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COMPARATIVE STUDIES ON KINETICS OF ANAEROBIC AND AEROBIC BIODEGRADATION OF LIPIDS FROM OLIVE OIL MILL WASTEWATERS WITH MIXTURE OF *Bacillus spp.* CELLS

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Abstract

The experiments on the olive oil mill wastewater treatment for removing the lipids by anaerobic and aerobic biodegradation with mixed *Bacillus spp.* culture indicated that the rate of the aerobic process is up to two times higher than that of the anaerobic one. In this context, it was found that the influence of the dissolved oxygen concentration on the lipids biodegradation process is significant, but the kinetic model given in literature does not consider this parameter. Therefore, using the dependence between the ratio of specific rates, for aerobic and anaerobic processes, and the concentration of oxygen dissolved in medium, the new model $-\frac{dC_{TL}}{dt} = k_d \cdot C_{O2}^{0.62} \cdot C_{TL}$ was established for describing the kinetics of aerobic biodegradation with *Bacillus spp.* This model is more adequate for the studied systems of lipids-rich wastewaters bacterial treatment, offering the maximum error of 12.6% and the average one of ± 6.84%.

Key words: Bacillus spp., biodegradation, kinetics, lipids, specific rate

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1. Introduction

Lipids are organic water insoluble biomolecules produced in different amounts by microbial, vegetal, and animal cells. Among the complex lipids, a particular class includes the triglycerides, namely fats and oils.

According to the Global Industry Analysts Report, the worldwide production of vegetable oils increased from 130 millions tonnes in 2010 to 144 millions tonnes in 2011, being estimated at 169 millions tonnes for 2015 (GIA, 2010). This evolution is related to the population and, implicitly, consumption increase, as well as to the diversification of the vegetable oils utilization from food to chemical industry.

The producers of vegetable oils are important sources of wastewaters, the characteristics of pollutants depending on the used raw materials and technologies. The olive oil represents about 3% from the worldwide production of vegetable oils, most of this quantity being consumed in Europe. The wastewaters resulted from olive oil mills are important pollutants, due to their high organic content (lipids 0.2-1%, carboxylic acids 0.5-1.5%, sugars 1-8%, polyphenols and pectins 1-1.5%, tannins, polyalcohols etc.) (Azbar et al., 2004; Benitez et al., 1997). Most of the difficulties

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associated with the pollution generated by olive oil mills wastewaters are related to the insoluble organic biopolymers, mainly lipids, and polyphenols, because these compounds affect drastically the activity of aquatic microorganisms or organisms (Aggelis et al., 2003; Mekki et al., 2007).

Several physical and chemical methods (decantation, concentration by evaporation, filtration and ultrafiltration, reverse osmosis, flotation, adsorption, oxidation and photo-oxidation etc.) (Cammarota and Freire, 2006; Deschamps et al., 2003; Khoufi et al., 2007; Mameri et al., 2000; Moazed and Viraraghavan, 2005; Mysore at al., 2005; Sun et al., 2002), as well as biological or enzymatic ones (Aggelis et al., 2003; Asses et al., 2009; Benitez et al., 1997; Daims et al., 2006; Galaction et al., 2014; Gavrilescu and Chisti, 2005; Lan et al., 2009; Martinez-Garcia et al., 2009; Mechri et al., 2010; Peixotoa et al., 2008; Ucisik and Henze, 2008; Zeeman and Sanders, 2001) have been tested for the treatment of lipids-rich wastewaters. The bio-treatment is based on aerobic or non-aerobic processes using free or immobilized bacteria (*Burkholderia cepacia, Phormidium spp., Oscillatoria spp., Chroococcus spp., Enterobacter aerogenes, Mucor racenosus*), yeasts (*Candida oleophila, Candida tropicalis*, *Yarrowia lipolytica*), fungus (*Apergillus niger, Phanerochaete crysosporium, Lentinus edodes, Pleorotus ostreatus, Funalia trogii, Geotrichum candidum, Mucor rouxii, Absidia coerulea, Penicillium restrictum, Penicillium verucosum*) or enzymes (*lipases*). In the biological systems, the triglycerides are bioconverted to long chain fatty acids, which are finally oxidated to acetate or propionate and used for energy production or biomass growth (Chipasa and Medrzycka, 2006; Galaction et al., 2014; Gavrilescu and Chisti, 2005; Rinzema, 2003; Zeeman and Sanders, 2001). Because the lipids are water insoluble and, implicitly, inaccessible for the microorganism metabolism, they are initially hydrolyzed to low molecular weight compounds (fatty acids) by the microbial enzymes. These enzymes secretion is induced after the contact with the pollutants. Generally, the lipids are more rapidly consumed in the presence of oxygen, but the choice of aerobic *vs*. anaerobic operating conditions for wastewaters treatment at industrial scale depends on the costs of equipments and energy for aeration.

