

**UNIVERSIDADE FEDERAL DA FRONTEIRA SUL
CAMPUS ERECHIM
CURSO DE ENGENHARIA AMBIENTAL E SANITÁRIA**

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**Production of Biocomposites derived from fermented *Chlorella* sp. with
Wickerhamomyces sp. UFFS-CE-3.1.2**

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Trabalho de Conclusão de Curso apresentado ao Curso de Engenharia Ambiental e Sanitária da Universidade Federal da Fronteira Sul (UFFS), como requisito para obtenção do título de **Engenheira Ambiental e Sanitarista**.

Orientadora: Prof. Dra. Helen Treichel

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Dissertação apresentada ao Programa de Pós-Graduação em Engenharia Ambiental e Sanitária da Universidade Federal da Fronteira Sul (UFFS) como requisito parcial para obtenção do grau de Engenheiro Ambiental e Sanitarista.

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A quem sonhou comigo, mesmo quando eu
duvidei.

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Este trabalho carrega um pouco de cada um de vocês.

ABSTRACT

The transition toward a circular economy and the pursuit of environmental sustainability are driving humanity to develop alternative technologies for producing various bioproducts. In this context, fermentation processes mediated by microorganisms have gained prominence. Although yeasts are well recognized for their ability to produce alcohols, they are also capable of generating a wide range of value-added byproducts. In parallel, microalgae emerge as an advantageous non-conventional feedstock, as their cultivation does not require arable land, thereby avoiding competition with food production. Addressing this demand, the present study aimed to produce biocompounds through submerged fermentation using biomass from the microalga *Chlorella* sp. Enzymatic hydrolysis was optimized through a Central Composite Rotational Design (CCRD) 2², with algal biomass and enzyme mass as independent variables. This step was followed by fermentation with the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2. The enzyme alpha amylase employed is of commercial origin, commonly used in the brewing industry, characterized by its easy accessibility and lower environmental impact compared to chemical hydrolysis methods. The results demonstrated that the combination of microalgal biomass with enzymatic preparation led to the production of several compounds of interest, such as glycerol and acetic acid. These products have broad industrial applications and market demand, supporting the potential of yeast–microalgae synergy for the sustainable production of high-value biocompounds.

Keywords: Yeast, Enzyme, Glycerol, *Chlorella* sp., Acetic Acid

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1. INTRODUCTION

Microalgae are a low-cost biomass with high potential for the practical production of environmentally relevant compounds. They can serve as energy sources and do not require arable land for cultivation; moreover, they grow rapidly and with relatively low water demands (Samoylova et al., 2024; Konwar et al., 2025). Microalgal biomass is composed primarily of carbohydrates, mainly polysaccharides such as glycogen, starch, and cellulose, which can be further processed in industry to produce target compounds such as enzymes, glycerol, and organic acids, including acetic acid (Samoylova et al., 2024; Farkas et al., 2019).

Typically, these fermentation processes are carried out using strains of *Saccharomyces cerevisiae*, characterized by rapid growth and reproduction, a short metabolic cycle with minimal byproducts, ease of cultivation and separation, and tolerance to specific concentrations of ethanol, acetic acid, and other inhibitors. However, several studies have shown that the exclusive use of *S. cerevisiae* reduces the biochemical complexity of the process, ultimately increasing the diversity of compounds and biocompounds, since its metabolism is known to produce different products (Padilla et al., 2018). Fermentations using non-*Saccharomyces* yeasts, such as *Wickerhamomyces* sp., produce a wide range of specific extracellular enzymes, including proteases, pectinases, cellulases, glycosidases, and lipolytic hydrolases, that act on relevant substrates for the production of alcohol, esters, fatty acids, terpenes, and other metabolites (Chen et al., 2021; Sun et al., 2022; Lai et al., 2023), in addition to having a significant environmental impact.

Glycerol is a simple polyol widely used in the food and pharmaceutical industries for the production of cosmetics, paints, medicines, paper, textiles, leather, and explosives (Semkiv et al., 2017). It is expected to become a key platform compound for the development of biopolymers and other high-value chemicals in the future (Pagliaro, 2017). An alternative method for glycerol production involves microbial cells converting carbohydrates or CO₂ into

glycerol. New strains of yeast, algae, and cyanobacteria, found in nature or genetically modified, can be used (Zhao et al., 2015). Under anaerobic conditions, glycerol production is consistently associated with the generation of byproducts such as ethanol, acetate, acetic acid, and acetoin, all of which have industrial value (Semkiv et al., 2020). Acetic acid is an essential organic acid, widely used in the food, pharmaceutical, and chemical industries (Solieri & Giudice, 2009). It is produced through an initial fermentation step and is prepared for use with starchy or sugary raw materials (Saichana et al., 2015). Other compounds are generated during fermentation, such as enzymes, which can be applied in various areas, including sanitation, energy production, herbicide production, and hydrolysis (Kubeneck et al., 2025).

The integration of biotechnology with microalgae biomass can significantly contribute to the development of a low-carbon circular economy, thereby promoting sustainability. Therefore, this study aimed to evaluate the production of targeted biocompounds using microalgae as a fermentation substrate in a stirred tank bioreactor (STR) with *Wickerhamomyces* sp., employing an alternative enzyme during the hydrolysis step.

2. OBJECTIVES

2.1 GENERAL OBJECTIVES

This work aims to optimize the sugar production process and evaluate the technical feasibility of expanding its production scale.

2.1 SPECIFIC OBJECTIVES

The specific objectives of this work include obtaining glycerol, acetic acid, ethanol, and the production of different enzymes.

3. MATERIALS AND METHODS

3.1 MICROALGAL BIOMASS

Commercial microalgae of the genus *Chlorella* sp. were purchased online from the Zona Cerealista store website, manufactured by the company Brasbol, and identified by lot number 10271. They were stored at -60°C until use. The moisture content was determined to standardize the residual moisture content of the biomass using a moisture analyzer. According to the supplier, the microalgae biomass contains 2 g of carbohydrates, 5 g of protein, and 1.3 g of total fat per 10 g of biomass.

