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Sustainable Co-Production of Xylanase, Cellulase, and Pectinase through Agroindustrial Residue Valorization Using Solid-State Fermentation: A Techno-Economic Assessment

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Abstract: This work describes the evaluation of the solid-state fermentation (SSF) bioprocess utilizing brewery spent grain (BSG) and apple pomace (AP) as carbon sources and matrices for microorganism growth to produce xylanase, pectinase, and cellulase. The process was assessed at a larger scale by designing a packed column-type bioreactor equipped with sensors for monitoring critical parameters such as CO_2 concentration, humidity, and temperature. Then, process simulation was used to evaluate the techno-economic feasibility of the bioprocess at an industrial scale. The analysis centered on evaluating which formulation, primarily containing xylanase (scenario 1), pectinase (scenario 2), or cellulase (scenario 3), yielded the most promising results for advancing to the commercial stage. Additionally, a sensitivity analysis was conducted to explore the influence of variations in raw material costs and enzyme prices. The obtained results at a higher scale were within the expected results obtained under optimum conditions. Scenario 1 exhibited strong economic viability with further optimization potential (base case: 5000 kg/batch with an ROI of 37.59%, payback time of 2.66 years, IRR of 26.8%, and net present value of USD 7,325,537). The sensitivity analysis revealed that changes in enzyme prices, particularly xylanase, could significantly influence the process's profitability. This study also demonstrated the potential for cost optimization by selecting a more cost-effective inoculum media and optimizing water usage to enhance process efficiency and sustainability.

Keywords: techno-economic analysis; circular economy; biowaste; solid-state fermentation

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1. Introduction

Throughout the food supply chain, a large amount of organic waste is generated. Recycling, re-use, and re-valorization of these materials are actions that contribute to a circular economy process. Biowaste is defined as those organic fractions of forestry, agricultural, livestock, or industrial origin from which some benefit can be obtained after the use for which it was acquired has been fulfilled. Some of these benefits are the generation of energy, fertilizer, livestock feed, and raw materials for bioprocesses. Its utilization is important because biowaste is considered one of the main renewable resources of the future.

Plant biowaste is generated from the primary source, such as leaves, straws, seeds, stems, and wood chips. During processing, pomace, bagasse, pomace, seeds, and husks are generated. The commercialization process also generates materials that have suffered some damage and are no longer suitable or desirable for consumption, not to mention the

biowaste of animal origin, which also generates large quantities of material such as poultry manure, leather, feathers, hair, blood, cartilage, and whey.

Of the possible uses of biowaste, one of the most versatile is its use as a raw material in bioprocesses. This cost is considered the main one, consuming more than 50% of the total bioprocess cost. The use of biowaste as a source of nutrients for microorganisms allows them to be converted into value-added compounds through an economical, renewable, and clean source. Biowaste has been used for solid, liquid, semi-solid, anaerobic, and aerobic fermentation, and for growth of bacteria, fungi, yeasts, and microalgae; this indicates that they are versatile and easily applicable materials. However, once fermentation processes are proposed and growth models, material and energy balances, production yields, and downstream processes are described, a technical–economic analysis of the bioprocess is essential to scale up production and determine the economic parameters that will indicate its viability.

Of all of the value-added biocompounds that can be obtained, this work focuses on obtaining enzymes by solid fermentation using biowaste as substrate support.

The global market for enzymes is growing every year [1], making enzyme production a prominent area in biotechnology research. Scientists search for new enzymes and applications for existing ones, as well as extremophilic microorganisms and the enzymes they produce. However, it is crucial to consider not only the production or application of the biomolecules but also the technical and economic factors involved in obtaining enzymes.

Enzymes find a wide range of applications across various industries such as food, feed, pharmaceutical, cosmetics, water treatment, and biosensors [2]. Even though the use of enzymes can increase production costs, they are still widely used due to their effectiveness. The level of enzyme purity required varies depending on the application, with medical and food applications requiring highly purified enzymes, while bioremediation or water treatment may use less purified enzyme extracts. Although several laboratory-scale uses of enzymes have been described, their feasibility at the industrial scale is yet to be determined.

Enzymes used in various industries are sourced from animals, plants, and microorganisms, with the latter being the primary source. Out of all of the microorganisms, fungi contribute 50% of the enzymes, while bacteria contribute 35%. Enzymes are predominantly produced in Western Europe, the USA, Canada, and Japan. There are only a few companies that focus on enzyme production, namely, Novozymes, DSM, BASF, and DuPont, which together supply 75% of all enzymes used worldwide [3].

The production of industrial enzymes involves both solid and submerged fermentation techniques. With the help of biotechnology and engineering, enzyme production has greatly improved using modeling, optimization, and the development of bioreactors. In terms of enzyme production, solid-state fermentation has proven to be advantageous over submerged fermentation because it has higher yields, is more feasible for fungal growth, and can use inexpensive lignocellulosic materials as a carbon source and solid matrix for microorganism growth [4].

Latin America and the Caribbean are globally acknowledged for their significant contribution to the production of agricultural raw materials and food. However, the conventional perspective toward the enormous amount of residues generated by the agro-food industry still leads to a pollution problem. There is a growing interest in the productive use of biowaste in the region, which has great potential. Countries like Mexico, with a developing economy, generate a large amount of biowaste which can be utilized as low-cost substrates for the industrial production of enzymes using solid fermenters. This could be an economic trigger for the country.

This study aimed to develop a cost-effective bioprocess for the bioproduction of xylanase, cellulase, and pectinase. To achieve this, the solid-state fermentation (SSF) bioprocess that had been previously optimized was scaled up to a bioreactor level, and BSG (brewery spent grain) and AP (apple pomace) were used as carbon sources and matrices for microorganism growth. A packed column-type bioreactor was designed for the large-scale production of enzymes using solid-state fermentation (SSF) via biowaste valorization. The

packed-bed column was equipped with sensors to measure important parameters such as the concentration of CO_2, humidity, and temperature. Finally, the economic feasibility of producing the multi-enzymatic cocktail comprising xylanase, pectinase, and cellulase through SSF using biowaste was evaluated.

2. Materials and Methods

2.1. Raw Materials and Inoculum Preparation

Apple pomace (AP) and brewery spent grain (BSG) were sourced from a cider producer and a craft brewery, respectively. BSG and AP were underwent drying at 60 °C for 48 h in a convection oven (CEB-2600, Guadalajara, México). BSG was milled using a cutting mill (Retsch SM 100, Burladingen, Germany), and AP was provided already milled. The microorganism selected for solid-state fermentation was *Aspergillus* sp.; for inoculum preparation, a spore solution of 50 µL (1×10^6 spores mL^{-1}) was inoculated into the center of a Petri dish with Sabouroud dextrose agar (SDA) and incubated at 30 °C for 5 days. The inoculum consisted of mycelial plugs with 0.5 cm diameter.

