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Enzymatic Pretreatment of Slaughterhouse Wastewater: Application of Whole Lipolytic Cells of *Rhizopus oryzae* Produced from Residual Vegetable Oil

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Abstract: This study assessed the application of whole lipolytic cells in the pretreatment of slaughterhouse wastewater to reduce its lipid content. The fungal biomass of *Rhizopus oryzae* was evaluated in the hydrolysis of slaughterhouse wastewater containing high lipid concentrations, focusing on the biomass's concentration and the effect of using an emulsifier and surfactant. The use of the whole-cells lipase of *Rhizopus oryzae* grown in a residual vegetable oil medium proved effective in the hydrolysis of slaughterhouse wastewater, generating concentrations of free fatty acids (FFA) ranging from 40.36 to 90.14 mM. The action of lipase in the hydrolysis of slaughterhouse residues indicated its effectiveness in pretreating lipid-rich liquid residues, potentially boosting the microbiota of this anaerobic treatment. The results showed that lipase activity without surfactant exhibited a similar performance to that of Triton X-100 in the hydrolysis of liquid residues. However, the combination of lipase and surfactant could represent a promising strategy to optimize free fatty acid production from slaughterhouse residues, strengthening anaerobic treatment processes and potentially enhancing the overall efficiency of waste management systems.

Keywords: whole cell; lipase; hydrolysis

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1. Introduction

The growth of the world's population, coupled with improving economies in developing countries, is rapidly driving global food demand [1]. The global supply of meat is expected to increase to meet rising demand, reaching 377 million tons by 2031, with Brazil as one of the primary leaders in the global export of poultry and beef. This growth is driven by a favorable exchange rate and an abundance of grains for feed. Brazil is predicted to maintain and expand its position as the leading exporter of poultry and beef throughout the projected period, until 2031 [2]. The growth in meat production is driving the increase in slaughterhouse waste, raising concerns about waste generation and pollution.

Slaughterhouses are places where animals are slaughtered to meet the growing demand for meat for human consumption. The waste generated in these places is mostly byproducts from animal processing [3,4]. The meat processing industry is a major consumer of fresh water (2.5 to 40 m^3 of water per metric ton of produced meat), generating large volumes of effluents. Their composition includes fats, proteins, and fibers from the slaughtering process. These residues contain contaminants, primarily blood, stomach, and intestinal mucus, along with high levels of organic products, microorganisms, and cleaning agents [5,6].

The wastewater discharged after proper treatment must comply with local regulations for the safe disposal of effluents. In the case of organically rich wastewater, and especially effluents from slaughterhouses, biological treatment is generally preferred over other treatment options like electrocoagulation, membrane separation, and advanced oxidation [7]. However, waste from dairies, slaughterhouses, and meat processing, while rich in nutrients and organic matter, also contains high levels of fats and oils, which are challenging for biological treatments. A prior separation of fats and oils is essential to optimize this treatment [8].

Anaerobic digestion is a biological treatment method employed for wastewater from slaughterhouses. It involves a community of microorganisms performing different tasks to convert organic matter into biogas. Initially, organic macromolecules (carbohydrates, lipids, proteins) undergo hydrolysis into monomers. These monomers are subsequently converted into volatile fatty acids during the acidogenesis phase. Following acidogenesis, acetogenesis occurs, where volatile fatty acids are transformed into acetic acid, carbon dioxide, and hydrogen gas (H_2). Finally, in methanogenesis, acetic acid and a portion of H_2 are converted into methane and carbon dioxide [9–11].

The primary reason for the removal of oils and fats is that an excess of these elements can accumulate on the sludge surface, reducing the transfer rates of soluble substrates to biomass and oxygen due to aerobic microorganisms. This inhibits sludge activity and the development of filamentous microorganisms, hindering sludge settlement and leading to biomass losses due to reactor overflows. Additionally, long-term challenges such as blockage occurrence and the generation of unpleasant odors caused by fats and oils in wastewater are evident [8,12,13]. Therefore, the pretreatment process is necessary to hydrolyze fats and oils, enhancing efficiency of the subsequent biological treatment of wastewater.

The inhibitory effects of lipids during the biodigestion process are also associated with interference in the electron transport chain, by compromising nutrient uptake, inhibiting specific enzymatic activities, or generating peroxidation products. Since inhibition is reversible, it is partially driven by the adsorption of lipid onto biomass. Methanogenic activity can resume once the lipids accumulated in the biomass are progressively metabolized through pretreatments [14,15].