3.2 PRETREATMENT AND SACCHARIFICATION

To optimize enzymatic saccharification, an experimental design was carried out with algal biomass mass and the volume of commercial alpha-amylase from the Prodooze brand (200 U/mL) as independent variables. The experimental design was performed using a Central Composite Rotational Design (CCRD) (Table 1), with the total amount of reducing sugars as the response variable..

Table 1 – CCRD for saccharification (absolute and coded values).

Assay	Mass (g)	Volume of enzyme (mL)
1	20 (-1)	2 (-1)
2	20 (-1)	7 (1)
3	40 (1)	2 (-1)
4	40 (1)	7 (1)

5	30 (0)	0,96 (-1,41)
6	30 (0)	8,04 (1,41)
7	15,86 (-1,41)	4,50 (0)
8	44,14 (1,41)	4,50 (0)
9	30 (0)	4,50 (0)
10	30 (0)	4,50 (0)
11	30 (0)	4,50 (0)

The procedure began with a physical pretreatment to release the carbohydrates present in the microalgae. The experimental biomass masses (Table 1), corrected for moisture content, were diluted to 100 mL in 0.2 M sodium phosphate buffer (pH 5.5). The solution was frozen at -20 °C for 24 h, then incubated at 4 °C for 24 h, and finally placed in a thermostatic water bath at 100 °C for 10 min, as described by Rempel et al. (2018).

Subsequently, saccharification was performed by adding α -amylase to the microalgal biomass (Table 1) and maintaining the mixture at 50 °C and 150 rpm in an orbital shaker. Samples were collected to determine available reducing sugars using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959).

3.3 MICROORGANISM AND INOCULUM

The yeast used in the fermentation was *Wickerhamomyces* sp. UFFS-CE-3.1.2, known for its ability to produce ethanol (Bazoti et al., 2017). It was maintained in YPD culture medium, composed of 1% yeast extract, 2% peptone, 2% glucose, and 2% agar. The strain was transferred to liquid YPD medium (without agar) and incubated at 30 °C for 24 hours before inoculation into the wort at 10% (v/v) (Zanivan et al., 2022)..

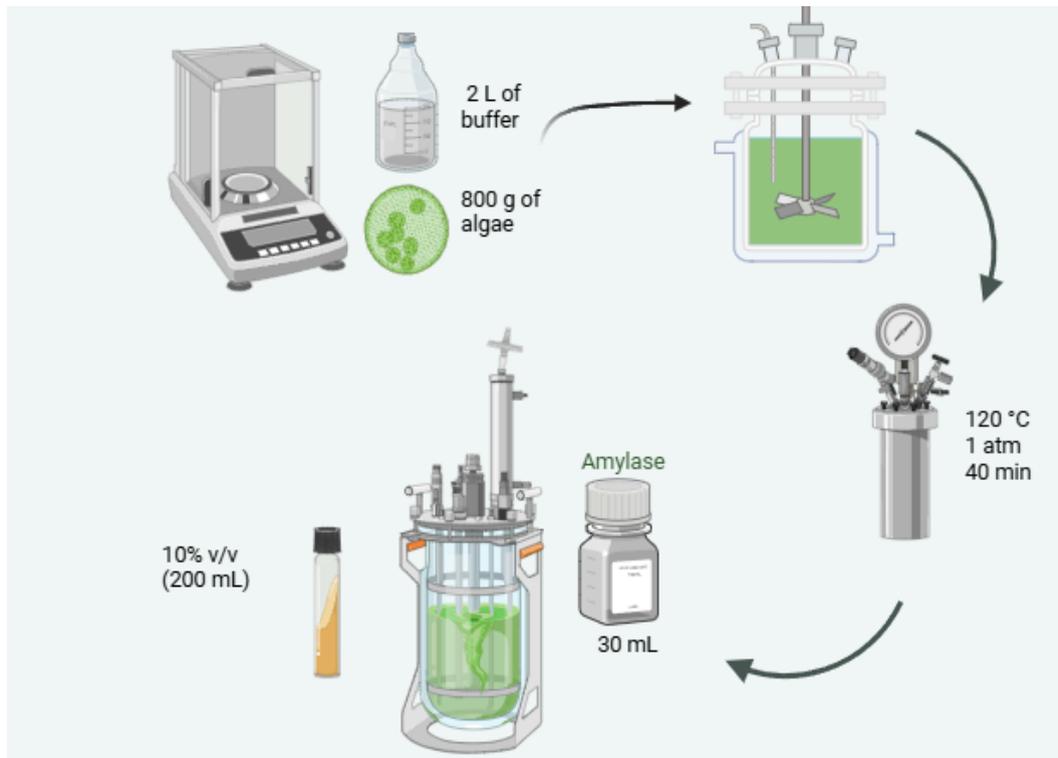
3.4 SHAKE-FLASK FERMENTATION

The wort, with a volume of 100 mL, was adjusted to pH 5.0. After inoculation with *Wickerhamomyces* sp. UFFS-CE-3.1.2, the experiments were subjected to fermentation at 30 °C and 120 rpm for 48 hours. (Bonatto et al., 2021). Aliquots were collected at 0, 12, 24, and 48 h for quantification of sugars, glycerol, carboxylic acids, and ethanol.

3.5 SCALE-UP

To scale up the process and assess the impact of volume on compound production, the experiment yielding the best results was fermented in a stirred tank reactor (STR) (BIO TEC, Tecnal) with a total volume of 7 L, as shown in Figure 1. A total of 800 g of microalgae was weighed and diluted into 2 L of 0.2 M sodium phosphate buffer (pH 5.5). The wort was autoclaved at 121 °C and 1 atm, with the dual objective of sterilizing the medium and pre-treating the biomass. Due to the high temperature, the sterilization process also served as a pretreatment step, fractionating the structural polymers to enhance the accessibility of hydrolytic enzymes to carbohydrates in the subsequent step (hydrolysis/saccharification).