2.2. Packed-Bed Column Bioreactor Design

A packed column-type bioreactor was designed for the large-scale production of enzymes through biowaste valorization using solid-state fermentation. The bioreactor is composed of a cylindrical glass vessel with a 500 mL capacity, equipped with various inlets and ports for instrumentation, as illustrated in Figure 1. To maintain the desired moisture level within the column, the bioreactor is connected to an air humidifier that supplies air with a relative humidity ranging from 95% to 100% at the bottom of the column. At the top, the bioreactor is fitted with a gas outlet, which is linked to a collector and a CO_2 sensor for monitoring purposes. Additionally, the bioreactor features three ports on the side of the column for sensor instrumentation, such as humidity and temperature measurements.

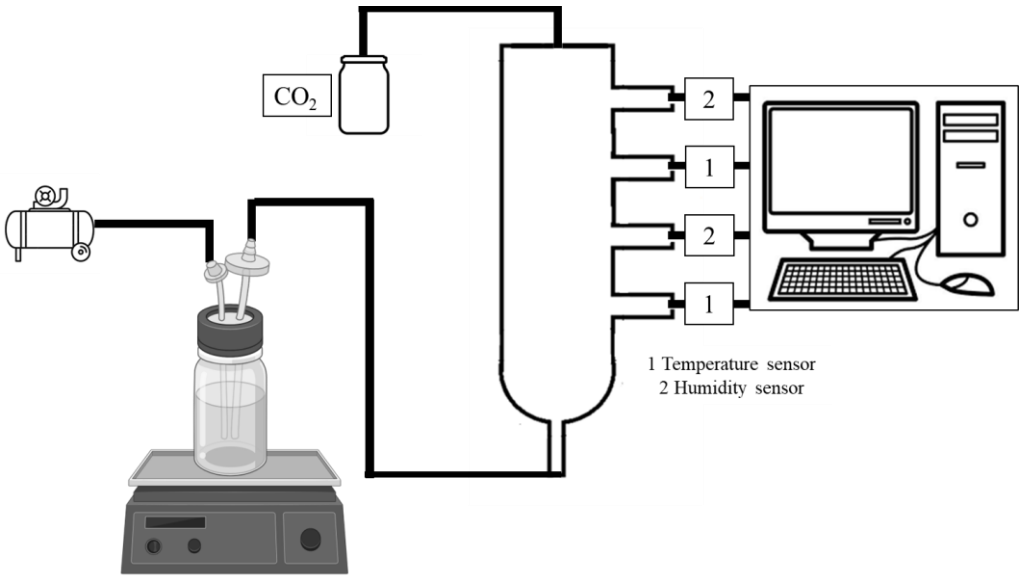


Figure 1. Packed-bed column for solid-state fermentation.

2.3. Packed-Bed Column Bioreactor Instrumentation

As mentioned, the bioreactor is equipped with sensors to measure the concentration of CO_2 at the gas outlet, as well as to monitor humidity and temperature within the bioreactor. A calibrated CO_2 sensor, as shown in Figure 2a, was employed. This sensor operates on a 5V power supply and generates an analog output voltage that corresponds to the CO_2 concentration. The specific relationship between CO_2 levels and the analog voltage output was detailed in the manufacturer's datasheet.

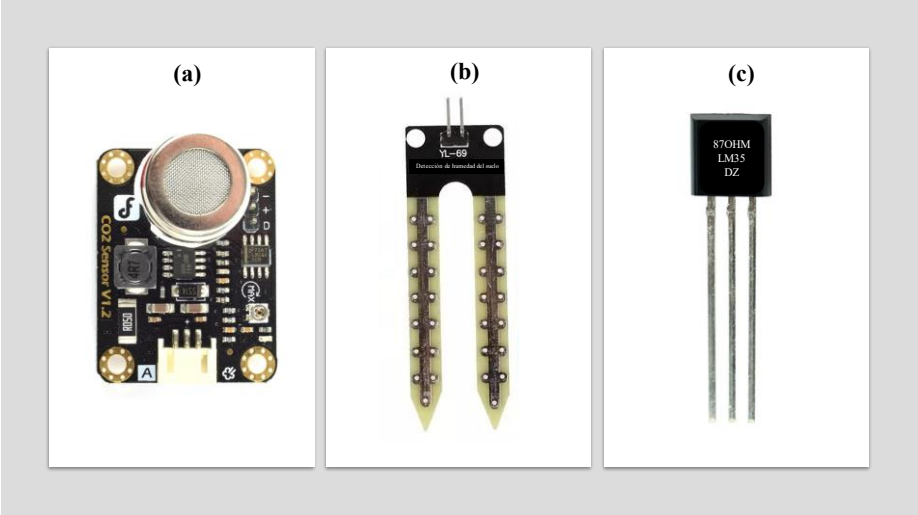


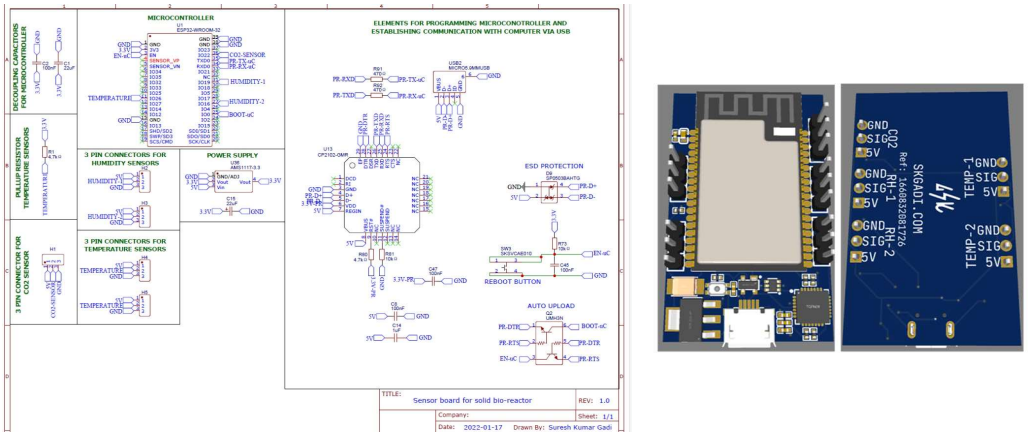
Figure 2. Sensors used to monitor (**a**) CO_2, (**b**) humidity, and (**c**) temperature in packed-bed column bioreactor.

For monitoring humidity levels within the bioreactor, a soil moisture sensor was employed, as illustrated in Figure 2b. This sensor operates on a 5V power supply and delivers an analog output voltage that varies inversely with the moisture content of the solid bed. To calibrate the sensor, it was positioned within a solid bed with a precisely known humidity level, and the analog voltage signal at the output was recorded. This procedure was replicated across a range of known humidity levels to establish a calibration curve, mapping humidity against voltage.