An alternative treatment method involves utilizing a biochemical pathway, and particularly lipases, due to their clean application and low environmental impact. Lipases can be applied to a large variety of biotechnological applications, including the treatment of effluents with high fat contents. The biological anaerobic treatment of this kind of effluent has some problems, which are caused by high fat contents [16]. Thus, a pretreatment with enzymes has been proposed as an alternative or a complement to conventional biological processes. Another alternative is the use of biosurfactants that can make fats and oils more available to microbial biomasses [17].

The application of lipases in the treatment of wastewater with a high lipid content faces challenges such as high production costs, purification issues, and stability concerns under ideal conditions. Specific operational conditions and factors like a high organic load, temperature, pH, and the presence of metallic ions can limit the effectiveness of lipases in treating industrial effluents. Although enzyme immobilization may enhance their stability, its scalability for wastewater treatment is still underexplored [16]. Low-cost enzymatic products for wastewater treatment are crucial because high-cost commercial lipase products would render the process economically unfeasible. As an alternative, there is the production of whole-cell biomass from filamentous fungi with high lipolytic activity, which can be used for wastewater treatment, eliminating the need for the recovery and purification of these enzymes [18].

Given the scarcity of studies involving the application of commercial lipases and/or lipolytic cells in the pretreatment of slaughterhouse wastewater, the current study aims to produce whole cells with high hydrolytic activity and evaluate their application in the pretreatment of poultry and swine slaughterhouse wastewater (*SSW*) to hydrolyze the

present oils and fats. The fungus *Rhizopus oryzae* has been highlighted as one of the most promising fungal species in the production of whole cells with mycelium-bound lipase, as well as for its efficient application in the hydrolysis reactions of substrates containing different compositions of fatty acids. This underscores the potential of this fungus to be applied as a biocatalyst in the pretreatment of slaughterhouse wastewater.

2. Materials and Methods

2.1. Microorganism

The strain used was *Rhizopus oryzae* CCT3759, obtained from the André Tosello Tropical Research and Technology Foundation (Campinas, SP, Brazil). In order to obtain and maintain culture spores, fungal cells had been previously inoculated on Sabouraud agar medium under aseptic conditions. The cultures were incubated at 30 °C for 72 h or until they reached their highest sporulation status.

2.2. Materials

Sabouraud agar (HIMEDIA®, Kennett, MI, USA); soy peptone (HIMEDIA®, Kennett, MI, USA); magnesium sulfate heptahydrate (Vetec®, Duque de Caxias, Brazil); olive oil (Carbonell®, Córdoba, Spain); gum arabic powder (Dinâmica®, Indaiatuba, Brazil); disodium phosphate dibasic (Dinâmica®, Indaiatuba, Brazil); sodium hydroxide (Vetec®, Duque de Caxias, Brazil); ethyl alcohol; Triton™ X-100 (70% v/v) (Vetec®, Duque de Caxias, Brazil); acetone (Synth®, Diadema, Brazil); phenolphthalein (Synth®, Diadema, Brazil). Slaughterhouse wastewater (SWW) was obtained from a local slaughterhouse farm focused on poultry and pork production (Alfenas, Minas Gerais, Brazil). *SWW* samples were collected before and after, from a flotation tank located at the treatment plant itself. Slaughterhouse waste samples were then frozen and maintained at −20 °C prior to utilization.

2.3. Physicochemical Characterization of SWW

For the characterization of the raw effluent, its pH, total solids (TSs), fixed solids (TFSs), volatile solids (TVSs), free fatty acids (FFAs), acidity index, and saponification were analyzed. All analyses were conducted following procedures outlined in the Standard Methods for the Examination of Water and Wastewater [19]. Fatty acid composition of both synthetic and real effluents was determined as fatty acid methyl ester (FAME) by gas chromatography according to the American Oil Chemists' Society (AOCS) official method [20].

2.4. Enzymatic Activity

Mycelium-bound lipase activity was assessed in terms of its dry biomass concentration (g L^{-1}) and hydrolytic activity (U g^{-1}), using the method of olive oil emulsion hydrolysis. Enzyme activity (U g^{-1}) is defined as the amount of dry biomass required to release 1 µmol of free fatty acids per minute under experimental conditions (0.1 g of biomass at a 37 °C reaction temperature, 100 mM sodium phosphate buffer, and at a pH of 7.0 for a 5 min reaction) [18].