Although most of bacteria are able to use a large spectrum of natural compounds or pollutants as carbon and energy sources, one single bacterial culture does not posses the ability to metabolize all pollutants existing in the wastewater. However, the mixed cultures exhibit an amplified biodegradative potential against the xenobiotic compounds (Fritsche and Hofrichter, 2000). The use of active sludge for wastewaters treatment is more convenient from the point of view of operating cost. But, in this case, the phenomenon of flotation cannot be avoided, due to the adsorption of lipids in sludge, its intensity becoming important at high oils content (Chipasa and Medrzycka, 2006; Rinzema, 2003).

This phenomenon affects the efficiency of biological treatment. For this reason, although they are rather expensive, the treatments systems for wastewaters containing lipids with selected microorganisms or enzymes become more and more preferred, owing to their superior efficiency and productivity (Caşcaval et al., 2012).

The bacterial biodegradation of lipids can be kinetically described by the model developed by Pavlostathis and Giraldo-Gomez, 1991. This model is rather simple, being related only to the lipids concentration in the media. For this reason, its relevance for the aerobic processes is limited, due to the significant influence of oxygen content on the lipids biodegradation rate.

In this context, our studies are focused on the analysis of the performances of the anaerobic and aerobic biological treatments of olive oil mill wastewaters using mixed *Bacillus spp.* culture. By means of the experimental results, a new and more complex kinetic model for aerobic bacterial bioconversion of lipids has been proposed and compared with that from literature.

2. Experimental

The experiments were carried out in 2 L laboratory stirred bioreactor (Fermac, Electrolab), provided with computer-controlled and recorded parameters. The bioreactor mixing system consists of one turbine impeller and three baffles. The bioreactor and impeller characteristics are given in a previous paper (Caşcaval et al., 2012).

For the anaerobic biodegradation process, the dissolved oxygen amount from medium was not controlled during the process, varying free from its initial value of 1.6 mg/L. For the aerobic process, the bioreactor was provided with the sparging system consisting of a perforated tube with 7 mm diameter, placed at 15 mm from the vessel bottom, having 4 holes with 1 mm diameter. The air volumetric flow rate was varied between 5 and 30 L/h, in order to maintain the dissolved oxygen concentration at a prescribed constant value in the domain of 1.6 - 5.9 mg/L. The rotation speed was maintained at 150 rpm.

The bioreactor contained 1 L of olive oil - water emulsion (the oil concentration was 10 mg/L emulsion). According to the previous studies on olive oil biodegradation with *Bacillus spp.* cells (Caşcaval et al., 2012), the pH and temperature have been adjusted and maintained at the optimum values, namely 8 and 40 ^oC, respectively.

In the experiments, mixture of *Bacillus spp.* has been used (*Bacillus subtilis, Bacillus megaterium, Bacillus licheniformis*, and *Bacillus ortoliquefaciens* in equal ratios). The concentration of bacteria cells was 1 g d.w./100 ml medium. The fermentation end has been considered when either the olive oil was completely consumed or its concentration remained constant for 12 h. The process evolution has been analyzed by means of the variation of total lipids, using the spectrophotometric method with triolein (Levy, 1972).

3. Results and discussion

The variation of total lipids concentration from wastewater during the anaerobic and aerobic biodegradation process with free *Bacillus spp.* cells is plotted in Fig. 1.

It can be observed that the concentration of lipids is more rapidly decreased in the aerobic process, the rate of lipids consumption being accelerated by increasing the concentration of oxygen in the medium. Therefore, for the anaerobic biodegradation, the total lipids content is reduced to 1.3 g/L after 60 h, while for aerobic process it is possible to become 0 g/L after 40 h, depending on the oxygen concentration.

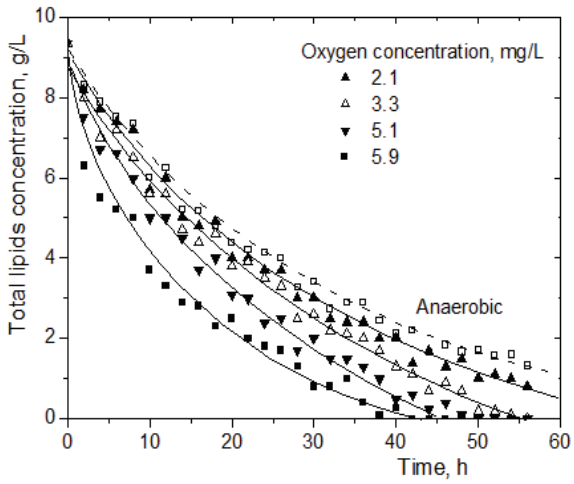

Fig. 1. Variation of total lipids concentration during their anaerobic and aerobic bacterial biodegradation