For temperature monitoring within the packed-bed column, an encapsulated temperature sensor DS18B20 was employed (Figure 2c). The sensor is capable of measuring temperatures in the range from −55 °C to +125 °C with an accuracy of 0.5 °C. It receives a 5V power supply to measure the temperature on demand. Communication with the sensor is carried out through a single yellow wire which should be pulled high with a 4.7 kΩ resistor. Each sensor is uniquely identified by a manufacturer-assigned identification number, allowing for the connection of multiple sensors to the same pin of a microcontroller. In this study, two such sensors were utilized.

A printed circuit board (PCB) was designed using EasyEDA to facilitate the connection of all five sensors to the microcontroller. An ESP32 microcontroller was employed as the data logger, and communication between the ESP32 and a computer was established using the CP2102 chip. The CP2102 chip serves as a universal serial bus (USB) bridge, bridging the USB communication device class (CDC) of the computer to the Universal Asynchronous Receiver/Transmitter (UART) of the ESP32.

The schematics and 3D model of the PCB are illustrated in Figure 3. For each sensor, three pins are provided, and a USB-MICRO port is included to connect a USB cable to enable communication with the computer. The CO_2 and humidity sensors are linked to dedicated pins on the microcontroller to read the sensors' output analog voltage. Meanwhile, both temperature sensors are connected to the same port, with a 4.7 kΩ pull-up resistor. Additionally, electrostatic discharge (ESD) protection has been incorporated for the USB port.



(**a**) (**b**)

Figure 3. Schematics showing the designed printed circuit board (**a**) and 3D model (**b**).

The microcontroller was programmed to periodically retrieve data from the sensors every 5 min, format it into a human-readable American Standard Code for Information Interchange (ASCII) package, and transmit it to the computer via USB-CDC. Two special ASCII codes \r and \n are added to indicate the end of the package.

Within each package, data from the five sensors are presented with commas as separators. The logging of sensor data is carried out on a personal computer using serial terminal software called Termite v3.4. Termite facilitates the establishment of communication with USB-CDC. It is configured to save the received data in a comma-separated values (CSV) format. This CSV file can be subsequently opened and analyzed using various software applications such as Python, MATLAB, and Microsoft Excel.

2.4. Cultivation Conditions in a Packed-Bed Column

Optimized process conditions for the simultaneous production of xylanase, pectinase, and cellulase by *Aspergillus* sp. were validated in the bioreactor previously described [5]. The selected conditions were those that enhanced the synthesis of xylanase.

The column was packed with a mixture of biowaste (AP and BSG) at a ratio of 81/19. The working volume was maintained at a maximum of 90% of the total volume capacity. Initially, a humidity level of 50% was set by adding Czapeck-Dox mineral medium with a pH of 4.5 ± 0.1 to the biowaste. The column was inoculated with 3 mycelial discs for every 10 g of residue from the previously incubated inoculum, which had been cultivated on SDA at 30 °C for 5 days.

After 100 h, the crude extract was recovered. Recovery of the enzymatic extracts and quantification of the enzyme activities followed established methodologies [6]. The recovery process involved mixing distilled water with the fermented biowaste samples at a ratio of 5:1, followed by agitation at 200 rpm for 60 min. Subsequently, samples were centrifuged at 8000 rpm for 15 min, followed by filtration (Whatman 1 filter paper).

Carbohydrase enzymatic activities (U mL^{-1}) were assessed by quantifying the release of reducing sugars from pectin, xylan, and cellulose substrates utilizing the enzymatic extracts. Briefly, the crude extract (200 µL) was combined with each substrate (200 µL, 1%) comprising pectin (citrus peel pectin, Sigma Aldrich, St. Louis, MO, USA), xylan (beechwood xylan, TCI American), and cellulose (crystalline cellulose, Sigma Aldrich) for the assessment of pectinase, xylanase, and cellulase activities, respectively.

Samples were incubated at 50 °C for 30 min and then immediately transferred to a cold bath to end the reaction. The released reducing sugars were quantified by the 3,5-dinitrosalicylic acid (DNS) method [7]. For each enzymatic activity, one unit was defined as the quantity of enzyme needed to yield one µmol of galacturonic acid, xylose, or glucose for pectinase, xylanase, and cellulase, respectively.

2.5. Simulation Description

The techno-economic feasibility of industrial-scale production of a multi-enzymatic cocktail comprising xylanase, pectinase, and cellulase through solid fermentation using biowaste was evaluated using SuperPro Designer 10.3® (Intelligent Inc., Scotch Plains, NJ, USA).

For this analysis, the facility's location was assumed in northern México. The construction and study year was set to 2023, with a project lifespan of 15 years. The plant was anticipated to operate at full capacity for 30 months, following a startup period of 4 months. The operational mode was configured as batch operation, with 330 days of operation per year. This schedule considered 35 days each year for maintenance and quality control procedures within the plant. SuperPro Designer was operated in design mode, whereby process scale is established through the input of raw materials.

2.6. Process Description

This study involves the comparison of three distinct scenarios for the co-production of an enzymatic complex consisting of xylanase, pectinase, and cellulase through solid fermentation using biowaste. The optimal conditions, as previously determined [5].(, were compared for each enzyme's enhancement:

- Scenario I. ECEX (U mL^{-1}): X = 582.39, P = 22.86, and C = 26.10. Optimum conditions to enhance xylanase activity, using a BSG/AP ratio of 81/19 and an initial humidity of 50%.
- Scenario II. ECEP (U mL^{-1}): X = 135.46, P = 61.73, and C = 10.54. Optimum conditions to enhance pectinase, using a BSG/AP ratio of 72/28 and an initial humidity of 66%.
- Scenario III. ECEC (U mL^{-1}): X = 0, P = 1.01, and C = 69.90. Optimum conditions to enhance cellulase, using a BSG/AP ratio of 88/12 and an initial humidity of 66%.

The three scenarios were simulated at an industrial scale, considering a base production scale utilizing 5000 kg of biowaste, which combines BSG and AP.

The overall process flow diagram is depicted in Figure 4, highlighting four key process stages: (i) inoculum preparation, (ii) raw material pre-treatment, (iii) solid-state fermentation, and (iv) product recovery.

Raw material compositions, product yields, mass transfer data, equipment, and technical parameters such as timing, temperature, and mass flows were sourced from the experimental data. As for the recovery and concentration of enzymatic extracts in stage iv, the simulation incorporated data from the experimental section and relevant literature [8].

The batch process begins with the inoculum preparation (P1/IF1) in a solid culture using SDA medium, and the fermentation lasts 8 days (total time). Raw material pre-treatment also begins at the batch's outset, with BSG and AP being separately dried using a tray dryer (P5/TD1 and P8/TD2, respectively). Both dryers have an evaporation rate of 0.5 kg/m^2h and a relative sweeping gas (air) per mass of evaporating cake components of 1.0 gas/evaporated. After drying, BSG undergoes grinding (P6/G1) and subsequent storage (P7/S1), while AP is solely stored (P9/S2), as it is supplied pre-milled by the supplier. AP and BSG are blended in varying ratios within a drum, depending on the simulated scenario (P10/83).

