of nitrate to winery wastewaters to control the formation of VFA in order to prevent odours during storage and treatment was studied in batch bioreactors at different $\text{NO}_3^-/\text{chemical oxygen demand (COD)}$ ratios and at full scale in natural evaporation ponds ($2 \times 7000 \text{ m}^2$) by measuring olfactory intensity. In the absence of nitrate, butyric acid (2304 mg L^{-1}), acetic acid (1633 mg L^{-1}), propionic acid (1558 mg L^{-1}), caproic acid (499 mg L^{-1}) and valeric acid (298 mg L^{-1}) were produced from reconstituted winery wastewater. For a ratio of $\text{NO}_3^-/\text{COD} = 0.4 \text{ g g}^{-1}$, caproic and valeric acids were not formed. The production of butyric and propionic acids was reduced by 93.3% and 72.5%, respectively, at a ratio of $\text{NO}_3^-/\text{COD} = 0.8$, and by 97.4% and 100% at a ratio of $\text{NO}_3^-/\text{COD} = 1.2 \text{ g g}^{-1}$. Nitrate delayed and decreased butyric acid formation in relation to the oxidoreduction potential. Studies in ponds showed that the addition of concentrated calcium nitrate (NITCALTM) to winery wastewaters (3526 m^3) in a ratio of $\text{NO}_3^-/\text{COD} = 0.8$ inhibited VFA production, with COD elimination (94%) and total nitrate degradation, and no final nitrite accumulation. On the contrary, in ponds not treated with nitrate, malodorous VFA (from propionic to heptanoic acids) represented up to 60% of the COD. Olfactory intensity measurements in relation to the butanol scale of VFA solutions and the ponds revealed the pervasive role of VFA in the odour of the untreated pond as well as the clear decrease in the intensity and not unpleasant odour of the winery wastewater pond enriched in nitrates. The results obtained at full scale underscored the feasibility and safety of the calcium nitrate treatment as opposed to concentrated nitric acid.

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1. Introduction

The heavy biodegradable organic load that characterises food industry wastewaters has multiple impacts on treatments and on the environment: excessive sludge production, oxygen requirements and nutrient deficiency linked to aerobic processes, risk of acidification as a result of anaerobic digestion and long-term storage of seasonal wastewaters in order to prevent overload. The noxious odours during storage and treatment of food industry wastewaters are a major

environmental problem today (ADEME, 2005). Winery wastewaters clearly fall within this framework (Guillot et al., 2000; Bories, 2006; Chrobak and Ryder, 2006). Worldwide wine production—a total of $26.5 \times 10^9 \text{ L year}^{-1}$ (OIV, 2005), of which 69% takes place in Europe—consumes approximately $0.8 \text{ L water L}^{-1}$ wine and generates large volumes of wastewater on a seasonal basis (Rochard et al., 1996; Duarte et al., 1998; OIV, 1999; Picot and Cabanis, 1998; ITV, 2000; Rochard, 2005). Natural evaporation in ponds, a rustic and economical technique (Duarte et al., 1998; TV, 2000; Le Verge and Bories,

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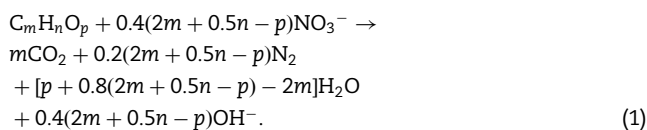
2004), well adapted to discharge variations and seasonal production, is a widely used treatment method in the largest wine production region in France, the Languedoc-Roussillon (approximately 1.6×10^9 L of wine/year, 10% of the overall European production) where 179 evaporation ponds were identified (Lambert and Lecharpentier, 2006, personal communication).

Volatile fatty acids form the major products resulting from the fermentation of carbon compounds in winery wastewaters (Bories et al., 2005; Bories, 2006), and are responsible for characteristic foul odours as a result of their low olfactory perception threshold (Le Cloirec et al., 1991). Other odorous compounds such as esters, mercaptans and aldehydes were also identified from winery wastewater treated in ponds (Guillot et al., 2000).