Figure 1 – Scale-up of the process using a STR bioreactor.



Source: The authors (2025).

Then, 45 mL of the enzyme was added to the bioreactor to initiate saccharification. The amounts of microalgae and enzyme corresponded to the ideal conditions obtained in the shaken flask fermentation, but were scaled up to a final volume of 2 L. An additional volume of 1 L, totaling 3 L, was added to improve the homogenization of the medium and facilitate subsequent steps due to viscosity. After one hour, the yeast was inoculated at 10% (v/v) and transferred to the fermentation tank, as described by Bonatto et al. (2020). The fermentation was conducted for 72 hours at 30°C and 80 rpm. Samples were collected at 0, 12, 24, 48, and 72 hours for further analysis.

3.6 ENZYMATIC ANALYSES

To analyze and quantify the enzymes present in the reactor fermentation, the enzymes were quantified using the methodologies described by Kubeneck et al. (2025). For amylase (with starch as the substrate) and cellulase (cellulose as the standard substrate), the methodology adopted was based on the quantification of total reducing sugars, with readings taken on a spectrophotometer at 540 nm using the DNS method. The enzymatic activities of amylase and cellulase were calculated from the glucose standard curve and expressed in U/mL.

Peroxidase activity, with 8% hydrogen peroxide as the standard substrate, was determined by measuring the absorbance at 470 nm and expressed in units per milliliter (U/mL). The substrate for the laccase enzyme reaction was 2,2'-azino-di-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and U was defined as the amount of enzyme capable of forming one μmol of ABTS⁺ per minute under the reaction conditions. Protease activity, with casein as the substrate, was determined by spectrophotometry at 660 nm, and U was defined as the amount of enzyme required to release one μg of tyrosine per minute under the test conditions.

Catalase activity, using 0.0125M hydrogen peroxide as a substrate, was determined in a spectrophotometer at 240 nm for 3 minutes, with readings taken every 30 seconds, in order to measure the oxidized compounds. For ascorbate peroxidase, a wavelength of 290 nm was used, with readings taken every 15 seconds for 1 minute, using 0.008M ascorbic acid as the substrate. Finally, superoxide dismutase activity was measured under light exposure, using a 15 kW fluorescent lamp, except for the control, which was kept in the dark. Enzymatic activity was quantified at 560 nm every 1 minute for 15 seconds. Superoxide dismutase activity is determined by the amount of enzyme required to inhibit 50% of the photoreduction of NBT.

3.7 ANALYTICAL METHODS

Quantification of glucose, cellobiose, arabinose, and fructose, as well as ethanol, glycerol, citric acid, and acetic acid, was performed by High-Performance Liquid Chromatography (HPLC). Samples were diluted with 0.005 M sulfuric acid, vacuum-filtered using a Millipore® 0.45 µm membrane, and degassed in an ultrasonic bath for 15 min (Zanivan et al., 2022). The chromatographic system consisted of a Shimadzu instrument equipped with a refractive index detector (RID-10A) and an Aminex Biorad HPX-87H column. Chromatographic conditions were: mobile phase (H₂SO₄) flow rate of 0.6 mL/min and temperature of 45 °C (Bazoti et al., 2017).

3.8 STATISTICAL ANALYSIS

Statistical analysis of the CCRD 2² was performed using Protimiza Experimental Design software, with a 95% confidence level ($p < 0.05$).

4. RESULTS AND DISCUSSION

4.1 OPTIMIZATION OF ENZYMATIC HYDROLYSIS

The results of the experimental trials demonstrated the high potential of commercial amylase to convert the polysaccharides present in the biomass into sugars, as shown in Table 2, being able to catalyze the transformation of starches and other α -glucans into fermentable carbohydrates (Siqueira et al., 2020). In trial 3, with 40 g of microalgae biomass and 2 mL of enzyme, approximately 31.8 g/L of total reducing sugars (TRS) were obtained after saccharification. This condition was selected for scale-up, as it was identified as the most promising for the production of compounds of interest through fermentation in a bioreactor.

Considering the amount of sugar available and an interesting condition in relation to the enzyme concentration, this represents a matter of cost and availability. It was observed that, among the compounds analyzed, the ethanol concentration was not promising, while acetic acid reached 23 g/L in 48 hours of fermentation, which can be considered interesting, despite the cost of the product. Compounds such as citric acid and arabinose were not detected at any time.

Table 2 – Results of the enzymatic hydrolysis optimization, with responses regarding the production of total reducing sugars.

Assay	Mass (g)	Volume of enzyme (mL)	Total reducing sugars (g/L)
1	20	2	29,86
2	20	7	26,36
3	40	2	31,83
4	40	7	30,72
5	30	0,96	18,14
6	30	8,04	15,42
7	15,86	4,50	23,62
8	44,14	4,50	30,01
9	30	4,50	39,82
10	30	4,50	34,22
11	30	4,50	37,46

Trial 1 showed an attractive sugar content; however, these sugars were not consumed during fermentation, which does not indicate the formation of byproducts of this consumption, such as sugars and acids.

The optimized model was evaluated through Analysis of Variance (ANOVA), as shown in Table 3, where the calculated F value exceeded the tabulated F value, and the R² was 86%. These results indicate that the experimental data are well explained by Equation 1, where X represents the enzyme volumes and Z represents the algal biomass mass.

