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Enzymatic hydrolysis and anaerobic biological treatment of fish industry effluent: Evaluation of the mesophilic and thermophilic conditions

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ABSTRACT

Enzymatic hydrolysis and anaerobic treatment of effluent similar to that generated in the fish processing industry were evaluated at 30 °C and 50 °C. Hydrolysis used lipase produced by fungus *Penicillium simplicissimum* in solid state fermentation with babassu cake as substrate, which has optimal activity at 50 °C. Hydrolysis kinetics was conducted with mixtures of effluent (containing 1500 mg oils and greases/L) and different lipase activities (0–0.67 U/ml of effluent), verifying that with 0.16 U/ml of effluent, 9.69 μmol/ml of free acids were produced after 4 h at 50 °C. Anaerobic biodegradation assays were conducted with effluent submitted to three different treatments: thermophilic (hydrolysis and anaerobic treatment at 50 °C), mesophilic (hydrolysis and anaerobic treatment at 30 °C) and hybrid (hydrolysis at 50 °C and anaerobic treatment at 30 °C). The best results (97.5% of chemical oxygen demand [COD] removal and 105.4 ml CH_4/g $COD_{removed}$) were obtained with the hybrid treatment in only 68 h. The thermophilic hydrolysis not only reduced the amount of enzyme and the hydrolysis time but also reduced the time and the cost of mesophilic anaerobic treatment, favoring the application of this treatment on an industrial scale.

1. Introduction

Fish industry effluents have high levels of chemical oxygen demand (COD), temperature, oil and grease, and total suspended solids (TSS). They are generally discharged into water bodies after receiving treatment that is incompatible with their degree of pollution, causing serious environmental damage [1].

Although aerobic processes are traditionally used in the treatment of industrial effluents, anaerobic processes are gaining prominence due to the advantages they bring, such as the removal of organic matter and methane generation from high-load effluents (COD > 4000 mg/L), such as those produced by the fish industry [2–4].

However, anaerobic processes are adversely affected by the high oil and grease concentrations in these effluents, which can form agglomerates or pellets in the sludge flocs, hindering sludge sedimentation and reducing the efficiency of the treatment [1,5,6].

Alternatives to the pretreatment of effluents rich in fats to reduce their oil and grease content and aid the subsequent biological treatment should therefore be investigated. One such potential alternative is enzymatic pre-hydrolysis with lipases (glycerol ester hydrolase, E.C. 3.1.1.3), enzymes that generally act in the aqueous–organic interface, catalyzing the hydrolysis of triacylglycerols [7–11].

Enzymatic pretreatment is a way of improving the activity of the microbial population in the subsequent biological treatment, since it prevents the accumulation of fats in the sludge. In addition, it permits the conversion of complex organic compounds in the form of fats and proteins, which would be discarded as problematic solid waste, into methane, which can be used as a source of energy in the same industry.

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The application of these enzymes is growing steadily because of their ability to catalyze a wide range of reactions, including the hydrolysis of oils and greases in effluents from fish industry [7]. However, for the enzymatic pre-hydrolysis step in effluent treatment to be economically feasible on an industrial scale, less costly ways of producing these enzymes, such as solid state fermentation (SSF), should be adopted.

SSF can use agroindustrial waste as a support and substrate for the growth of microorganisms that not only reduce the total cost of the process, but are also biodegradable. Fermented solids containing enzymes called solid enzyme preparations (SEP), obtained by SSF, can be used directly in the treatment of effluents, bypassing the enzyme extraction and/or recovery steps. Thus, SSF assigns values to a residue that would be discarded into the environment, transforming it into a byproduct of great interest to various industries [11,12].

This study evaluated the anaerobic biological treatment of a fish industry effluent at 30 °C and 50 °C, with and without preliminary hydrolysis at 30 °C and 50 °C, using a pool of enzymes rich in thermophilic hydrolase obtained by SSF.

2. Material and methods

2.1. Synthetic effluent

In view of the extreme variability of the composition of the industrial effluent used in a prior study [27], we chose to use a synthetic effluent whose composition was similar to the industrial one. The synthetic effluent was prepared following a methodology proposed by Rollón [1] using 160 g heads, viscera, bones, and tails of fresh sardines (*Sardinella* sp.) and 400 ml distilled water. The mixture was homogenized in a blender at the slowest speed for 1 min, then sieved (1 mm average pore size) to obtain a concentrate. An aliquot of this concentrate was removed to characterize its COD and oil and grease content, with the remainder being stored at −20 °C. At the time of use, the concentrate was diluted in distilled water to obtain the desired oil and grease concentration, and had its pH, COD, BOD_5, total Kjeldahl nitrogen, total phosphorus, oil and grease, and total solids determined.

