

Integrated Process for the Production of Lipase and Methane from Olive Mill Wastewaters

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Abstract

The continuous system for olive oil production can be operated by three and two-phase extraction technologies, diverging in the water supplies. The three-phase extraction process has a slightly better yield, leading to less amount of olive cake but a significant production of olive mill wastewater (OMW).

The aim of the present work is the OMW valorization by producing high-value compounds while degrading this waste. The effluents are submitted to a lipase producing aerobic fermentation that contributes for a partial OMW degradation, followed by an anaerobic methanogenic degradation process.

The aerobic treatment, with production of lipase, is useful for a better yield of methane production. The lipase produced on the aerobic step had loss some activity from 60% to 95%. Best results were achieved for 5 g OMW COD L⁻¹ in terms of methane production (78% of methanisation) and lipase activity decay (60%).

Keywords

anaerobic digestion; biodegradability; lipase; methane; olive mill wastewater; toxicity

INTRODUCTION

The world's olive oil is mainly produced in Mediterranean countries. Spain is the most important producer, followed by Italy, Greece, Tunisia, Turkey, Syria (Tziotzios et al., 2007) Morocco and Portugal. The olive oil is obtained by two main extraction methods: press (traditional) and continuous (solid-liquid centrifuge) processes. The continuous system can be operated by three and two-phase extraction technologies, diverging in the water supplies. The three-phase extraction process has a slightly better yield, leading to less amount of olive cake but a significant production of olive mill wastewater, OMW (Roig et al, 2006). The incorrect disposal of this effluent causes serious environmental problems, such as colouring natural waters, threat to the aquatic life, surface and ground water pollution, soil quality, plant growth and odours (Akdemir and Ozer, 2008). In fact, OMW contains fats, sugars, phosphates, phenols and metals (Scioli and Vollaro, 1997) and it is also characterized by high values of acidity, organic load, solids content (Eusebio et al, 2002) and a characteristic lack of nitrogen.

Conventional wastewater treatment methods are relatively ineffective to remove OMW pollutants and, instead of disposal solutions, an approach of using this waste as a renewable resource is of great interest. The aim of the present work is the OMW valorization by producing high-value compounds while degrading this waste by a two-step process. The effluents are submitted to a lipase producing aerobic fermentation that contributes for a partial OMW degradation, followed by an anaerobic methanogenic degradation process.

MATERIALS AND METHODS

The OMW samples used in this work were from the 2008/2009 and 2009/2010 campaigns and were collected from different three phases' olive oil mills from the north region of Portugal. They were stored in the same day of collection at -20 °C. This effluent was characterized for pH, total solids (TS), volatile solids (VS), total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), total organic carbon (TOC), reducing sugars, phenols and lipids (Table 1).

Table 1. Characterization of OMW's used

	OMW A	OMW B	OMW C
	2008/2009	2008/2009	2009/2010
pH	4.91	4.52	4.95
TS/(g L ⁻¹)	118.4 ± 0.0	155.3 ± 0.6	58.0 ± 0.8
VS/(g L ⁻¹)	77.5 ± 28.4	116.2 ± 6.3	46.1 ± 2.5
TSS/(g L ⁻¹)	34.3 ± 8.2	55.4 ± 8.8	31.0 ± 0.4
COD/(g L ⁻¹)	178.2 ± 14.0	261.2 ± 24.6	96.7 ± 1.7
TN/(mg L ⁻¹)	284.7 ± 47.4	198.3 ± 9.5	3.4 ± 0.1
TOC/(g L ⁻¹)	46.8 ± 2.5	45.6 ± 0.0	15.3 ± 3.4
Reducing sugars/(g L ⁻¹)	68.2 ± 2.7	68.5 ± 1.2	9.3 ± 0.3
Phenols/(g L ⁻¹)	6.0 ± 1.2	7.9 ± 1.9	2.7 ± 0.2
Lipids/(g L ⁻¹)	11.2 ± 7.1	31.9 ± 15.1	3.5 ± 0.6

The pH of the OMW (Table 1) was adjusted to 7.2 and supplemented with of yeast extract (as source of nutrients) and NH₄Cl (because of the lack of nitrogen). The phosphate buffer 1 M (pH 7.2) and anti-foam were also added to the media before sterilization. A part of the prepared OMW media was aerobically treated (27° C; 500 rpm) on the 2-L bioreactor (Biolab, B. BRAUN), during 9 days, and the remaining medium was stored at -20 °C, to be used as non-treated OMW. Yeast strains of *Candida rugosa* (PYCC 3238, CBS 2275), *Candida cylindracea* CBS 7869 and *Yarrowia lipolytica* (CBS 2073, W29 ATCC 20460, IMUFRJ 50682) were used. Extracellular lipase was measured in the samples supernatant using p-nitrophenyl-butyrate (pNPB) in sodium acetate buffer 50 mM at pH 5.6 as a substrate, at 37 °C for 15 min. One unit of activity was defined as the amount of enzyme that produces 1 μmol of p-nitrophenol per minute under essay conditions. The operating conditions were optimized and the most adapted strain selected.