Before the solid-state fermentation stage, the mineral medium is prepared (P2/MB1) and sterilized (P3/HS1). Once cooled, the mineral medium is mixed with the inoculum (P4/M1). Subsequently, the packed-bed column is filled with the biowaste (BSG/AP), followed by the addition of the mineral medium and inoculum. The process conditions align with those optimized in previous experiments [5] to maximize either xylanase, pectinase, or cellulase. The stoichiometry balance considers mass coefficients.

The enzymatic extract is recovered through solid/liquid extraction (P15/L1) using water at a solid/liquid ratio of 1:3, and ideal conditions with a 98% total recovery yield in the liquid phase are considered. The crude extract undergoes further centrifugation (P16/C1) and filtration (P17/DEF1) to eliminate any remaining solid residues and fine particles. Finally, the extract is concentrated using a membrane diafiltration unit (P18/DF1). Distilled sterile water is added to facilitate the removal of membrane-permeating species, and product denaturation of 5% is considered.

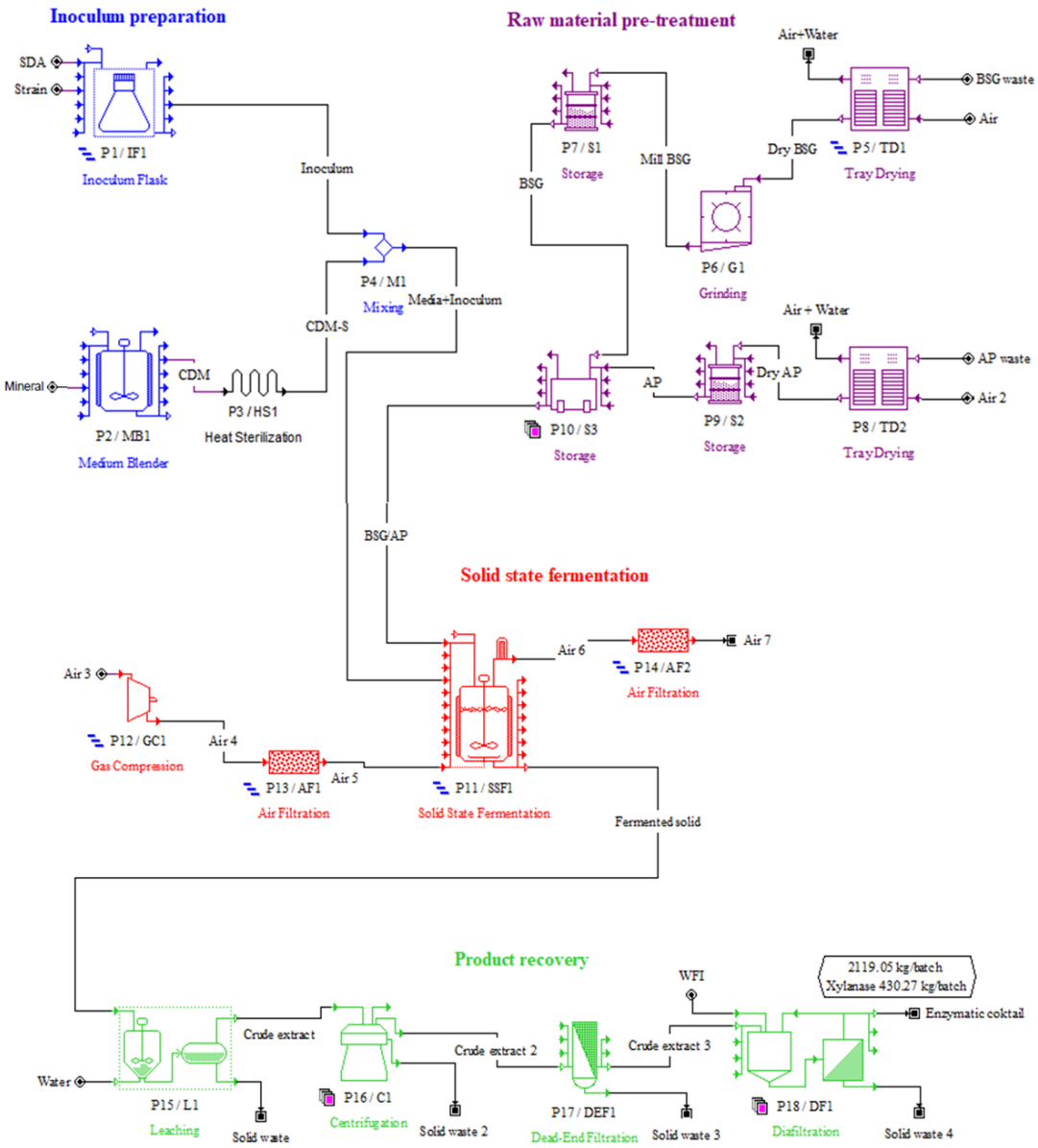


Figure 4. Process flow diagram of base scenario for production of multi-enzymatic extract.

2.7. Techno-Economic Assessment

The economic evaluation involved calculating the total capital investment and annual operating costs. To assess the project's financial viability and potential returns, several key indicators such as gross margin (%), return on investment (ROI, %), payback time (in years), internal rate of return (IRR, %), and net present value (NPV, USD) were considered. These metrics offer a comprehensive analysis of the project's profitability.

The base price for BSG and AP were 28 and 100 USD/ton, respectively. Such prices were provided by local suppliers. Regarding the selling price of the enzymatic extracts, the cost of commercially available enzymes can vary significantly, and there is not a universally agreed-upon selling price [9]. However, for the purposes of this analysis, we assigned market-competitive selling prices based on the individual enzymatic activities of the extract. The selected selling prices were determined by the specific enzymatic activity units and were set at USD 12 per 1000 U for xylanase, USD 15 per 1000 U for pectinase, and USD 55 per 1000 U for cellulase.

Total capital investment encompasses the fixed costs linked to the project, encompassing fixed capital (comprising plant equipment and facilities), working capital to cover

wages and raw material expenses, and other items that necessitate direct payment. The software calculates the direct fixed capital investment by relying on the total equipment purchase cost, employing the following equation:

$$\text{Cost} = C_0 \, (Q/Q_0)^a \quad (1)$$

C_0 represents the base cost, Q denotes the capacity, Q_0 signifies the base capacity, and 'a' is a fitting parameter. The prices and base capacity values were derived from data gathered through the Alibaba platform (URL: www.alibaba.com) and local suppliers. The parameter 'a' was determined through non-linear regression (Excel, Microsoft v2401).

The capital investment allocated for raw materials, working capital, and other expenditures was calculated to provide coverage for a period of 30 days. As for the operating costs, these encompassed expenses associated with raw materials, facility maintenance, and labor, and also factored in additional costs such as laboratory expenses, consumables, utilities, disposal costs, and miscellaneous expenses. The costs associated with raw materials were also determined based on data sourced from www.alibaba.com and local suppliers.