Table 3 – ANOVA for enzymatic saccharification

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-test
Regression	601.7	5	120.3	6.1
Residual	98.6	5	19.7	
Lack of fit	97.7	3		
Pure error	0.9	2		
Total	700.3	10		

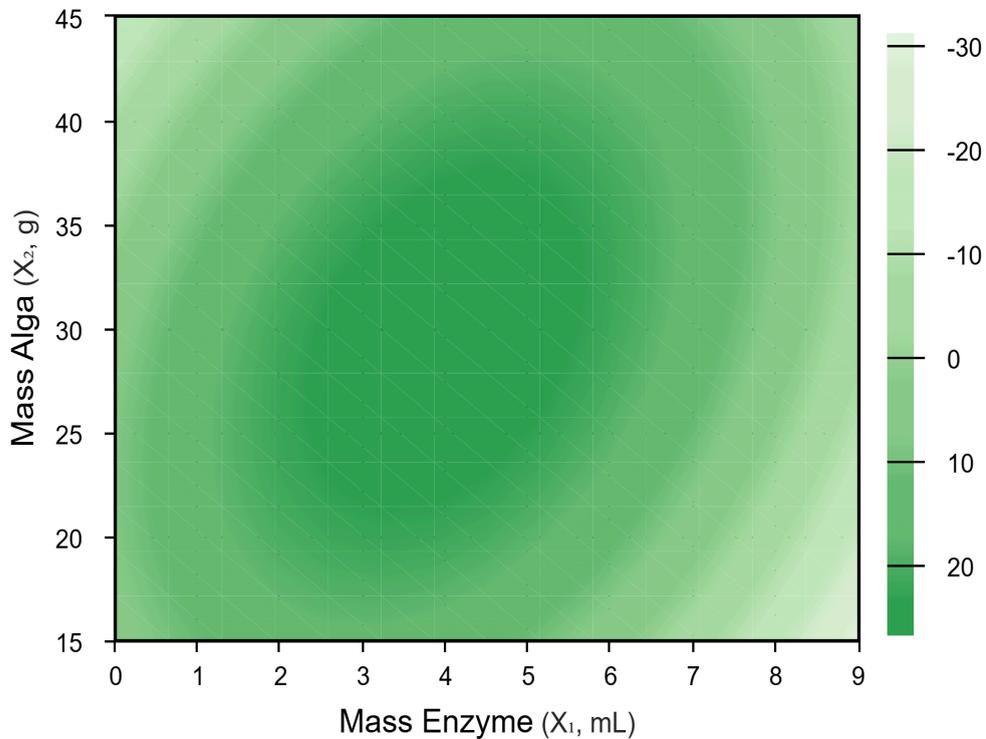
Regression Coefficient: $R = 0.85$; $F_{0,95;5;5} = 5.05$

Equation 1

$$TRS = 26.55 - 3.14 \times X - 8.16 \times X^2 + 0.41 \times Z - 5.52 \times Z^2 + 4.62 \times X \times Z$$

In the relationship between enzyme concentration and algal biomass, it was observed that intermediate amounts were favorable to the process. In this study, the enzyme used was readily available, allowing feasible concentration ranges; however, using smaller amounts of enzyme is preferable. A contour plot was constructed based on the model validation, as shown in Figure 2, highlighting the central region.

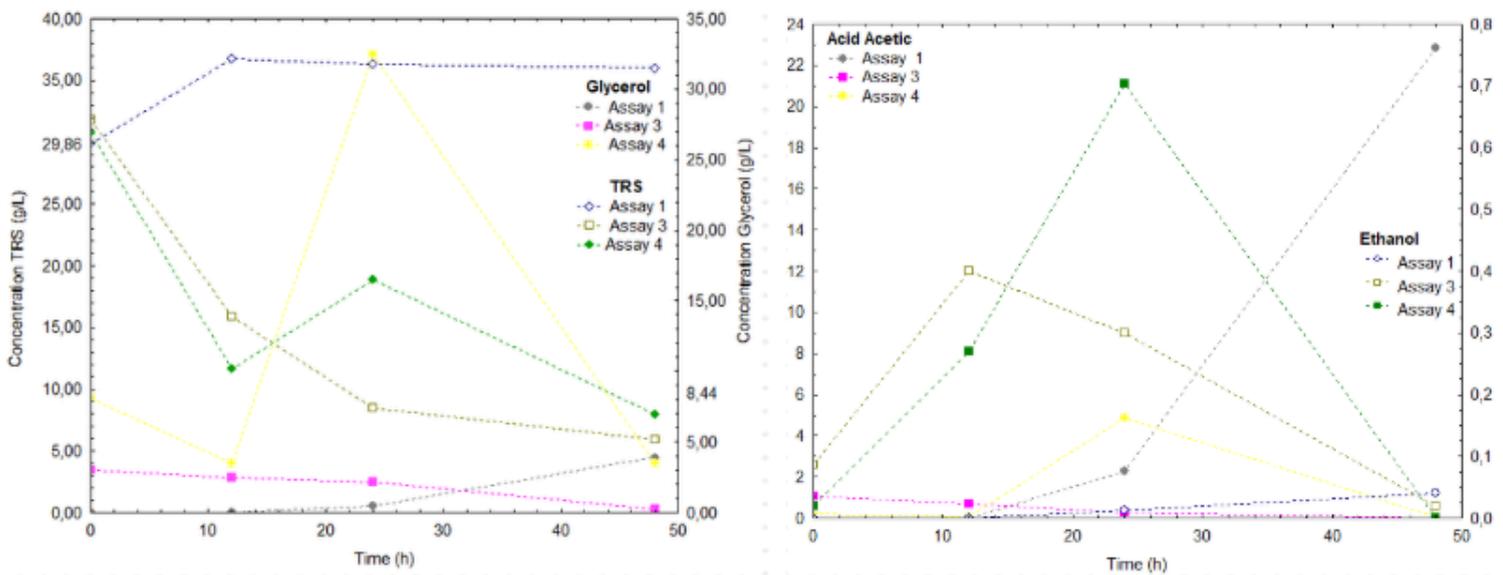
Figure 2 – Reducing sugar obtained by enzymatic hydrolysis, represented by CCRD 2² response surface.