2.5. Culture Medium and Conditions

The culture medium consisted of 30 g L^{-1} of residual frying oil after vacuum filtration, 70 g L^{-1} of soy peptone, 1 g L^{-1} of $NaNO_3$, 1 g L^{-1} of KH_2PO_4, and 0.5 g L^{-1} of $MgSO_4 \cdot 7H_2O$, all previously autoclaved (121 °C for 15 min) [18]. After autoclaving, Erlenmeyer flasks were cooled and olive oil (3% w/v) was added in a sterile manner. The cultures were established in 250 mL Erlenmeyer flasks containing 100 mL of autoclaved medium and inoculated with a suspension of 1×10^6 spores at 30 °C, with orbital agitation at 180 rpm. The spore concentration was determined by counting the cells in a Neubauer chamber using an Olympus® binocular microscope (Hicksville, NY, USA). At the end of the cultivation, the produced biomass was separated from its medium by vacuum filtration,

washed with water and acetone and quantified for its hydrolytic activity [21]. Subsequently, the fungal biomass was stored at 4 °C before use.

2.6. Enzymatic Pretreatment of Slaughterhouse Wastewater in Stirred Tank Reactors

In 250 mL glass-lined reactors, 100 mL of SWW was used as substrate for a hydrolysis reaction that used 2.0 g of dry biomass. The tests were carried out at 40 °C and 600 rpm of mechanical agitation, which was carried out using a suspended agitator motor with a steel helical impeller for up to 24 h. A 50:50 (v/v) mixture of acetone and ethanol was added to the aliquots (0.5 g), which were removed periodically, and the FFA concentration was quantified by titration with 20 mM of sodium hydroxide (NaOH) solution using phenolphthalein as the indicator. The concentration of free fatty acids (FFAs) (mM) was calculated using Equation (1).

$$\text{FFA (mM)} = \frac{(V_a - V_b) * C_{NaOH} * 1000}{m} \quad (1)$$

where V_a is the volume of NaOH in the sample (mL); V_b is the volume of NaOH in the control (mL); C_{NaOH} is the molar concentration of NaOH (20 mM); and m is the sample mass (0.5 g).

Initial reaction rates were analyzed by the formation of FFAs (mM) in the initial 12 h of the reaction. Results were plotted using the software Origin Pro version 5.0 so as to obtain a linear equation for the initial hydrolysis reaction rates of the *SWW*.

2.7. Variation in Biomass Mass during the Enzymatic Pretreatment of Slaughterhouse Wastewater

Different initial masses of fungal biomass were evaluated as biocatalysts in the enzymatic pretreatment of *SWW*. The assessed masses were 0.5, 1.0, and 2.0 g of dry biomass, corresponding to an average hydrolytic activity of 100, 200, and 400 U, respectively. The produced lipase was evaluated for its ability to generate free fatty acids (FFAs) by hydrolyzing the triglycerides present in the *SWW* pretreatment.

2.8. Evaluation of pH Adjustment in the Enzymatic Pretreatment of SWW

The need for a pH adjustment was assessed to determine the influence of the natural pH of slaughterhouse wastewater on the performance of the produced lipase. The pH was adjusted using a concentrated HCl solution to reach a pH of 6.0, known as the optimal pH for the activity of the lipase bound to the mycelium of the fungus *Rhizopus oryzae* CCT3759.

2.9. Influence of Surfactants and Emulsifiers on the Enzymatic Pretreatment of SWW

The influence of a surfactant and an emulsifier was evaluated in the enzymatic pretreatment in association with lipase-catalyzed hydrolysis from *Rhizopus oryzae* biomasses. Both the surfactant and the emulsifier were assessed at a concentration of 3% (w/v), with Triton X-100 evaluated as the surfactant and arabic gum as the emulsifier. Both were applied individually and in combination.

3. Results and Discussion

3.1. Characterization of Slaughterhouse Wastewater

The meat industry utilizes significant amounts of water in its industrial processes, generating considerable volumes of effluents characterized by high organic loads and concentrations of suspended solids. The characteristics of these effluents vary depending on the type of industrial process adopted [3,5]. In this study, effluent from slaughterhouses (birds and pigs), collected from two sectors of the slaughterhouse treatment plant, was used. One sample was taken from raw slaughterhouse wastewater (*RSWW*), prior to it entering the main flotation tank, and another sample from within the flotation tank (*SWWF*). The characterization of both samples is described in Table 1.