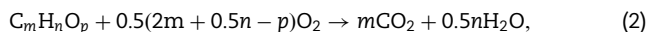
The degradation of VFA by denitrifying microorganisms was studied and used to eliminate nitrate in wastewaters (Min et al., 2002; Elefsiniotis et al., 2004; Sponza and Atalay, 2004). More generally, the denitrification treatment of wastewaters has been investigated with various carbon sources, microbial systems and models (Bolzonella et al., 2001; Chiu and Chung, 2003; De Lucas et al., 2005; Sage et al., 2006).

However, the outlook for curative treatment of VFA by means of denitrification in winery wastewater evaporation ponds appears to be compromised because of the massive quantities of VFA already accumulated and the emission of odours previous and/or subsequent to the curative treatment. On the other hand, the prevention of VFA formation by orienting the degradation of organic matter in wastewater into odourless products through anaerobic respiration with nitrate as the electron acceptor (denitrification) was studied in winery wastewaters to which nitrate had been added in the form of concentrated nitric acid (Bories et al., 2005).

The stoichiometric requirement in nitrate depends on the degree of carbon reduction, according to the following Eq. (1):



Since the chemical oxygen demand (COD) is a function of the degree of carbon reduction according to the following Eq. (2):



the nitrate requirement in relation to the COD is defined as a molar ratio $NO_3^-/O_2 = 0.8 \text{ mol mol}^{-1}$, resulting in a weight ratio of $NO_3^-/COD = 1.55 \text{ g g}^{-1}$.

In this study, the influence of the different NO_3^-/COD ratios in relation to VFA formation from a winery wastewater was studied, and the effectiveness of the preventive treatment of a pond using calcium nitrate was assessed by measuring olfactory intensity in addition to physico-chemical analysis. The addition of nitrate in increasing quantities to reconstituted winery wastewater was studied on the basis of the evolution of the redox potential and VFA formation kinetics in order to underscore competition between acidogenic fermentation pathways and denitrification, and to determine the NO_3^-/COD ratio required to inhibit VFA formation.

Since the general aim was to prevent odours, it was necessary to study and to compare winery wastewater evaporation ponds both treated and not treated with calcium nitrate under real conditions, with discharges over a long period of time and involving microbial systems.

2. Materials and methods

2.1. Winery wastewaters

For the study of the NO_3^-/COD ratios, reconstituted winery wastewater was prepared with a mixture of red grape must and red wine, in equal proportions and then diluted 10-fold with distilled water. The composition is presented in Table 1. The pH of the reconstituted wastewater was then adjusted to pH 6.5 with sodium hydroxide.

The reconstituted winery wastewater was divided between four 1-L closed glass bioreactors, equipped with a gas exhaust pipe (i.d.: 6 mm) and butyl septa for sampling and reagent addition, thermostatically controlled at 25 °C by water circulating through double-walled tubing, stirred at a speed of 250 rpm with a magnetic stirrer. The pH was measured with an Ingold electrode linked to an INGOLD 2300 pH metre, and adjusted to pH 6.5 by the addition of 10 N sodium hydroxide.

2.2. Study of the NO_3^-/COD ratio

Three bioreactors containing reconstituted wastewater were supplemented with nitrate in the form of soluble concentrated calcium nitrate (50% w/w), according to the quantities corresponding to the NO_3^-/COD mass ratios (w/w): 0.4; 0.8; 1.2. The bioreactors supplemented with nitrate and a fourth without nitrate (control) were inoculated to a volume rate of 5% (vol/vol) with a suspension of sediments taken from evaporation ponds whose microbial activity was tested beforehand in the laboratory. The wastewaters were incubated for 21 days and samples (4 mL) were regularly taken with a syringe through a septum and maintained at -18°C before being analysed.

Table 1 – Composition of the reconstituted winery wastewater

	Concentration (g L ⁻¹) ^a	Theoretical COD (g O ₂ g ⁻¹)	COD balance (%)
pH	3.5		
COD	22.3		100
Ethanol	4.5	2.09	42.2
Glucose	5.2	1.07	24.9
Fructose	4.9	1.07	23.4
Glycerol	0.52	1.22	2.8
Tartaric acid	0.25	0.53	0.6

^a Except pH.

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