2.8. Sensitivity Analysis

The sensitivity analyses were conducted to assess the impact of varying raw material costs (inoculum medium and biowaste) and selling prices on the process's profitability across different batch sizes (measured in kilograms of biowaste per batch). The modified variables and their corresponding tested values were as follows:

- BSG cost: USD 19, USD 28, and USD 36 per ton.
- AP cost: USD 70, USD 100, and USD 130 per ton.
- Inoculum media cost: USD 5, USD 30, and USD 60 per kilogram.
- Selling price of cellulase: USD 20, USD 45, USD 55, and USD 80 per 1000 units.
- Selling price of pectinase: USD 11, USD 15, and USD 38 per 1000 units.
- Selling price of xylanase: USD 10, USD 12, USD 24, and USD 30 per 1000 units.

These variations were tested to assess how changes in these factors would impact the overall profitability of the process under different batch sizes. For the biowaste, the price limits considered a ±30% from the base price. Meanwhile, for the inoculum media cost, the prices varied considering other medium commonly used for fungi propagation such as potato dextrose agar (PDA) and malt extract agar (MEA). Regarding the prices of the enzymatic extracts, the variables' limits were based on various suppliers. The variables were simulated one at a time by setting the others at the baseline levels.

3. Results

3.1. Hydrolytic Enzyme Production in a Packed-Bed Column

The crude extract obtained had an enzymatic activity of X = 593.05 ± 5.58, P = 29.72 ± 6.60, and C = 28.70 ± 5.37. Additionally, Figure 5 displays the values recorded by the CO_2, humidity, and temperature sensors.

The data presented in Figure 5 provide crucial insights into the bioreactor system's performance. The temperature values were found to be consistent at both the inlet and outlet of the bioreactor, indicating that the air flow was efficiently eliminating heat buildup. On the other hand, while the output sensor's humidity levels remained stable, the input humidity levels varied.

The moisture retention characteristics of materials are intricately linked to their compositional attributes. Certain substrates exhibit enhanced moisture retention owing to the presence of non-cellulosic sugars. In the specific context of the examined materials, AP displayed a composition of 10% hemicellulose and 13% cellulose, with an absence of lignin [5]. Notably, this bioresidue comprised 65% carbohydrates, predominantly consisting of 80% glucose, 18% mannose, and 1% xylose. In contrast, BSG exhibited a composition comprising 19% hemicellulose, 5% cellulose, and 3% lignin. The carbohydrate content of

BSG was measured at 29%, with quantifiable amounts of 83% glucose, 11% mannose, 3% xylose, and 1% galactose [5].

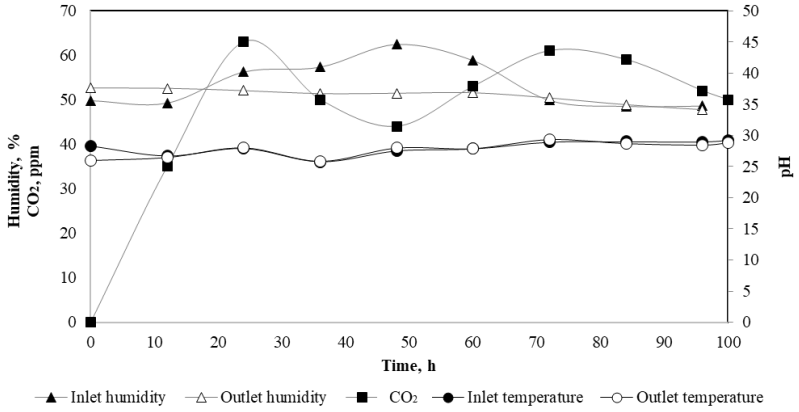


Figure 5. Humidity, temperature, and CO_2 production values in packed column bioreactor inoculated with *Aspergillus* sp. growing in BSG/AP.

After 24 h, the humidity levels began to rise steadily, reaching 62% by hour 48. Moreover, the growth of microorganisms was observed to decrease after 24 h, coinciding with the increase in humidity at the bioreactor inlet. This suggests that the accumulation of water at the system's inlet obstructed the microorganisms' growth. To address this issue, it is recommended to introduce dry air during the initial stages of the process.

3.2. Techno-Economic Assessment

The next step involved performing a techno-economic evaluation to determine which formulation, primarily focusing on xylanase, pectinase, or cellulase, produces the most promising results for reaching the commercial stage. For the three scenarios, the recipe batch time was around 390 h, but the cycle time was 210 h. This resulted in only ≈35 batches being produced yearly, making the process unprofitable.

Hence, the initial assessment primarily focused on addressing process bottlenecks within a base capacity of 5000 kg per batch of biowaste employed in the SSF process. Various simulations were conducted for each of the three scenarios to identify and subsequently mitigate bottlenecks as they surfaced.

In this process, the primary bottlenecks were identified as the main bioreactor (P11/SSF1) and the inoculum step (P1/IF1). These bottlenecks were addressed by implementing a staggered operational mode, which involves executing procedures on identical equipment out of phase with one another. After conducting a series of simulations, we gradually introduced additional fermenters, including compressors and air filters, until another bottleneck was encountered, which happened to be the dryer for the BSG (TD1). Consequently, the dryer was also set to operate in staggered mode.

However, it is noteworthy that eliminating process bottlenecks at this stage would necessitate a significant increase in investment, without a significant increase in the number of batches produced [10]. As a result, only the main bioreactor, the inoculum step, and the dryer were operated in staggered mode. A total of 20 SSF bioreactors (including compressors, filters, and inoculum steps) and 2 dryers were required. This strategic approach led to a reduced cycle time of 10.70 h, resulting in an increased number of batches produced annually (704 batches/year) and, consequently, an overall augmentation in annual production.

Table 1 summarizes the economic performance of each scenario at the selected batch size that showed the best economic indexes. The enzyme production shown (U mL^{-1}) represents the enzymatic yields achieved after fermentation (P11/SSF1), which decrease due to the losses and denaturation after the recovery unit operations.

Table 1. Economic parameters for simulated scenarios for xylanase, pectinase, and cellulase.

Project Indices	Scenario Xylanase	Scenario Pectinase	Scenario Cellulase
Enzyme production, U/mL	X = 582.39 P = 22.86 C = 26.10	X = 135.46 P = 61.73 C = 10.54	X = 0 P = 1.01 C = 69.90
Investment, USD	4,523,678	4,425,109	4,481,762
Annual operating cost, USD/yr	3,365,109	3,357,599	3,316,886
Unit production cost, USD/kg	2.25	2.31	2.30
Unit production revenue, USD/kg	3.72	1.92	2.22
Annual revenues, USD/yr	5,544,254	2,800,335	3,201,315
Gross margin, %	39.90	−19.90	−3.61
ROI, %	37.59	−3.92	6.10
Payback time, yr	2.66	N/A	16.39
IRR (After tax)	26.80	N/A	N/A
NPV at 7.00%, USD	7,325,537	−5,791,384	−2,813,314

The scenarios have similar capital investments and annual operating costs. The main difference lies in the process conditions required to produce enzymatic cocktails with varying proportions of xylanase, pectinase, or cellulase. However, annual revenues from xylanase-based enzymatic cocktails are almost twice those from pectinase and cellulase-based ones. This is because the unit production costs are similar (ranging from 2.25 to 2.31 USDkg^{-1}), but the unit production revenue is significantly higher for the enzymatic cocktail primarily containing xylanase (3.72 USDkg^{-1}). As stated earlier, the cost of enzymes can vary significantly in the commercial market, and there is no standard price agreed upon across the industry.