Table 1. Physical–chemical characterization of raw slaughterhouse wastewater (*RSWW*) and slaughterhouse wastewater obtained from the flotation tank (*SWWF*).

Parameter	*RSWW*	*SWWF*
pH	6.15 ± 0.06	7.59 ± 0.35
Total solids (mg L^{-1})	18,609.17 ± 661.98	13,530 ± 1300.34
Total fixed solids (mg L^{-1})	3665.83 ± 195.26	2545.33 ± 139.91
Total volatile solids (mg L^{-1})	14,943.33 ± 698.49	10,984.67 ± 57.18
Free fatty acids (%)	0.75 ± 0.031	0.51 ± 0.023
Acidity index (mgKOH g^{-1})	1.64 ± 0.689	1.11 ± 0.051
Saponification (mgKOH g^{-1})	5.38 ± 0.39	4.32 ± 0.77
Fatty acids composition (%)		
Caprylic acid—C8:0	4.30	-
Myristic acid—C14:0	4.49	-
Palmitic acid—C16:0	30.00	30.71
Stearic acid—C18:0	0.34	-
Oleic acid—C18:1	31.30	35.92
Linoleic acid—C18:2	29.56	33.37
Average molecular weight of FFAs (g mol^{-1})	273.79	265.68

The results in Table 1 highlight that the evaluated *RSWW* presented higher concentrations of solids, as previously reported in the literature [7,22]. A lower concentration of solids is evident in the effluent collected directly from the flotation tank (*SWWF*), showing a decrease in total solids of 27.29% (from 18,609.17 to 13,530 mg L^{-1}), total fixed solids of 30.57% (from 3665.83 to 2545.33 mg L^{-1}), and total volatile solids of 26.49% (from 14,943.33 to 10,984.67 mg L^{-1}). Similarly, the measurements of the free fatty acids, acidity index, and saponification also decreased due to their treatment in the flotation tank. The primary flotation treatment system is well known for separating suspended and/or fatty particles from the effluent, directly influencing its physicochemical parameters that are related to oils and fats [23].

Regarding the distribution of fatty acids within the triacylglycerols present in the lipids of *RSWW*, the raw effluent contained saturated fatty acids that were not detected in the *SWWF*, such as caprylic acid (4.30%), myristic acid (4.49%), and stearic acid (0.34%); whereas the most concentrated saturated fatty acid, palmitic acid (30%), was also quantified in the *SWWF* with similar concentrations (30.71%). Despite the low percentage of fatty acids present in the flotation tank wastewater, higher concentrations of unsaturated fatty acids were found in this sample; 35.92% and 33.57% for oleic and linoleic acids, respectively. The higher proportions of oleic (31.30–35.92%) and palmitic (30–30.71%) acids were expected, as these acids are reported to be the most abundant in food processing effluents, such as slaughterhouse effluent [13].

3.2. The Influence of Biocatalyst Mass on the Hydrolysis of Slaughterhouse Wastewater

In order to evaluate the influence of the mass of catalytic biomass added on the hydrolysis process, that is, how much hydrolytic activity will be generated, different masses of biomass were evaluated, using 0.5, 1.0, and 2.0 g of dry mycelium. The obtained results are shown in Figure 1.

The higher hydrolytic activity provided was effective for the RSSW (Figure 1A), with a maximum FFA formation of 85.34 ± 2.59 mM. However, a better performance was observed in the system with 1.0 g of biomass, showing a profile similar to the 2.0 g system up to 10 h of operation. However, after this time, the FFA formation stabilized for 1.0 g of the biocatalyst, reaching only 68.12 ± 0.36 mM, whereas the reaction with 2.0 g of biomass increased from 60.84 to 85.34 mM.