In order to evaluate the aerobic step usefulness, to improve the anaerobic digestion, biodegradability and toxicity tests were performed using OMW samples with and without aerobic treatment. The methanogenic toxicity tests and biodegradability assays were performed with OMW in concentrations ranged from 5 g COD L⁻¹ to 50 g COD L⁻¹. The basal medium used in these experiments was described elsewhere [6]. All batch tests are performed in triplicate and incubated at 37 °C and 120 rpm. Biogas was daily monitored by a pressure transducer to record the increase in pressure and headspace biogas was sampled periodically to assess, by gas chromatography, the methane content. These assays were performed with a granular sludge previously selected through anaerobic methanogenic activity tests. The activity of this granular sludge in presence of acetate was 50 mL CH₄/g VSS.day, approximately.

The cumulative methane production at the end of the batch essays (maximum plateau), divided by the OMW COD, and the slope of each curve were determined for each vial, for biodegradability and toxicity tests, respectively. The methane production of the blanks (vials without substrate) was considered.

RESULTS AND DISCUSSION

Aerobic treatment results show that all strains were able to grow on the OMW-based medium and to consume sugars and COD. However, phenols degradation was quite difficult, particularly when more easily degradable carbon sources were still present in the medium. Lipase production by *Y. lipolytica* W29 in flasks was improved, by approximately 60%, at C/N ratio of 15 and pH 7.0, in the presence of sodium phosphate buffer. These results were improved with the increase of agitation (from 400 rpm to 500 rpm) and aeration rates (from 1.5 L min⁻¹ to 2.0 L min⁻¹). Under these conditions, lipase production by *Candida cylindracea* was enhanced, achieving a lipase activity of 8.0 U mL⁻¹, approximately.

Lipase was not extracted after aerobic treatment so its influence should be considered. Cirne et al. (2006) had studied the influence of lipase on methane production and concluded that the higher the enzyme concentration, the more accentuated was the inhibition of methane production. This was not possible to observe since no inhibition neither lag-phases was noticed on the treated OMW. On non-treated OMW no lag phases were also detected. In table 2 is shown the maximum plateau achieved for each vial and the initial methane production rate.

Table 2. The maximum plateau achieved and the initial methane production rate for OMW with and without aerobic treatment, with OMW concentration ranged from 5 g L⁻¹ to 50 g L⁻¹, for toxicity and biodegradability tests

	Treated OMW	Non treated OMW
	<i>Initial methane production rate</i> mg COD-CH ₄ /(batch.day)	<i>Initial methane production rate</i> mg COD-CH ₄ /(batch.day)
Control	3.5 ± 0.1	3.5 ± 0.1
5 g OMW COD L ⁻¹	0.3 ± 0.2	2.7 ± 0.6
10 g OMW COD L ⁻¹	1.2 ± 0.2	1.9 ± 0.8
20 g OMW COD L ⁻¹	2.1 ± 0.5	2.8 ± 1.2
50 g OMW COD L ⁻¹	3.2 ± 1.0	4.6 ± 0.5
	<i>Maximum plateau/COD added</i> g COD-CH ₄ /g COD added	<i>Maximum plateau/COD added</i> g COD-CH ₄ /g COD added
<i>Biodegradability tests</i>		
5 g OMW COD L ⁻¹	0.78 ± 0.10	0.15 ± 0.07
10 g OMW COD L ⁻¹	0.23 ± 0.02	0.11 ± 0.04
20 g OMW COD L ⁻¹	0.15 ± 0.04	0.08 ± 0.02
50 g OMW COD L ⁻¹	0.02 ± 0.01	0.02 ± 0.01

In the toxicity results, the initial methane production rate was superior for the non-treated OMW. For 50 g OMW COD L⁻¹ and for the control (acetate) was observed that it's possible to achieve similar values of production rate. These values suggest that easily biodegradable organic matter is present in OMW. In these tests was also observed that the conditions that allow a better initial methane production rate lead to a lower production of methane.