Table 2 presents different examples of techno-economic assessments (TEA) for producing enzymes using biowaste. Only studies conducted from 2020 to 2023 are included in the table. Ferreira and colleagues conducted a thorough review and summary of TEA analyses for producing lignocellulose-degrading enzymes before 2020 [9]. Included details regarding the production scale, plant capacity, and any economic indexes in the table depend on the available data reported in the article.

The studies shown in Table 2 focused on the production of cellulases, but other enzymes such as xylanase, amylase, and other lignocellulose-degrading enzymes were also included. The production scale varied significantly, ranging from less than 100 kg per batch to more than 350 tons per batch, depending on the processed biowaste. All of the reviewed processes show promise for higher-scale implementation, with positive NPV, ROI, and IRR indexes and a payback time of less than 5 years.

In this work, despite the higher price of cellulase units under consideration, the yield of the enzymatic extract containing xylanase significantly contributes to the revenues in scenario 1. Consequently, only scenario 1 exhibited a positive gross margin. Moreover, the ROI for scenario 1 is the highest (37.59%) compared to scenarios 2 and 3 (−3.92% and 6.10%, respectively) which translates into a payback time of 2.66 years for scenario 1. Notably, this payback period aligns closely with that reported for cellulase production using *Trichoderma reesei* (≈2 years), although such bioprocesses were carried out using submerged fermentation [11,12].

Table 2. Techno-economic assessment studies for enzyme production through fermentation and biowaste valorization.

Enzyme	Microorganism	Biowaste/Carbon Source	Production Scale/Plant Capacity	Economic Index/Product Cost	Sensitivity Analysis	Reference
Cellulase	* *Trichoderma reesei*	Coffee husk	1893 tons/year and 5.7 tons/batch (processed biowaste); 20,000 L (fermenter).	PBT: 2.27 years ROI: 44.08% NPV: USD 32,958,000 Cost: 28.68 USD/kg Selling price: 42 USD/kg	-	[11]
Cellulase	*Trichoderma reesei*	Glucose	12,785.97 tons/year (processed biowaste).	PBT: 1.88 years IRR: 36.95% NPV: USD 140,328,000 Cost: 20.71 USD/kg	*Aeration rate* Yield Energy demand Productivity	[12]
Amylase Cellulase Xylanase	*Trichoderma reesei*	Wheat chaff	375 tons/batch (processed biowaste); 2803 m^3 (fermenter).	PBT: 2.75 years IRR: 27.93% NPV: USD 29,895,000	-	[13]
Endo-β-1,4-Glucanase	* *Escherichia coli*	Oil palm empty fruit bunch	8.5 tons/batch (processed biowaste); 82.27 m^3 (fermenter).	PBT: 3.29 years IRR: 31.64% NPV: USD 32,121,000 Selling price: 250 USD/kg	Raw material cost *Cellulase price* Labor cost Overall operating cost	[14]
Lacasse	*Pycnoporus sanguineus*	Oil palm empty fruit bunch	50 tons/batch.	ROI: 28.70% NPV: USD 2.86 million Cost: 14.256 USD/kg	*Raw materials* Direct-fixed capital	[15]
Laccase Manganese Peroxidase Lignin peroxidase	*Aspergillus iizukae*	Pumpkin peels	66.6 kg/batch (processed biowaste); 2.7 m^3/batch (fermenter).	IRR: 50% Cost: 0.107 USD/cm^3 Selling price: 1 USD/cm^3	-	[16]
β-glucosidase β-xylo- sidase, endo-β-1,4-glucanase Endo-β-1,4-xylanase	* *Trichoderma reesei*	Sugarcane molasses	300 m^3 (fermenter).	Cost: 2.86 to 3.59 USD/kg protein. Selling price: 4.24 USD/kg	-	[17]
Cellulase Pectinase Xylanase	*Aspergillus* sp.	Brewery spent grain/apple pomace	6825 kg/residue per batch 8 m^3 (fermenter).	PBT: 2.2 years ROI: 45% IRR: 26.80% NPV: USD 7,325,537.00 Cost: 2.25 USD/kg	Raw material cost *Inoculum medium cost* Selling price	This work

* Indicates genetically modified. PBT, payback time; ROI, return of investment; IRR, internal rate of return; NPV, net present value. Italics in sensitivity analysis indicates significant parameter.

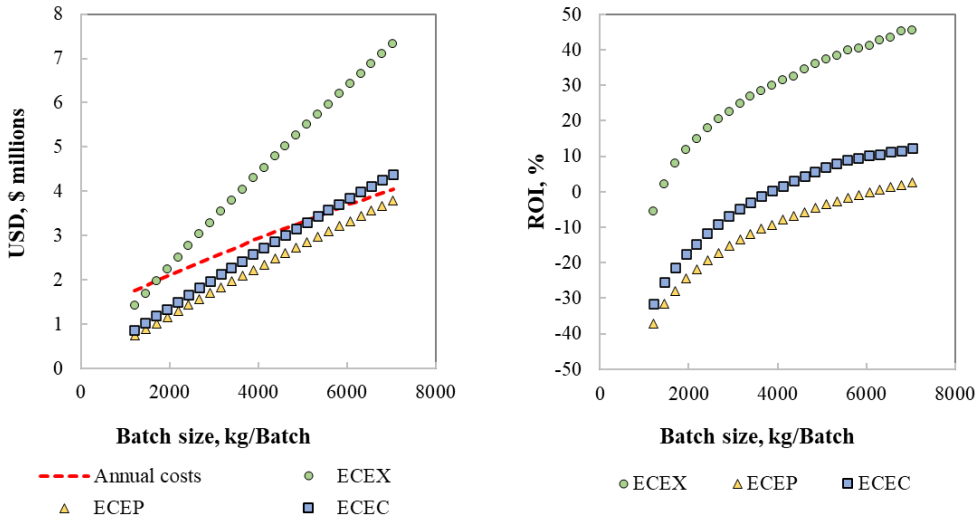
In addition, only scenario 1 exhibits a positive IRR at 26.8%, resulting in a favorable NPV. This value is comparable to an enzymatic extract containing amylase, cellulase, and xylanase produced by *Trichoderma reesei* using wheat chaff in [13]. IRR is a widely used metric for assessing the profitability of potential investments, representing the discount rate that equates the NPV of all cash flows from a particular project to zero. A higher IRR signifies a more promising project and is therefore a critical factor in investment decision making [12,18,19]. Hence, when considering a processed biowaste of 5000 kg/batch, it is evident that scenario 1, primarily focused on xylanase production, emerges as the most profitable option.