These results can be suggestively underlined by plotting in Fig. 2 the dependence between the average rate of lipids biodegradation and the value of dissolved oxygen concentration. The average rate of process is defined by (Eq. 1).

$$\bar{r}_d = \frac{C_{TL0} - C_{TL}}{t} \qquad (1)$$

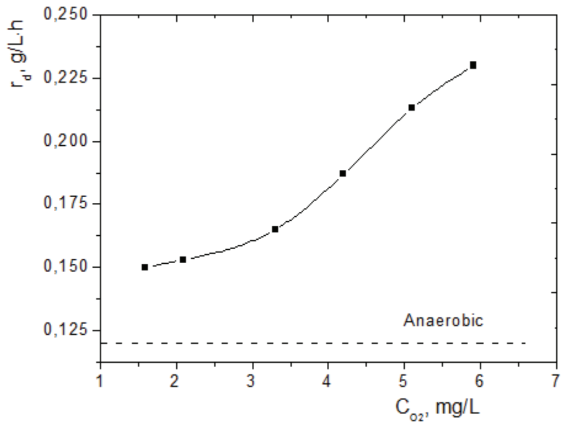

Fig. 2. Influence of oxygen concentration on average rate of lipids bacterial biodegradation

By increasing the concentration of oxygen dissolved in medium from 1.6 to 5.9 mg/L, Fig. 2 indicates that the average rate is accelerated for about 1.5 times. Compared to the anaerobic process, at 5.9 mg/L oxygen concentration the average biodegradation rate becomes for over 1.9 times higher.

The kinetic analysis of the lipids bacterial biodegradation under anaerobic conditions considers initially the model proposed by Pavlostathis and Giraldo-Gomez, 1991 (Eq. 2).

$$-\frac{dC_{TL}}{dt} = k_d \cdot C_{TL} \qquad (2)$$

For establishing the value of the specific biodegradation rate for the anaerobic process, k_d, the straight line resulted by solving Eq. (2) has been plotted in Fig. 3.

$$\ln\frac{C_{TL0}}{C_{TL}} = k_d \cdot t \qquad (3)$$

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In the case of aerobic consumption of lipids, Eq. (2) was adapted by including the modified specific rate, k_d', which is a function on oxygen concentration (Eq. 4).

$$-\frac{dC_{TL}}{dt} = k_d' \cdot C_{TL} \qquad (4)$$

Because the oxygen concentration inside the medium is controlled and maintained at a constant level during the aerobic biodegradation process, the value of kd' could be calculated at different oxygen concentrations similarly to the above presented algorithm, by plotting the corresponding straight lines (Fig. 3).

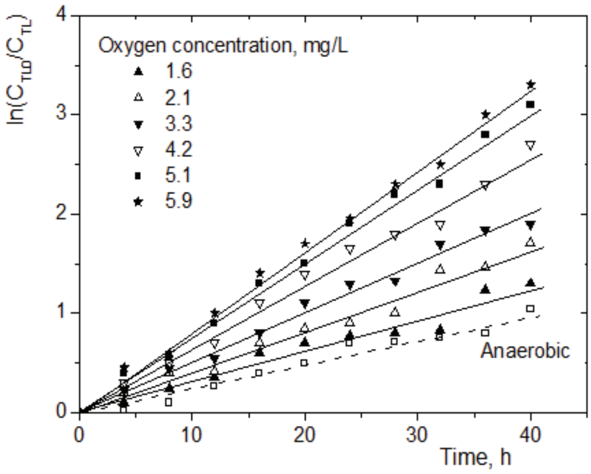

Fig. 3. Graphical calculation of specific biodegradation rate (for anaerobic process) and apparent specific biodegradation rate (for aerobic process)

The values of specific rate and modified specific rates at different concentrations of oxygen in medium are given in Table 1.

Table 1. Values of specific rate, k_d, and modified specific rate, k_d', of lipids biodegradation with cells of *Bacillus spp.*

C_{O2}, mg/L	Anaerobic	1.6	2.1	3.3	4.2	5.1	5.9
k_d, $k_d' \times 10^2$, h^{-1}	2.61	3.53	3.7	4.76	6.4	7.55	8.12

For quantifying the influence of aerobic conditions on the rate of lipids biodegradation with *Bacillus spp.*, it was analyzed the variation of the ratio k_d'/k_d with the increase of oxygen concentration. This dependence, plotted in Fig. 4, suggests the following exponential correlation (Eq. 5). The values of coefficient α and exponent β can be calculated graphically by liniarizing the Eq. (5), according to Fig. 4. Therefore, Eq. (5) becomes Eq. (6). Consequently, the aerobic degradation of lipids from olive oil using *Bacillus spp.* can be described by the kinetic model expressed by Eq. (7).