Some trials, such as trials 5 and 6, demonstrated significant sugar consumption during fermentation, 80% and 84%, respectively. However, in trial 5, the low availability of sugars released during hydrolysis suggests a potentially lower product yield. On the other hand, trial 6, which consisted of 30 g of biomass and 8.04 mL of enzyme, achieved considerable sugar conversion; however, its enzyme volume was four times that of trial 3, making the process less economically viable.

As shown in Figure 3, trial 3 produced 2.44 g/L of glycerol and 0.67 g/L of acetic acid in 12 h, representing the best condition for total reducing sugars (TRS), as it contained 15.84 g/L at the start of fermentation. Next, trial 4 stands out for producing 32.5 g/L of glycerol in 24 h and 18.86 g/L of TRS, while trial 1 produced 22.8 g/L of acetic acid in 48 h with 36 g/L of TRS.

Figure 3 – Sugar consumption and production of different compounds during fermentation.



Studies demonstrate a relationship between high glycerol production and low ethanol yield, caused by the diversion of dihydroxyacetone phosphate from glycolysis toward glycerol-3-phosphate via the enzyme glycerol-3-phosphate dehydrogenase. This process consumes NADH and reduces the fermentative flux, thereby decreasing ethanol production. However, due to redox imbalance, acetic acid accumulates (Remize et al., 1999), one of the main fermentation inhibitors that impairs yeast growth and metabolism (Guaragnella & Bettiga, 2021; Li et al., 2022). Consequently, in experiment 1, ethanol production was hampered by the high concentration of acetic acid derived from sugar degradation (Han et al., 2024).

4.2 SCALE-UP

During scale-up, it was observed that 2 L of buffer was insufficient for fermentation, as the medium became dense, hindering reactor agitation and other operational parameters. Therefore, an additional 1 L of buffer was added before sterilization and fermentation. It is

important to note that scale-up is not necessarily linear; bioprocess scaling typically relies on empirical and semi-empirical approaches to maintain consistency in key process variables (Garcia-Ochoa & Gomez, 2009).

The fermentation results are shown in Annex A. The main values found were: 36.34 g·L⁻¹ of TRS at zero h, 2.10 g·L⁻¹ of glycerol at zero h, 1.47 g·L⁻¹ of ethanol at 24 h, and 0.68 g·L⁻¹ of acetic acid at 48 h. The ethanol concentration was lower compared to optimized fermentations with *Chlorella* sp., where values above 10 g·L⁻¹ were reported (Ngamsirisomsakul et al., 2020) and up to 20 g·L⁻¹ with efficient pretreatment and the use of *Saccharomyces cerevisiae* (Condor et al., 2022). This difference may be related to the limited availability of fermentable sugars.

Regarding acetic acid, the accumulation of 0.68 g·L⁻¹ is considered moderate. In fermentations with *S. cerevisiae* or *Zymomonas mobilis*, concentrations between 5–10 g·L⁻¹ already exert strong inhibitory effects, while under extreme conditions (7.5–15 g·L⁻¹), significant reductions in growth and sugar consumption occur. Thus, the value obtained in this study is below the critical threshold, suggesting it did not substantially compromise fermentation (Sammakarn et al., 2024; Chen et al., 2016; Zhao et al., 2008).

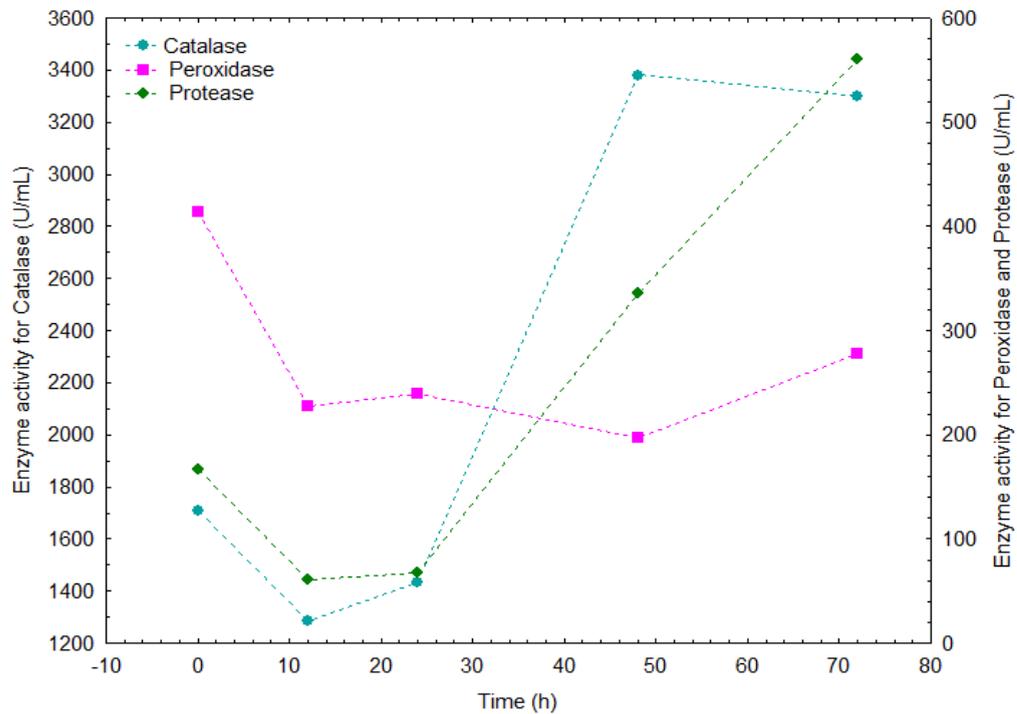
4.3 EVALUATION OF ENZYMATIC ACTIVITIES

This study successfully documented the presence of various enzymes during fermentation in a reactor using the added microorganism. The yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 has been previously used for ethanol production; however, different studies are expanding the range of products derived from its metabolism. This study highlights its potential for producing a fermented composite rich in enzymes with diverse applications. A significant increase in the production of various enzymes was observed after 24 hours of

fermentation. This is because decomposition processes drive the synthesis and secretion of enzymes (Yang et al., 2025).