When it comes to upstream processing, it is crucial to determine whether the material will be used as it is for the bioprocess or if specific substrates need to be recovered from the biowaste. For instance, in the production of Endo-β-1,4-Glucanase using oil palm empty fruit bunch as a raw material by using an engineered *Escherichia coli*, the biowaste was subjected to various pre-treatment processes such as alkaline and sequential acid–alkaline treatments and steam explosion [14]. The study concluded that the alkaline pre-treatment was the most feasible for recombinant cellulase production. Similarly, in the cellulase production using coffee husk as a raw material in submerged fermentation by using an engineered *Trichoderma reesei*, the biowaste required the utilization of commercial enzymes for an enzymatic hydrolysis step and the process was still profitable [11]. In this work, extra unit operations were not required to recover the fermentable sugars.

Additionally, different systems and modes have been compared for the bioreactor stage. Specifically, studies have compared submerged and solid systems, as well as batch and fed-batch modes [11,13]. An economic analysis was performed to determine the best production process for an enzymatic extract containing amylase, cellulase, and xylanase by using *Trichoderma reesei* and wheat chaff [13]. It was found that the solid-state process is more effective than the submerged process. In addition, to produce cellulases by using *Trichoderma reesei* under submerged fermentation, two fermentation modes were evaluated: batch and fed-batch processes [12]. The authors concluded that the fed-batch process increased the process's profitability. Furthermore, simulation-based TEA was used to incorporate process kinetics and evaluate the most promising scenario in the economic analysis of the production of hydrolases by using *Aspergillus awamori* through SSF using babassu cake [20]. It was demonstrated that a fermentation time of 96 h was more suitable than 144 h due to increased batch throughput (batches/year). In this work, we used 192 based on previously evaluated process kinetics [5].

Concerning downstream processing, there have been various recommendations proposed to increase the profitability of recovery and purification processes. For instance, in the case of cellulase production through submerged fermentation, researchers evaluated two product presentations, liquid vs powder form, and concluded that the liquid presentation had better economic indices [11]. Additionally, for the recovery process of an enzymatic extract composed of amylase, cellulase, and xylanase produced by *Trichoderma reesei*, it was recommended to reuse the remaining liquid after separating it from the solid phase (recovered medium from SSF). Evaluating the volume of liquid required to extract the enzymes from the SSF medium after fermentation and its effect on process economics is also recommended [13].

In any case, despite scenario 1 (ECEX) being the most profitable, we also assessed the break-even point for all three scenarios, as illustrated in Figure 6a. The break-even point signifies the estimated economic equilibrium, which represents the processed biowaste needed per batch to generate annual revenues exceeding the operating costs. Figure 6b depicts the annual revenues for scenarios ECEX, ECEP, and ECEC, with annual costs presented exclusively for ECEX due to their similarity across all three scenarios.



(**a**) (**b**)

Figure 6. (**a**) Break-even point of the annual revenues and costs at each scenario and (**b**) ROI as a function of batch size (kg/batch).

It is observed that for scenario 1 (ECEX), annual revenues surpass processing costs when processed biowaste exceeds 2000 kg/batch. Conversely, in scenario 2 (ECEP), within the assessed biowaste range, revenues fail to overcome processing costs. Finally, scenario 3 (ECEC) shows annual revenues exceeding processing costs at around 6000 kg/batch. These findings underscore the necessity to scale up process capacity to achieve profitability.

Figure 6b illustrates the increase in ROI concerning batch size. It is readily apparent that within the assessed range, scenarios 2 and 3 are not cost-effective. This conclusion is drawn from the negative ROI in scenario 2 and the ROI below 10% in scenario 3, which would correspond to a payback time of 10 years. Furthermore, as the processing of biowaste increased across all three scenarios, the capacity of batches per year diminished due to the emergence of new process bottlenecks. Moreover, a surge in capital investment was observed beyond 5000 kg/batch [10]. This increase can be attributed to equipment capacity and associated costs which are closely tied to batch throughput. Notably, equipment costs escalate in correlation with capacity. However, most equipment costs and sizing are constrained by the maximum available industrial capacity. Consequently, above specific batch capacities, the software assumes the inclusion of an additional piece of equipment to achieve the desired batch throughput.

Thus, scenario 1 was selected for further analysis as the base case (5000 kg/batch) due to the obtained better economic indexes, even though the break-even point is at 2000 kg/batch. As an example, it is worth noting that the largest brewery company in Mexico, situated in the northern region, produces approximately 25 million hectoliters of beer per year [21]. It is estimated that for every 100 L of brewed beer, approximately 20–30 kg of wet BSG is generated [22]. On the other hand, 58 thousand liters of cider are produced in Mexico each year (SIAP, 2022). When apples are processed, 40 kg of pomace are generated per 100 L of juice produced [23,24]; this juice is then fermented to produce cider.

Consequently, the availability of raw materials should not pose a significant obstacle. Furthermore, integrating this type of bioprocess into breweries could play a pivotal role in fostering a circular economy.

Table 3 summarizes the major equipment specifications for the selected scenario (ECEX, scenario 1). Notably, it necessitates 20 packed-bed columns, each with a capacity of 8 m^3. It is worth emphasizing that the practice of employing multiple bioreactors operating in staggered mode has been previously recommended, particularly in the context of cellulase production [25]. Indeed, the choice between a single large bioreactor and multiple smaller ones is contingent upon specific process requirements, the scale of production, and the constraints imposed by available resources. Nevertheless, the utilization of multiple

bioreactors brings several advantages to the process. Notably, it mitigates the risk of contamination and offers a higher degree of flexibility when it comes to production scheduling. These considerations emphasize the importance of process simulation when assessing the techno-economic feasibility of a bioprocess.

Table 3. Major equipment specifications for the production of enzymes (ECEX, scenario 1).

Quantity	Name	Description	Unit Cost, USD	Cost, USD
1	MB1	Blending tank vessel (1227.39 L)	14,000	14,000
1	HS1	Heat sterilizer (276.16 L/h)	3000	3000
2	TD1	Tray dryer (281.57 m^2)	10,000	20,000
1	G1	Grinder (4050.00 kg/h)	27,000	27,000
1	TD2	Tray dryer (69.36 m^2)	5000	5000
1	S2	Solids drum (1.40 m^3)	1000	1000
1	S1	Solids drum (5.97 m^3)	1000	1000
20	SSF1	Packed-bed column (8.03 m^3)	36,000	720,000
3	S3	Solids tote (1.7 m^3)	3000	9000
1	L1	Solids mixer (4.40 m^3/h)	16,000	16,000
2	C1	Centrifuge (1.74 m^3/h)	6000	12,000
1	DEF1	Dead-end filter (42.72 m^2)	1000	1000
2	DF1	Diafilter (60.06 m^2)	11,000	22,000
20	GC1	Centrifugal compressor (1.79 kW)	2000	40,000
20	AF2	Air filter (15.34 m^3/h)	1000	20,000
			TOTAL	1,126,000

Figure 7 displays the annual operating costs for scenario 1. Among these costs, labor expenses constitute the most substantial portion, making up 29% of the total. Following closely behind, raw materials account for 27% of the overall expenses. Consumables represent 26% of the total costs, while facility-related costs and utilities contribute 14% and 4%, respectively. These cost distributions are consistent with the findings of a study on the production of laccase using biowaste (oil palm empty fruit bunch) by *Pycnoporus sanguineus* [15]. In that study, the authors similarly identified the raw materials as a significant cost, comprising 31.68% of the total processing costs.