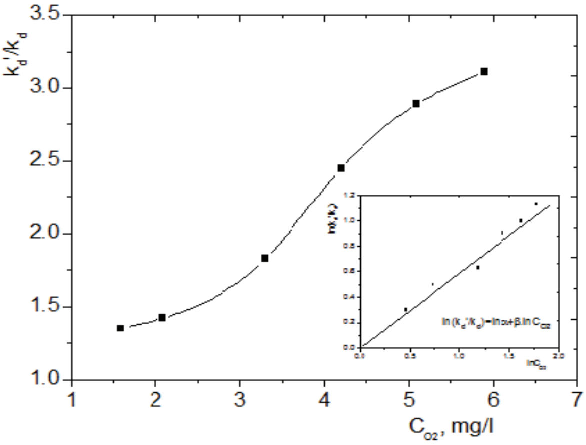

Fig. 4. Influence of oxygen concentration on ratio k_d'/k_d

$$\frac{k_d'}{k_d} = \alpha \cdot C_{O2}^{\beta} \tag{5}$$

$$\frac{k_d'}{k_d} = C_{O2}^{0.62} \tag{6}$$

$$-\frac{dC_{TL}}{dt} = k_d \cdot C_{O2}^{0.62} \cdot C_{TL} \tag{7}$$

The comparison between the experimental values of lipids biodegradation rate and those calculated with Pavlostathis and Giraldo-Gomez model and the proposed Eq. (7) is presented in Fig. 5, which indicates that the model proposed by Pavlostathis and Giraldo-Gomez is adequate for the anaerobic process, when the biodegradation occurs slowly. For the aerobic biodegradation, which corresponds to higher rate of process, the use of this kinetic model leads to important deviations from the experimental data, the calculated values being considerable lower than the real ones.

The kinetic model which considers also the dissolved oxygen concentration describes properly the aerobic biodegradation of lipids. Therefore, from Fig. 5 it can be observed the good concordance between the experimental values of aerobic process rate and those calculated by means of Eq. (7), the average deviation being ± 6.84%.

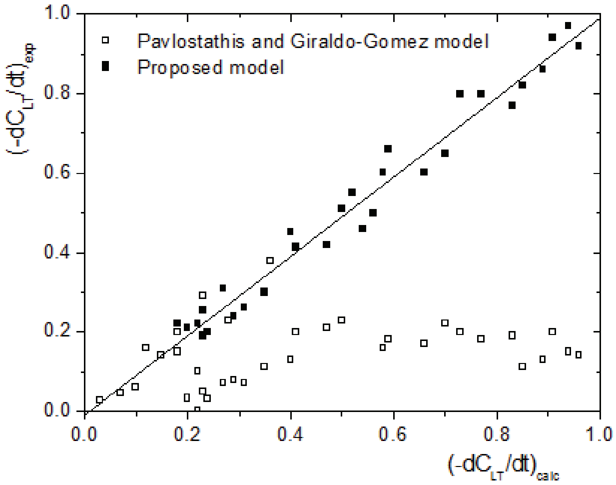

Fig. 5. Comparison between the experimental and calculated values of lipids biodegradation rate with *Bacillus spp.*

4. Conclusions

The studies on the biodegradation of lipids from olive oil mill wastewater with *Bacillus spp.* cells under anaerobic and aerobic conditions indicated that the aerobic process could be twice as fast.

Although the dissolved oxygen concentration in the medium exhibits an important influence on efficiency of biodegradation of lipids, the kinetic model proposed in literature does not include any term related to the oxygen concentration. Consequently, by means of the correlation between the modified specific rate, which includes the influence of oxygen concentration, specific rate, for the anaerobic biodegradation, and oxygen concentration, it was established a new kinetic model adequate for aerobic process.

This model offers a good concordance with the experimental results, the average deviation of the calculated values of lipids biodegradable rate from the experimental ones being ± 6.84%.

Notations

C_{O2}	dissolved oxygen concentration, mg/L
C_{TL}	total lipids concentration in wastewater, g/L
C_{TL0}	initial total lipids concentration in wastewater, g/L
k_d	specific rate of lipids biodegradation process, h^{-1}
k_d'	modified specific rate of lipids biodegradation process considering the dissolved oxygen concentration, h^{-1}
t	time, h

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