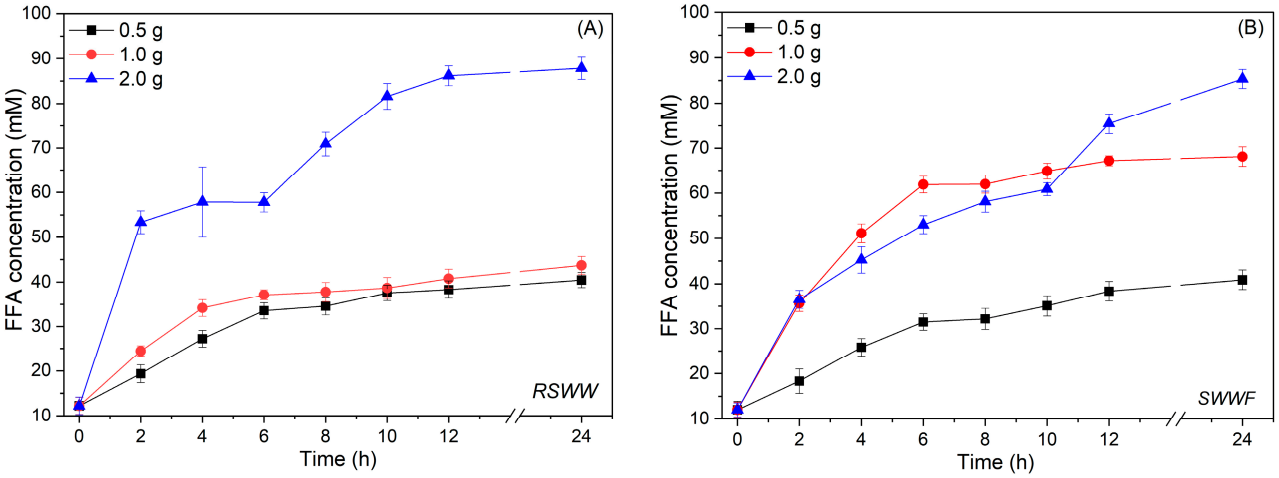

Figure 1. Evaluation of the initial mass of the biocatalyst in the formation of FFA from the hydrolysis of *RSWW* (**A**) and *SWWF* (**B**).

In the hydrolysis process of *SWWF* (Figure 1B), a higher initial supply of hydrolytic activity favored a higher formation of FFAs within the first 2 h of the reaction, with an FFA value of 53.33 ± 2.53 mM, which reached 87.85 ± 0.28 mM. For the masses 0.5 g and 1.0 g, similar hydrolysis profiles were observed, with maximum FFA formation values of 40.46 ± 0.12 mM and 43.71 ± 1.59 mM, respectively. From 8 h of reaction time onwards, the system containing 2.0 g of biomass managed to produce twice the results of the other systems (0.5 g and 1.0 g) by the end of the 24 h operation. This result was expected, due to the known influence that the mass of a biocatalyst has on the efficiency of lipase in the hydrolysis reaction of an effluent [24,25]. In a study by Valladão, Freire, and Cammarota (2007) [26], the influence of enzymatic load was also evaluated in the pretreatment of a slaughterhouse effluent using a solid enzymatic pool (SEP) for the subsequent application of an anaerobic treatment. Within the adopted conditions, it was observed that an increase in the SEP led to a higher formation of fatty acids; however, a lower SEP concentration favored organic matter removal.

3.3. Influence of the Initial pH of Slaughterhouse Wastewater on the Hydrolysis Process

A pH adjustment of the *SWW* was evaluated to determine its influence on the enzyme's performance, as the pH of the reaction medium directly affects the stability of the lipase's molecular structure and, consequently, its catalytic power and, furthermore, studies generally adjust the pH to the optimum pH for the enzyme that is to be used. The pH of the SWW was adjusted to pH 6.0, which is the optimal pH of *Rhizopus oryzae* CCT3759 whole-cell lipase [13,26,27].

Based on the results of the influence of pH shown in Figure 2, the hydrolysis profile presented by *RSWW* with an adjusted pH was slightly higher than that presented by the original pH (6.15) for up to 12 h of the reaction, but, after the first 12 h, the hydrolysis of *RSWW* without an adjustment of pH had a maximum FFA formation of 101.62 ± 0.75 mM, while the pH-adjusted system had a maximum conversion of 87.85 ± 0.48 mM. For *SWWF*, the hydrolyses without (pH 7.59) and with a pH adjustment were also similar, reaching values close to optimal FFA formation at the end of 24 h of reaction; values of 85.11 and 85.34 mM, respectively. The small differences in the hydrolysis process of the *SWW*s may have been due to the close pH values of the *SWW*s to the optimum pH of the enzyme, thus the lipase could act in a similar way in both study conditions; therefore, as the pH adjustment did not provide a considerable increase in the formation of FFAs, the other tests continued without pH adjustment.

