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# Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater

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#### Abstract

The biological treatment of a synthetic dairy wastewater containing high levels of oil and grease (200, 600 and 1000 mg/l) was investigated, using two identical UASB reactors. One reactor was fed with wastewater from an upstream enzymatic hydrolysis step and the other with raw wastewater. The hydrolysis was carried out at 35 °C for 14 h, using an enzyme preparation obtained through solid-state fermentation, presenting pronounced lipase activity. The reactors were continuously operated with each fat concentration. The performance of both reactors was similar up to the concentration of 600 mg/l. However, the benefits of the hydrolysis step became evident with the highest concentration (1000 mg/l). COD removals averaged 90% in the reactor fed with the hydrolyzed effluent and 82% in the control reactor. The results showed that UASB reactors are able to operate, even when fed with high levels of oil and grease in dairy wastewaters.

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Keywords: Dairy wastewater; Lipases; Enzymes; Anaerobic treatment; Enzymatic treatment; Oil and grease

#### 1. Introduction

Dairy industry is an important economic sector, but the pollution potential of this activity may be considered high, mainly when recovery of proteins, lipids and lactose is not performed. In many countries, small dairy industries contribute significantly to the national production of milk, cheese, cream and related products. These industries face difficulties to fulfill the requirements imposed by the stringent regulations for wastewater discharge and disposal. The cost of treatment systems may represent a barrier to achieve high quality wastewater standards. Thus, the search for low-cost, effective techniques may contribute to reduce the environmental impacts generated by the dairy industry.

The utilization of anaerobic treatment is widespread in food and agro industries; however, there are some concerns about its capability to assimilate variable loads of oil and grease [1]. The operational problems caused by oil and grease on up-flow anaerobic sludge blanket (UASB) reactors, such as sludge flotation and inhibitory and toxic effects of intermediate products were reported by Vidal et al. [2]. These detrimental effects of milk fat on the anaerobic treatment were also reported by Petruy and Lettinga [3]. These authors consider that the loss of process performance can be attributed to the low rate of fat hydrolysis in the anaerobic reactor.

Dharmsthiti and Kuhasuntisuk [4] have investigated the effect of addition of lipase to a biological system treating restaurant wastewater. These authors reported that the lipid content was totally removed after 48-h-incubation of the wastewater with the enzyme (lipase). However, the amount of enzyme preparation used by these authors was appreciably high. The utilization of enzymatic hydrolysis as a pre-treatment to the biological treatment of slaughterhouse wastewater was investigated by Massé et al. [5,6]. The enzymatic hydrolysis promoted a slight increase on COD removal attained in an anaerobic sequencing batch reactor. Hydrolysis of oil and grease in dairy wastewater by a solid preparation of lipase was firstly proposed by Cammarota et al. [7]. The results of that

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work showed that a pre-treatment with enzymatic hydrolysis improved the treatment of dairy wastewater in a bench-scale UASB reactor. A similar work was carried out by Leal et al. [8], using a liquid preparation of enzymes and batch operated anaerobic reactors. The highest level of oil and grease investigated by these authors was 1200 mg/l and the removal attained in reactors fed with the hydrolyzed stream was significantly higher than that reached in the control reactor without pre-treatment. Therefore, enzymatic pre-treatment seems to contribute to improve the performance of anaerobic reactors treating industrial wastewaters containing high fat concentrations.

Unfortunately, the utilization of commercial enzymes to perform the hydrolysis of fats is still very expensive. Thus, the viability of this technique is strongly dependent on the amount of enzyme necessary for the hydrolysis step and, mostly, the cost of the enzyme preparation. However, the utilization of solid-state fermentation (SSF) may contribute significantly to reduce enzyme costs. In a previous economic analysis performed by our research group [9], the SSF process provided unitary product costs 47% lower than the lipase market price. Furthermore, payback time was 1.5 years and the internal return rate was 62% for a 5-year project life. These economical data were obtained considering the production of a liquid lipase preparation (LLP). The production costs of a solid enzymatic preparation (SEP), like that used in this work, will be lower than the LLP production costs, since the final steps of micro and ultrafiltration will not be necessary.

This work investigates the utilization of solid-state fermented medium, called solid enzymatic preparation (SEP), as a source of enzymes for the hydrolytic step of dairy wastewater treatment. Hydrolyzed and raw wastewaters were fed to two identical bench-scale UASB reactors in order to evaluate the benefits promoted by the utilization of an enzymatic pre-treatment.

#### 2. Materials and methods

#### 2.1. Wastewater

The synthetic wastewater was prepared using 2 g/l of commercial powder milk in tap water plus an appropriate amount of fat from the flotation unit of a dairy industry (Lavras, MG, Brazil). The amount of fat incorporated to the wastewater varied in order to reach, approximately, 200, 600 and 1000 mg/l of fat. The incorporation of fat to the aqueous phase was performed using a mechanical impeller, which produced stable emulsions. The wastewater prepared was further sent to a conditioning stage (see Section 2.3) or to the hydrolysis treatment step.

#### 2.2. Enzymatic hydrolysis step

The enzymatic hydrolysis was carried out using solid medium from solidstate fermentation of the fungus *Penicillium restrictum*. The fermented material is called, henceforth, solid enzymatic preparation (SEP). The fermentation was conducted according to the procedure described by Leal et al. [10].

The hydrolysis was performed in stirred jacket vessels at three different temperatures (25, 35 and 45  $^{\circ}$ C) for wastewaters containing different fat contents, as indicated above. The amount of SEP was fixed at 0.1% (w/v), as recommended by Cammarota et al. [7]. The original lipase activity of the solid preparation was 21.8 U/g. The reaction lasted 24 h in the experiments performed to establish the most adequate hydrolysis conditions. The reaction progress was assessed through the determination of free fatty acids by titration (automatic titrator, Mettler, DL 21) with 0.01 M NaOH solution [8]. Determinations of oil and grease contents in the beginning and in the end of the reaction were made according to standard procedures [11].

#### 2.3. Biological treatment

Two identical bench-scale UASB reactors, made of Plexiglas (total height = 34 cm, diameter = 9 cm) and with a capacity of 1.8 l, were kept in a temperature-controlled room (35 °C) and fed with wastewaters either hydrolyzed or conditioned (Fig. 1). The former was produced after enzymatic hydrolysis carried out under selected conditions (see Section 3) in two larger stirred reactors (2 l), the latter was kept under the same conditions (14 h, 35 °C and mechanically stirred), but without enzyme (SEP) addition. The purpose was to submit both wastewaters to the same environmental conditions before feeding the UASB reactors.

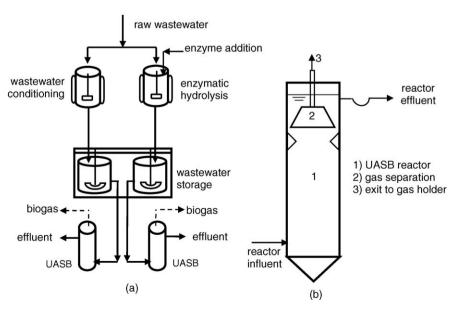


Fig. 1. Schematic representation of the treatment steps (a) and illustration of the UASB reactor (b).

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