The results demonstrate that the enzyme composite is rich in catalase, protease, and peroxidase, as shown in Figure 4. Crucial antioxidant enzymes, such as superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase, are vital for scavenging free radicals. Catalase is responsible for the degradation or reduction of hydrogen peroxide into water and oxygen (Gauthier et al., 2020; Ighodaro and Akinloye, 2018), reaching an activity of 3381 $\mu\text{mol}/\text{min mL}$ in 48 hours, highlighting that catalase activity was already high at the beginning of fermentation, demonstrating that the algae must have been already rich in terms of activity of this enzyme.

Figure 4 - Main enzymes with superior performance during fermentation

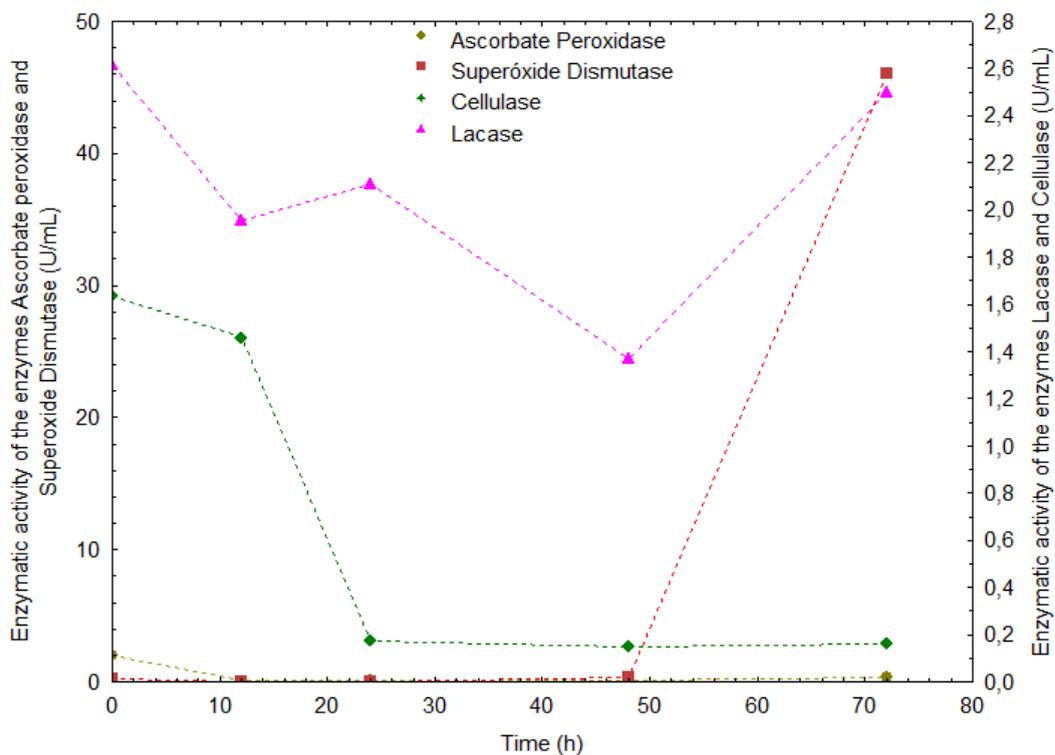


In this study, a protease production of 560.55 U/mL was achieved by the end of fermentation. Protease facilitates the hydrolysis of proteins into amino acids, making them more readily available for absorption (Egbune et al., 2023). The synergistic action of other

enzymes present in the fermentation matrix can enhance protease activity. Cofactors, coenzymes, or activators produced by plant extracts and yeast strains can facilitate protease activation and improve overall proteolytic activity (Rai et al., 2021). Different microbial species harbor proteases with distinct substrate specificities and optimal conditions, thereby increasing proteolytic activity (Abu-Khudir et al., 2019). Egbune et al. (2024) reported 39.2 U/mL of protease activity in their study. Consequently, the biomass and microorganisms used in this fermentation produced a substantially higher protease yield than in other studies.

Enzymes such as ascorbate peroxidase, cellulase, and laccase showed activities below 2 U/mL at all sampling times, indicating their absence in the biocompounds (Figure 5).

Figure 5 - Other enzymes quantified in fermentation



*Ascorbate peroxidase and superoxide dismutase refer to the left Y-axis, and the other enzymes to the right Y-axis.

The enzyme superoxide dismutase showed a significant activity level at 72 hours, with a value of 46 U/mL. Specifically, this enzyme facilitates the dismutation of the superoxide

anion (O_2^-) into O_2 and H_2O_2 , while peroxidase limits the accumulation of free radicals in plant cells, mitigating oxidative stress. Ascorbate peroxidase further detoxifies H_2O_2 , thus protecting plant cells from damage caused by oxidative stress (Egbune et al., 2022; Zhang et al., 2023).

For peroxidase, the highest activity was 277.78 U/mL at 72 hours of fermentation. This value is based on the initial amount of enzyme present in the must, which was not produced by the microorganism. This enzyme requires organic hydroperoxides or H_2O_2 as stimulating co-substrates for oxidative reactions (Demarche et al., 2012).

5. CONCLUSION

This study successfully demonstrated that the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 can be effectively used to convert microalgal biomass into a diverse range of high-value biocompounds.

Among the trials evaluated, the production of glycerol and acetic acid stood out, as both compounds are in high demand and have industrial relevance. Regarding the scale-up process, although non-linear behavior was observed compared to shaker trials, it was still possible to produce several enzymes with high activity levels, suitable for application in various fields. Therefore, this study highlights a viable and cost-effective alternative for the production of diverse compounds with broad applicability.