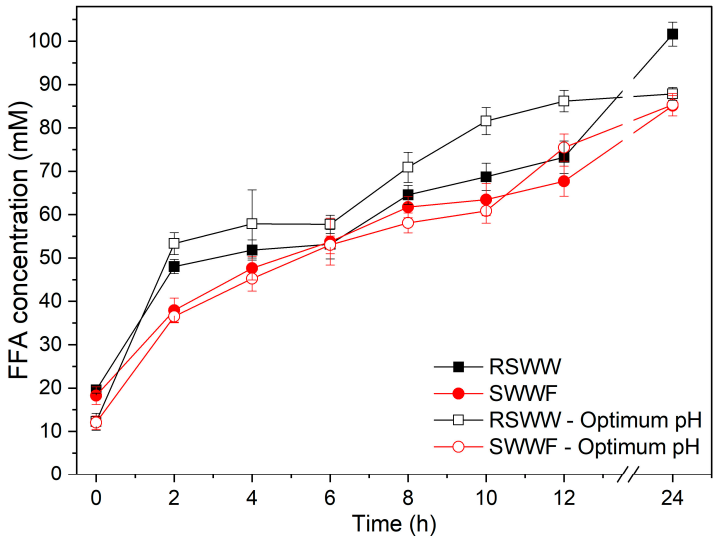

Figure 2. Influence of a pH adjustment on the enzymatic pretreatment.

3.4. Influence of Surfactants and Emulsifiers on the Enzymatic Pretreatment

Surfactants and emulsifiers can emulsify triglycerides, facilitating the action of lipases at the water/oil interface and thus accelerating the biodegradation process, eliminating the need for additional pretreatment processes to remove fats, which results in lower operating costs in the treatment of fatty effluents [28].

The surfactant Triton X-100 and the emulsifier arabic gum were evaluated in the enzymatic hydrolysis process of *RSWW* and *SWWF*. The formation of FFAs was evaluated during 24 h of reaction, as were the initial reaction rates of each system, and the results obtained are given in Figure 3 (*RSSW*) and Figure 4 (*SWWF*).

The results of the use of surfactant/emulsifier in the hydrolysis of *RSWW* (Figure 3A) show a higher production of FFAs with the use of Triton over 24 h of reaction. On the other hand, arabic gum was the system with the lowest FFA production among the systems evaluated, reaching 84.34 ± 0.50 mM. The hydrolysis generated only by the action of lipase linked to the mycelium of *Rhizopus oryzae* was superior to that achieved by arabic gum + lipase during the first 12 h of reaction, with a production of 88.97 ± 0.19 mM, which came close to the 24 h reaction value of FFA obtained by the arabic gum + lipase system, which was a maximum volume of FFAs of 90.14 ± 0.48 mM.

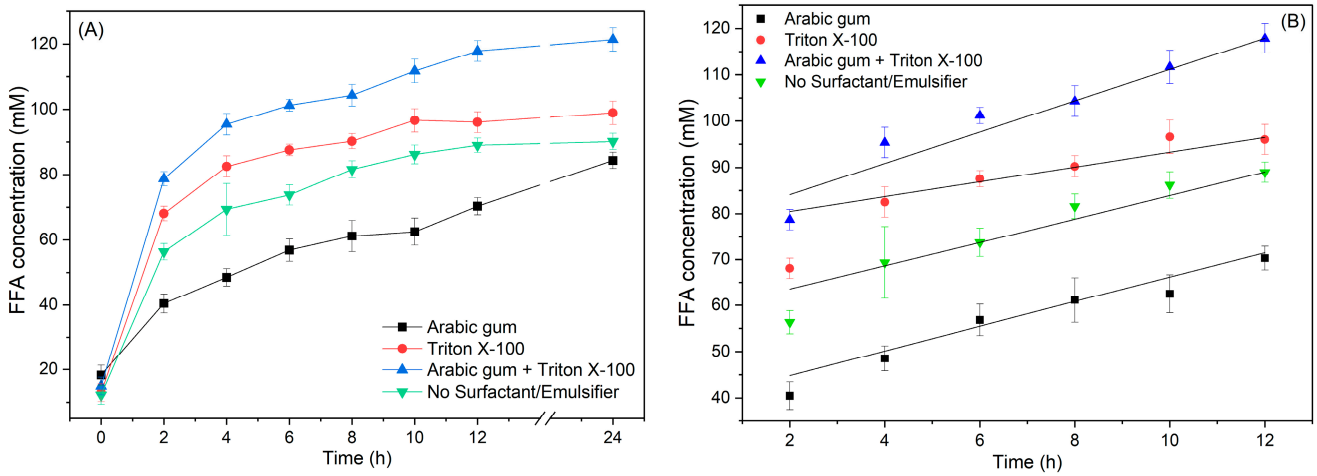

Figure 3. Influence of the use of surfactant and emulsifier on the *RSWW* pretreatment. (**A**) FFA concentration over 24 h; (**B**) FFA concentration in the first 12 h for initial hydrolysis rate analysis.