In the biodegradability tests 78 % of the 5 g COD L⁻¹ is converted to methane using treated OMW while in the same conditions using non-treated OMW only 15 % is achieved, justifying the need of the aerobic treatment step. For both OMW's the treatment of 50 g COD L⁻¹ was unsuccessful, implying some inhibition by OMW compounds. Therefore is required the dilution of the non-treated OMW or use of the aerobic treatment conditions that allows achieving lower values of OMW COD. The activity of the lipase of the treated OMW has decayed after toxicity and biodegradability tests. This decay was lesser to 5 and 10 g OMW COD L⁻¹, about 60 % and, than for higher concentrations, about 95%. These results could imply the need of lipase extraction after the aerobic treatment.

CONCLUSIONS

Lipase production was optimized (8 U mL⁻¹) with non-diluted OMW media inoculated with *Candida cylindracea*.

The biodegradability tests had shown that the aerobic treatment is useful for a better yield of methane production. The lipase produced on the aerobic step had loss activity. Best results were achieved for 5 g OMW COD L⁻¹ in terms of methane production (78% of methanisation) and lipase activity decay (60%).

References

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For each vial, the cumulative methane production at the end of the essays (maximum plateau), divided by the OMW COD, and the slope of each curve were determined, for biodegradability and toxicity tests, respectively. The methane production of the blanks (vials without substrate) was considered.

RESULTS AND DISCUSSION

The aerobic treatment results show that all strains were able to grow on the OMW-based medium and to consume sugars and COD. When more easily degradable carbon sources are still present in the medium, phenols degradation is particularly difficult. In the presence of sodium phosphate buffer, the lipase production by *Y. lipolytica* W29 improved by approximately 60%. Better results were achieved by increasing the agitation (from 400 rpm to 500 rpm) and the aeration rates (from 1.5 L min⁻¹ to 2.0 L min⁻¹). Under these conditions, lipase production by *Candida cylindracea* was enhanced, achieving approximately 8.0 U mL⁻¹ of lipase activity.

Lipase was not extracted after aerobic treatment so its influence should be considered. Cirne et al. (2006) had studied the influence of lipase on methane production and concluded that the higher the enzyme concentration, the more accentuated was the inhibition of methane production. This was not observed, since no inhibition neither lag-phases occurred in the treated OMW. Also, no lag-phases were detected in non-treated OMW. Table 2 presents the maximum plateau achieved for each vial and the initial methane production rate.

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50 g OMW COD L ⁻¹	0.02 ± 0.01	0.02 ± 0.01
	<i>Initial methane production rate</i>	<i>Initial methane production rate</i>
<i>Toxicity tests</i>	mg COD-CH ₄ /(batch.day)	mg COD-CH ₄ /(batch.day)
Control	3.5 ± 0.1	3.5 ± 0.1
5 g OMW COD L ⁻¹	0.3 ± 0.2	2.7 ± 0.6
10 g OMW COD L ⁻¹	1.2 ± 0.2	1.9 ± 0.8
20 g OMW COD L ⁻¹	2.1 ± 0.5	2.8 ± 1.2
50 g OMW COD L ⁻¹	3.2 ± 1.0	4.6 ± 0.5

In the toxicity results, the initial methane production rate was superior for the non-treated OMW. Similar values of production rate were achieved for 50 g OMW COD L⁻¹ and for the control (acetate). This may suggest that concentrated OMW is not toxic to acetate degradation or even that more easily degradable matter, than acetate, is present in OMW.

In biodegradability tests, 78 % of 5 g COD L⁻¹ is converted to methane using treated OMW, while only 15 % is achieved using non-treated OMW, in the same conditions, justifying the need of the aerobic treatment step. For both OMW's the treatment of 50 g COD L⁻¹ was unsuccessful, implying

some inhibition by OMW compounds. Therefore, the improvement of aerobic conditions to achieve lower values of OMW COD or OMW dilution is required.

Considering biodegradability and toxicity tests, the obtained results showed that the conditions that improve the initial methane production rate lead to a lower production of methane.

The lipase activity of the treated OMW decayed after toxicity and biodegradability tests. This decay was less significant to 5 and 10 g OMW COD L⁻¹ (about 60 %), than for higher concentrations (about 95%). These results may imply the need of lipase extraction after the aerobic treatment.

CONCLUSIONS

Lipase production, in the aerobic step, was optimized (8 U mL⁻¹) with non-diluted OMW media, inoculated with *Candida cylindracea*.

Biodegradability tests showed that the aerobic treatment is useful for a better yield of methane production, although lost of lipase activity occurs. Better results were achieved for 5 g OMW COD L⁻¹ in terms of higher methane production (78% of methanisation) and less lipase activity decay (60%).

References

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