6. REFERENCES

ABU-KHUDIR., S. M. et al. Production, partial purification, and biochemical characterization of a thermotolerant alkaline metallo-protease from *Staphylococcus sciuri*. **Applied Biochemistry and Biotechnology**, v. 189, n. 1, p. 87-102, mar. 2019. DOI: 10.1007/s12010-019-02983-6

BAZOTI, S. F. et al. Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain. **Bioresource Technology**, v. 244, p. 582–587, 2017. DOI: 10.1016/j.biortech.2017.08.007.

BONATTO, C. et al. Experimental data and modelling of 2G ethanol production by *Wickerhamomyces sp.* UFFS-CE-3.1.2. **Renewable Energy**, v. 145, p. 2445–2450, 2020. DOI: 10.1016/j.renene.2019.08.010.

BONATTO, C. et al. Utilization of seawater and wastewater from shrimp production in the fermentation of papaya residues to ethanol. **Bioresource Technology**, v. 321, p. 124501, Feb. 2021. DOI: 10.1016/j.biortech.2020.124501.

CHEN, L. et al. Effects of simultaneous and sequential co-fermentation on microbial interactions and aroma of rice wine. **Food Science & Nutrition**, v. 9, n. 11, 2021. DOI: 10.1002/fsn3.1899.

CHEN, Y. et al. Improved acetic acid resistance in *Saccharomyces cerevisiae* by overexpression of the WHI2 gene identified through inverse metabolic engineering. **Applied and Environmental Microbiology**, v. 82, n. 7, p. 2156–2166, Jan. 2016. DOI: 10.1128/AEM.03718-15

CONDOR, B. E. et al. Bioethanol production from microalgae biomass at high-solids loadings. **Bioresource Technology**, v. 363, Art. 128002, Nov. 2022. DOI: 10.1016/j.biortech.2022.128002.

DEMARCHE, P. et al. Harnessing the power of enzymes for environmental stewardship. **Biotechnology Advances**, v. 30, n. 5, p. 933-953, 2012. DOI: 10.1016/j.biotechadv.2011.05.013

EGBUNE, E. O. et al. Characterization of a surfactant-stable α -amylase produced by solid-state fermentation of cassava (*Manihot esculenta* Crantz) tubers using *Rhizopus oligosporus*: kinetics, thermal inactivation, thermodynamics, and potential application in laundry industries. **Biocatalysis and Agricultural Biotechnology**, v. 39, p. 102290, 2022. DOI: 10.1016/j.bcab.2022.102290.

EGBUNE, E. O. et al. *Rhizopus oligosporus* alkaline protease in cassava fermentation: characterization and detergent potential. **Biocatalysis and Agricultural Biotechnology**, p. 102954, 2023. DOI: 10.1016/j.bcab.2023.102954

EGBUNE, E. O. et al. Enhancement of biochemical parameters and enzyme activity in solid-state fermented and biofortified maize cobs utilizing yeasts and plant extracts. **Bioresource Technology Reports**, 2024, p. 101874. DOI: 10.1016/j.biteb.2024.101874.

FARKAS, C. et al. Microbial saccharification of wheat bran for bioethanol fermentation. **Journal of Cleaner Production**, v. 240, p. 118269, dez. 2019. DOI: 10.1016/j.jclepro.2019.118269.

GARCIA-OCHOA, F.; GOMEZ, E. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. **Biotechnology Advances**, v. 27, n. 2, p. 153–176, mar./abr. 2009. DOI: 10.1016/j.biotechadv.2008.10.006.

GAUTHIER, M. R. et al. Microalgae under environmental stress as a source of antioxidants. **Algal Research**, v. 52, p. 102104, 2020. DOI: 10.1016/j.algal.2020.102104

GUARAGNELLA, N.; BETTIGA, M. Acetic acid stress in budding yeast: From molecular mechanisms to applications. **Yeast**, v. 38, n. 7, p. 391–400, Jul. 2021. DOI: 10.1002/yea.3651.

HAN, J. et al. A practical regeneration approach and model simulation for poisoned-resin recycle to selectively detoxify acetic acid-like bio-inhibitors in acidic lignocellulose hydrolysate. **Separation and Purification Technology**, v. 328, art. 125110, 2024. DOI: 10.1016/j.seppur.2023.125110.

IGHODARO, O. M.; Akinloye, O. A. First line defence antioxidants—superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. **Asian Journal of Medical and Biological Research**, 2017. DOI: 10.1016/j.ajme.2017.09.001

KONWAR, G.; BANIK, R.; GEED, S. R. Bioprocess strategies for bioethanol production from algae-derived carbohydrates: scalable solutions and industry prospects in a circular bioeconomy. **Chemical Engineering Journal**, v. 515, p. 163456, jul. 2025. DOI: 10.1016/j.cej.2025.163456.

KUBENECK, S. et al. Characterization of enzymatic and metabolic properties of a biocomposite based on *Trichoderma koningiopsis* and *Chlorella* biomass. **Biocatalysis and Agricultural Biotechnology**, v. 65, p. 103542, abr. 2025. DOI: 10.1016/j.bcab.2025.103542.

LAI, Y.-T. et al. Application of aroma-producing yeasts and ageing technology in Kyoho-fortified wine. **European Food Research and Technology**, v. 249, n. 11, p. 2849–2860, jul. 2023. DOI: 10.1007/s00217-023-04332-8.

LI, Y.-C. et al. Tartary buckwheat protein hydrolysates enhance the salt tolerance of the soy sauce fermentation yeast *Zygosaccharomyces rouxii*. **Food Chemistry**, v. 342, art. 128382, 2021. DOI: 10.1016/j.foodchem.2020.128382.

MILLER, G. L. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. **Analytical Chemistry**, v. 31, n. 3, p. 426–428, 1 mar. 1959. DOI:10.1021/ac60147a030.