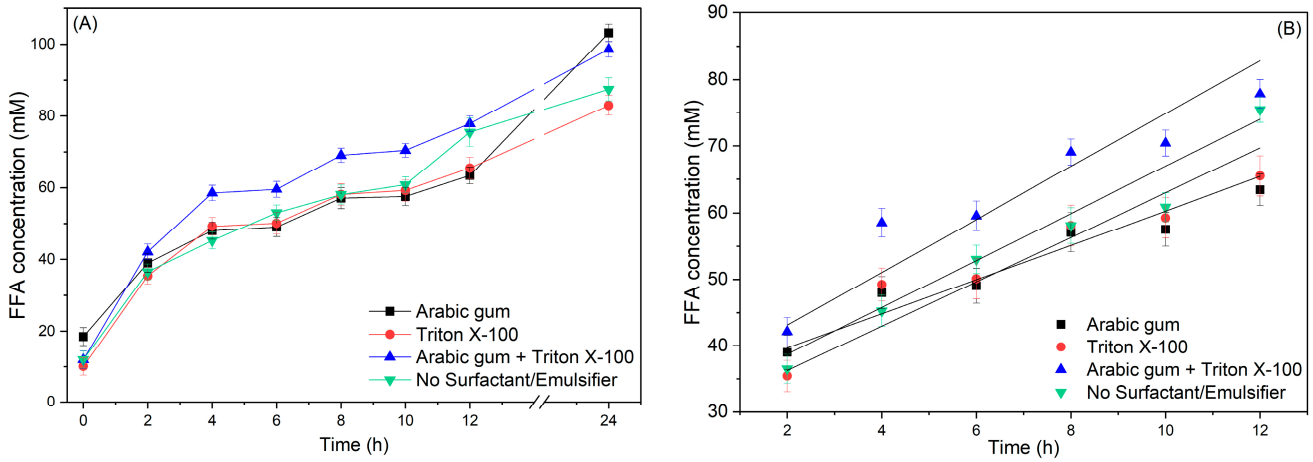

Figure 4. Influence of the use of surfactant and emulsifier on the *SWWF* pretreatment. (**A**) FFA concentration over 24 h; (**B**) FFA concentration in the first 12 h for initial hydrolysis rate analysis.

The combined action of Triton X-100 + arabic gum resulted in the highest FFA formation when compared to the use of the biosurfactant and emulsifier individually, with a maximum production of 121.38 ± 1.60 mM. This outcome may indicate that both components promoted a better stabilization of the formed emulsions, consequently enhancing the lipase-catalyzed reaction. The data also suggest that, at least within the initial 12 h of the reaction, Triton X-100 might have had a better role in stabilizing the water/lipid interface. This is supported by the fact that, when used individually, Triton X-100 led to higher FFA formation compared to the results of arabic gum alone.

The initial rates promoted in the *RSWW* (Figure 3B and Table 2) also indicate the positive effect of the combined use of Triton X-100 and arabic gum, as this showed the highest initial reaction rate (3.38 mM/h). It is worth noting that the reaction rates exhibited by the biosurfactant were the lowest obtained, with an FFA formation rate of 1.60 mM/h, possibly due to the smaller difference in FFA production over the reaction time (from 68.08 to 98.91 mM).

Table 2. Initial reaction rates of the hydrolysis of *RSWW* catalyzed by whole-cell *Rhizopus oryzae* CCT3759.

SWW—Pre-Flotator	Maximum FFA Concentration (mM)	*v* (mM/h)
Arabic gum	107.11 ± 0.50	2.66
Triton X-100	84.34 ± 1.60	1.60
Arabic gum + Triton X-100	121.38 ± 1.63	3.38
No surfactant/emulsifier	90.14 ± 0.48	2.55

When evaluating the action of the surfactant and emulsifier in the hydrolysis process of the *SWW* collected from the flotation unit (Figure 4A), a different reaction profile is observed. The systems containing Triton X-100, arabic gum, and the system without surfactant/emulsifier showed similar hydrolysis profiles during the initial 10 h of the reaction, differing only after 12 h of reaction, with a maximum FFA production of 82.95 ± 0.15, 103.17 ± 0.40, and 87.43 ± 2.59 mM, respectively. This result might be due to the lower complexity of the fatty acids in the *SWWF* (Table 1) compared to the fatty acids in the *RSWW*, which exhibited a higher proportion of various saturated fatty acids (C 8:0, C 14:0, C16:0, C18:0), while the *SWWF* presented only one type of saturated fatty acid (C 18:0). Despite showing higher FFA production values in the first 12 h of the reaction, the reaction system containing Triton X-100 and arabic gum also remained close in performance to the results obtained in the other assays.