2.2. Microorganism and propagation medium for the production of enzymes

The enzyme preparation for use in the hydrolysis was produced using a strain of *Penicillium simplicissimum* as an inoculum, selected for its capacity to produce lipases in different semi-synthetic mediums [13] and in a medium composed of babassu cake [14]. The strain was propagated at 30 °C for 7 days in a medium with the following composition (% w/v): soluble starch, 2.0; $MgSO_4.7H_2O$, 0.025; KH_2PO_4, 0.05; $(NH_4)_2SO_4$, 0.5; $CaCO_3$, 0.5; yeast extract, 0.1; olive oil, 1.0; and agar, 2.5. After 7 days of growth, spores were scraped and suspended in a sterile phosphate buffer (50 mM, pH 7.0), forming a spore suspension whose concentration was determined by counting in a Neubauer chamber.

2.3. Solid state fermentation (SSF)

The raw material used as a fermentation medium was babassu cake, a byproduct from the production of babassu oil (provided by Tocantins Babaçu S.A.), which was supplemented with 6.25% (w/w) molasses (waste product from sugar production). The cake obtained was ground in a Wiley mill to yield particles with diameters of up to 3 mm, although only particles of <1.18 mm were used in the production of the enzyme preparation. Fermentations were conducted in cylindrical tray-type reactors (15 cm height and 10 cm diameter). Each tray contained 15 g of the babassu cake forming a 1 cm-deep layer to achieve a good aeration and heat transfer between the cake and the surrounding space. The reactors were inoculated with 10^7 spores/g of dry babassu cake and incubated in a chamber with controlled temperature (30 °C) and humidity (90%) for 72 h. Part of the fermented cake was sampled to quantify protease and lipase activity, and the remainder – the solid enzyme preparation (SEP) – was stored under vacuum until use.

2.4. Submerged fermentation

Submerged fermentation was performed in 1000 ml Erlenmeyer flasks containing 240 ml of one of the two medium compositions. The synthetic medium (C:N ratio of 8.9:1) had the following composition (w/v): 1% meat peptone, 0.5% yeast extract, 0.05% $MgSO_4$, 0.05% KCl, 0.001% $FeSO_4 \cdot 7H_2O$, 1% glucose, 0.3% olive oil, diluted in 0.1 M sodium phosphate buffer at pH 7.6 [13]. The submerged fermentation with babassu cake used 2.5% (w/v) babassu cake with particle size <1.18 mm suspended in distilled water. In both fermentations, the medium was inoculated with 2×10^4 spores/ml, and the Erlenmeyer flasks were incubated in a shaker at 170 rpm and 30 °C [15]. Fermentation was monitored by measuring lipase activity, and was discontinued after 96 h. The fermented broth was filtered through Whatman filter paper (11 μm pore size) for biomass separation. An aliquot was used to determine lipase and protease activity, and the remainder (liquid enzyme preparation) was stored at 4 °C until use.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in 500 ml Erlenmeyer flasks containing 250 ml synthetic effluent with an initial concentration of around 1500 mg oil and grease/L, agitated in a shaker at 150 rpm and maintained at 50 °C (thermophilic hydrolysis) or 30 °C (mesophilic hydrolysis). Initial tests were conducted with lipase activity of 0.67 U/ml of effluent at 50 °C with and without the addition of 1% (w/v) sodium azide, an anti-microbial substance that acts as a respiratory chain uncoupler. The azide was added to prevent the growth of microorganisms and the consumption of the free acids produced so as to distinguish between the action of enzymatic catalysis and that of microbial biodegradation. The kinetics of the thermophilic hydrolysis was evaluated by determining the production of free acids over time by withdrawing 10 ml aliquots every 4 h up to 24 h. Having selected the best hydrolysis condition (at 50 °C), this was repeated without the addition of sodium azide for subsequent use in anaerobic biodegradability tests. Mesophilic hydrolysis was conducted for 8 h, as described by Alexandre et al. [7], for subsequent use in anaerobic biodegradability tests.

2.6. Anaerobic biodegradability tests

The enzymatically pretreated effluent and the crude effluent (without enzymes) were used in the anaerobic biodegradability tests. The tests were conducted in 100 ml penicillin flasks with 90 ml working volume of a mixture composed of anaerobic sludge and raw or pretreated effluent. The amount of sludge used in each biodegradability test was calculated to maintain an initial effluent COD-to-sludge volatile suspended solids (VSS) ratio of 1:1. The flasks were sealed with rubber stoppers and aluminum seals and incubated at 30 °C or 50 °C up to biogas volume stabilization.

The sludge was collected from a mesophilic upflow anaerobic sludge blanket reactor in operation at a poultry processing plant, and was adapted to the synthetic effluent simulating the effluent generated in fish industry. Sludge adaptation was conducted at