NGAMSIRISOMSAKUL, M. et al. Enhanced bio-ethanol production from *Chlorella sp.* biomass by hydrothermal pretreatment and enzymatic hydrolysis. **Renewable Energy**, v. 141, p. 482–492, Jun. 2019. DOI: 10.1016/j.renene.2019.04.008.

PADILLA, B.; GIL, J. V.; MANZANARES, P. Challenges of the non-conventional yeast *Wickerhamomyces anomalus* in winemaking. **Fermentation**, v. 4, n. 3, p. 68, 2018. DOI: 10.3390/fermentation4030068.

PAGLIARO, M. Glycerol. **Green Chemicals and Processes**. p. 109–132. 2017. DOI: 10.1016/B978-0-12-812205-1.00005-9.

PAUL, J. S. et al. Aspects and Recent Trends in Microbial α -Amylase: a Review. **Applied Biochemistry and Biotechnology**, 2021. DOI: 10.1007/s12010-021-03546-4

RAI, K. K. et al. Unlocking pharmacological and therapeutic potential of Hyacinth Bean (*Lablab purpureus* L.): Role of OMICS based biology, biotic and abiotic elicitors. **Legumes**. IntechOpen, 2021. DOI: 10.5772/intechopen 99345

REMIZE, F. et al. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in byproduct formation and to a stimulation of fermentation rate in stationary phase. **Applied and Environmental Microbiology**, v. 65, n. 1, p. 143–149, Jan. 1999. DOI: 10.1128/AEM.65.1.143-149.

REMPEL, A. et al. *Saccharification* of *Spirulina platensis* biomass using free and immobilized amyolytic enzymes. **Bioresource Technology**, v. 263, p. 163–171, set. 2018. DOI: 10.1016/j.biortech.2018.04.114.

SAICHANA, N. et al. Acetic acid bacteria: a group of bacteria with versatile biotechnological applications. **Biotechnology Advances**, v. 33, p. 1260–1271, 2015. DOI: 10.1016/j.biotechadv.2014.12.001.

SAMAKKARN, W. et al. New biomarkers underlying acetic acid tolerance in the probiotic yeast *Saccharomyces cerevisiae* var. *boulardii*. **Applied Microbiology and Biotechnology**, v. 108, p. 153, Jan. 2024. DOI: 10.1007/s00253-023-12946-x.

SAMOYLOVA, Y. V. et al. Use of microalgae biomass to synthesize marketable products: 4. Production of biofuels from microalgae using bioengineering approaches. **Catalysis in Industry**, v. 16, n. 1, p. 89–101, Mar. 2024. DOI: 10.1134/s2070050424010069.

SEMKIV, M. et al. Biotechnology of glycerol production and conversion in yeasts. **Biotechnology of Yeasts and Filamentous Fungi**. Springer, 2017. p. 117–148. DOI: 10.1007/978-3-319-58829-2_5.

SEMKIV, M. V. 100 years later, what is new in glycerol bioproduction? **Trends in Biotechnology**, v. 38, n. 7, p. 711–714, 2020. DOI: 10.1016/j.tibtech.2020.02.001.

SOLIERI, L.; GIUDICI, P. *Vinegars of the world*. Milan: Springer, 2009. p. 1–16.

SIQUEIRA, J. G. W. et al. Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: A review. **Biomass and Bioenergy**, v. 132, p. 105419, 2020. DOI: 10.1016/j.biombioe.2019.105419.

SUN, N. et al. Assessment of chemical constitution and aroma properties of kiwi wines obtained from pure and mixed fermentation with *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. **Journal of the Science of Food and Agriculture**, v. 102, n. 1, p. 175–184, 15 jan. 2022. DOI: 10.1002/jsfa.11344.

TREICHEL, H. et al. Waste biomass pretreatment methods. In: TREICHEL, H. et al. **Utilising Biomass in Biotechnology**. Springer, 2019. p. 19–48. (Green Energy and Technology). DOI:10.1007/978-3-030-22853-8_3.

YANG, Lijuan; CHENG, Jing; ZHAO, Yingchun; GUO, Fei. Quality control and law exploration of microbial oil production from spent grains fermented by *Wickerhamomyces*

anomalus CY2. *Journal of Environmental Management*, v. 380, p. 124854, abr. 2025. DOI: 10.1016/j.jenvman.2025.124854.

ZANIVAN, J. et al. Evaluation of bioethanol production from a mixed fruit waste by *Wickerhamomyces sp.* UFFS-CE-3.1.2. **Bioenergy Research**, v. 15, n. 1, p. 175–182, 7 abr. 2021. DOI: 10.1007/s12155-021-10273-5.

ZHANG, K. et al. The regulation of key flavor of traditional fermented food by microbial metabolism: a review. **Food Chemistry**, v. 19, p. 100871, 2023. DOI: 10.1016/j.fochx.2023.100871

ZHAO, J. et al. The inhibition of *Saccharomyces cerevisiae* cells by acetic acid quantified by electrochemistry and fluorescence. **Bioelectrochemistry**, v. 72, n. 2, p. 117–121, abr. 2008. DOI: 10.1016/j.bioelechem.2007.11.015.

ZHOU, Y. et al. Study on the control effect and physiological mechanism of *Wickerhamomyces anomalus* on primary postharvest diseases of peach fruit. **International Journal of Food Microbiology**, v. 413, p. 110575, Mar. 2024. DOI: 10.1016/j.ijfoodmicro.2024.110575

ANNEX

ANNEX A - Analysis results regarding the scale-up in the reactor

Compounds (g/L)	Time (h)				
	0	12	24	48	72
TRS	36,34	15,72	15,80	15,39	27,48
Cellobiose	1,67	0,01	0,19	0,17	0,04
Glucose	4,34	1,15	4,59	2,87	1,00
Citric acid	0,00	0,00	0,00	0,16	0,01
Glycerol	2,10	0,61	1,87	0,35	0,03
Acetic acid	0,29	0,08	0,47	0,68	0,26
Ethanol	0,45	0,34	1,47	1,38	0,54