A comparative study of the biodegradability of oleic acid by sludge from a reactor fed with olive mill wastewater and sludge from a reactor fed with oleic acid

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Abstract—In the present study, we investigate the biodegradability of oleic acid by sludge from anaerobic filter reactor fed with olive mill wastewater (sludge T). A comparative study was conducted using a second sludge from an expanded granular bed reactor exposed to oleic acid (sludge P). The performance of treating oleic acid and the influence of the composition of the culture media were investigated and compared. Methane production by sludge T started after a lag phase of 150 hours. However no lag phase was observed with sludge P. In contrast a higher methane production was obtained with the sludge T. The addition of nutrients (minerals and yeast extract) to culture medium reduced the lag phase of methane production by 40 hours.

Keywords—anaerobic digestion, oleic acid, olive mill wastewater, methane

1. INTRODUCTION

Lipids (fats, oils and greases) are one of the major organic matters in wastewaters. Certain contents of lipids are widely found in both municipal and industrial wastewaters. Olive mill wastewaters are a typical example of effluents containing lipids (0.02 -1%) [1]. In the Mediterranean area, particularly with the main producers of oil (Italy, Spain, Greece and Tunisia) olive oil plays a very important part in the economy. The tremendous amount of production is accompanied with huge quantities of highly polluted effluent and, consequently, leads to environmental impact. Although there have been many treatment strategies applied, anaerobic treatment processes represent a useful and cost effective alternative for developing countries. In the anaerobic digestion lipids are easily hydrolysed to Long Chain Fatty Acids (LCFA), which are further converted to acetate and hydrogen though β-oxidation mechanism by proton reducing aceticogenic bacteria [2]. LCFA are especially problematic compounds for anaerobic wastewater treatment. They are usually indicated as the responsible for the toxicity of the olive mill wastewaters towards both acetogenic and methanogenic bacteria in addition to phenolic compounds.

In the present study, we investigate the biodegradability of oleic acid by a sludge obtained from an anaerobic filter fed with diluted olive mill wastewater. A comparative study was conducted using a second sludge acclimated with oleic acid and obtained from an expanded granular bed reactor treating synthetic wastewater containing oleic acid. The influence of the composition of the culture media on the methane production from oleic acid was tested. Sodium olate was used as a LCFA model since it is, in general, the most abundant of all LCFA present in wastewater[3] as well as one of the more toxic and has a good solubility [4].

II. RESULTS AND DISCUSSION

A. Biodegradability of Oleic Acid

The biodegradability of oleic acid was tested with two different sludges. The first one, sludge T, was from an anaerobic filter fed with diluted olive mill wastewater and operated at mesophilic conditions (37 °C). An acclimated sludge (sludge P) obtained from an anaerobic expanded granular bed reactor fed with oleic acid was used for comparison. The oleic acid biodegradability assays were conducted in serum vials. Samples from each sludge were washed and centrifuged twice with anaerobic basal medium to remove the residual substrates. After incubation at 37 °C, 150 rpm for 24 hours in 25 ml-batch vials containing sample of sludges with anaerobic culture medium, 300 mg/l of sodium olate were added. The methane production was followed by measuring the pressure developed in each vial headspace, using a hand held pressure transducer (Fig. 1) capable of measuring a pressure variation of two bars (0 to ±20.26 kPa) over an output range of -200 to +200 mV [5].
As it can be seen in Fig. 2, methane production by sludge P started without any lag phase, whereas the methane production curve showed a lag phase of 150 hours for sludge T. The maximum plateau achieved in the methane production curve was 340.6 ml CH₄/stp/gVSS and 451.3 ml CH₄/stp/gVSS obtained for sludge P and sludge T respectively.

**B. Effect of the Composition of the Culture Media on the Methane Production**

To test the effect of the composition of the culture media on the methane production two basal media were used. Medium A was prepared under strict anaerobic conditions and contained per liter of demineralised water: KH₂PO₄, 0.3 g; KH₂HPO₄, 0.3 g; KCl, 0.1 g; CaCl₂·2H₂O, 0.1 g; MgCl₂·6H₂O, 0.2 g; NaCl, 0.6 g; NH₄Cl, 1 g; yeast extract, 0.5 g; cysteine-HCl, 0.25 g; NaHCO₃, 5 g; Na₂S, 0.05 g; resazurin, 0.001 g. The pH was adjusted to 7.2 and the medium was supplemented with 1.5 ml trace element solution [6]. The medium B was made up with demineralised water, was composed of cysteine-HCl (0.5 g/l), resazurin (0.001 g/l) and sodium bicarbonate (3 g/l), the pH was adjusted to 7.2 and prepared under strict anaerobic conditions. This assay was performed with sludge P in presence of 300 mg/l.

For medium A, the methane production started without any lag phase where it took 40 hours to begin for medium B (Fig. 3). However the maximum plateau achieved in the methane production curve obtained for medium B (455.2 ml CH₄/stp/gVSS) was higher than for medium A (306.1 ml CH₄/stp/gVSS). This phenomenon could be due to the accumulation of more toxic intermediates that inhibited oleic acid degradation through β-oxidation mechanism.

The decrease of the lag phase is probably related to the presence of nutrients in culture medium like minerals, growth factors and trace elements which stimulate bacterial growth. Besides, the addition of calcium in the medium reverses the inhibition of bacterial growth in batch cultures, mainly by forming insoluble salts.

**III. CONCLUSION**

When treating an olate based effluent, the sludge that had been acclimatized with this LCFA showed a methane production without any lag phase. The long term contact with olate induced adaptation to biodegradation to biodegradation of oleic acid. The ability of sludge acclimatized with olive mill wastewater to degrade oleic acid is probably due to the richness of this effluent in lipids.

Supplementing culture medium with growth factors and minerals stimulates methane production. The presence of calcium decreases the inhibitory effect of oleic acid by formation of insoluble salts.

**REFERENCES**