Observing the results presented in Figure 4 and Table 3, it is evident that similar initial reaction rates were obtained for the use of Triton X-100, Triton X-100 and arabic gum, and without the use of surfactant and emulsifiers, which show initial reaction rates of 3.34, 3.38, and 3.53 mM/h. It is noteworthy that higher reaction rates were achieved in the hydrolysis of *SWWF* than in *RSWW*, which could be attributed to the difference in the fatty acid composition of both wastewater types. The *SWWF*, besides having lower proportions of saturated fatty acids, also exhibits a higher proportion of unsaturated fatty acids. Studies on the lipase linked to *Rhizopus oryzae* CCT3759's mycelium demonstrate its higher selectivity for substrates with higher proportions of oleic and linoleic acid [29].

Table 3. Initial reaction rates of the hydrolysis of *SWWF* catalyzed by whole-cell *Rhizopus oryzae* CCT3759.

SWW—Flotator	Maximum FFA Concentration (mM)	v (mM/h)
Arabic gum	103.17 ± 0.40	2.57
Triton X-100	82.95 ± 0.15	3.34
Arabic gum + Triton X-100	98.59 ± 0.15	3.38
No surfactant/emulsifier	87.43 ± 2.59	3.53

The obtained results align with studies reported in the literature. In Damasceno et al.'s [28] study, the combined use of a biosurfactant produced from *Pseudomonas aeruginosa* PA1 and an enzymatic pool produced by solid-state fermentation with *Penicillium simplicissimum*, in the anaerobic treatment of a high-fat content effluent from a poultry slaughterhouse, resulted in higher lipid hydrolysis and subsequently led to higher specific methane production in the anaerobic treatment.

In studies by Alves et al. [18]; Braz et al. [27]; and Ferreira et al. [30], integral cells of filamentous fungi were evaluated for pretreating wastewater from the dairy industry. The mycelium of *Penicillium citrinum* and the fungus *Mucor circinelloides* were used as enzymatic catalysts in a hybrid treatment process during anaerobic treatment. It is also worth highlighting that filamentous fungi have a versatile ability to grow on a wide range of substrates and are able to produce lipolytic cells from several low-cost substrates, such as residual vegetable oil. The combined use of lipolytic integral cells not only increased the biodegradability of dairy waste but also promoted methane generation during treatment. These results highlight the potential of the biomass produced to act in tandem with the biodigestion process of oil- and fat-rich wastewater, such as that from slaughterhouses.

4. Conclusions

Whole cells of *Rhizopus oryzae*, with high hydrolytic activity, were effective in the hydrolysis process of slaughterhouse wastewater, resulting in FFA formations ranging between 40.36 and 90.14 mM. Adjusting the pH of wastewater to the optimal pH for lipase activity did not lead to a higher efficiency in FFA formation, as the original pH of the wastewater is close to the optimal pH for lipase. The performance of the lipase in the hydrolysis of *RSWW* and *SWWF* indicates its effectiveness in both residues, contributing to FFA production and potentially enhancing the anaerobic treatment microbiota. However, the use of a surfactant might be more suitable when combined with lipase in *RSWW* hydrolysis to increase FFA production, since our results indicated that the lipase's performance without the addition of surfactant was similar to its performance with Triton X-100 in *SWWF* hydrolysis. In conclusion, whole cells of *Rhizopus oryzae* demonstrate significant potential for application as an enzymatic pretreatment for slaughterhouse wastewater, aiming to enhance the biodegradability of the residue during the anaerobic digestion stage. Future studies will assess the optimization of the enzymatic pretreatment's efficiency at hydrolyzing slaughterhouse wastewater during the biodigestion process, as well as evaluate the toxicity parameters of slaughterhouse wastewater before and after the enzymatic

pretreatment. The operational stability of lipase and its reaction conditions will be vital parameters to be assessed for the feasibility of applying lipolytic cells of *R. oryzae* to the enzymatic pretreatment of wastewater with high levels of oils and fats.

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