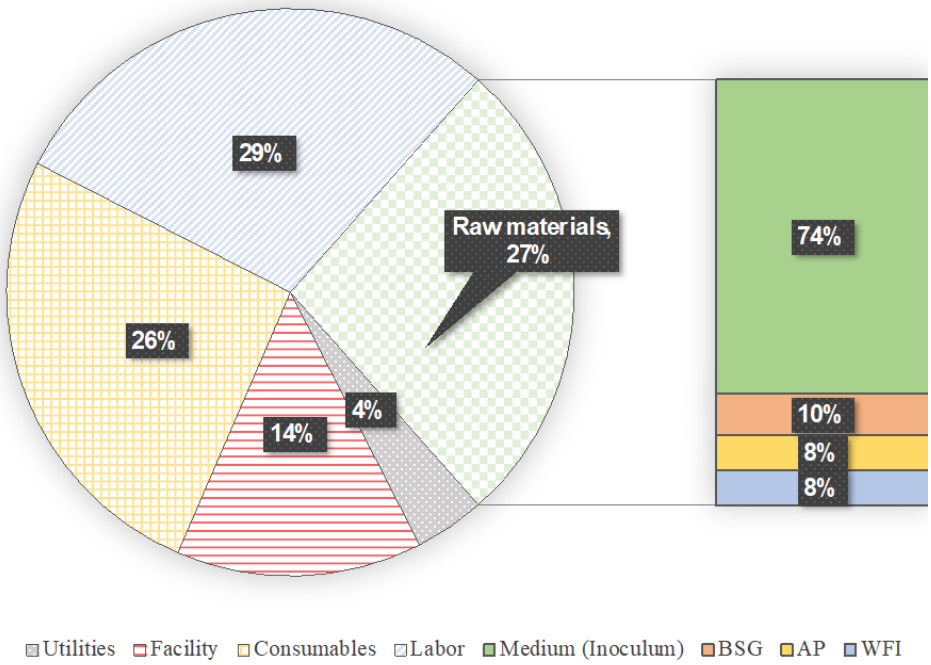


Figure 7. Annual operating cost breakdown for scenario 1.

Furthermore, the graph provides a breakdown of the raw materials utilized in the process. It is crucial to highlight that the costs associated with BSG and AP residues constitute only 10% and 8% of the total raw material expenses, respectively. What merits special attention is that the primary cost of raw materials arises from the medium employed for inoculum propagation, accounting for a substantial 74%.

Given this significant cost component, it might be beneficial to explore alternative culture medium options for microorganism propagation, similar to the approach taken in the production of amylases by *Aspergillus awamori* through SSF using babassu cake [20]. In their research, the authors highlighted the substantial impact of the inoculum propagation medium on processing costs, where PDA medium exhibited the most favorable cost–benefit ratio among the five alternatives.

Regarding the water used for recovery unit operations (WFI), although it represents a relatively minor portion at 8%, it offers an avenue for enhancing process sustainability in terms of both processing costs and environmental impact. Similarly, a study conducted on the production of amylase, cellulase, and xylanase by *Trichoderma reesei* using wheat chaff emphasized the necessity of exploring the optimal quantity of liquid required to extract enzymes from the SSF medium post-fermentation as well as the impact of this process on overall economics [13]. This highlights the potential for improving both the economic and environmental aspects of the process by optimizing water usage in recovery operations.

3.3. Sensitivity Analysis

Sensitivity analysis has also been useful in evaluating and managing the risks associated with fluctuating variables such as raw material costs, product prices, and other processing inputs [12,14,15]. This analysis is particularly important in industries where these variables are subject to frequent and significant fluctuations, such as the agricultural, energy, and manufacturing sectors.

Given that raw materials constitute a significant portion of the total processing costs, a sensitivity analysis was conducted to evaluate the impact of biowaste and inoculum medium costs on the economic indicators. In this sense, economic and technical variability risks must be avoided. This analysis also considered the influence of raw material costs by varying the batch capacity (kg/batch). As detailed in the Section 2.8, a deviation of ±30% from the base costs of BSG and AP was taken into account. Additionally, the cost of inoculum media was assessed, considering other commonly used mediums for fungi propagation, such as PDA and MEA. The outcomes of this analysis are presented in Figure 8, with the ROI % selected as the representative economic index.

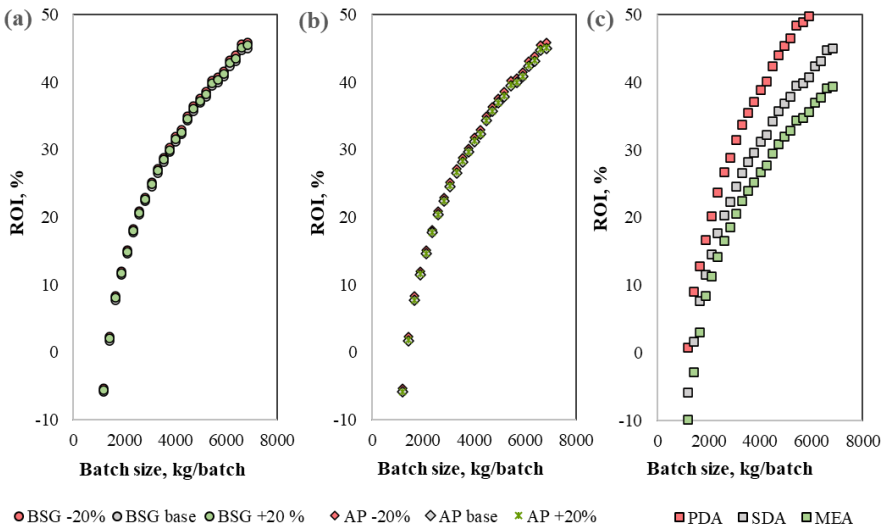


Figure 8. Sensitivity analysis of raw materials', (**a**) BSG; (**b**) AP; and (**c**) inoculum medium, cost effect on return of investment (ROI, %).

It is observed that a deviation of 30% from the base price of BSG or AP did not affect the ROI at different processing scales. Consequently, it is reasonable to conclude that a 30% increase in biowaste prices does not present a substantial risk to the process's profitability. It is advisable to exercise caution when considering the utilization of agroindustrial and food processing residues with significant cost fluctuations, despite their initial low cost, as these fluctuations can significantly impact process profitability. Nonetheless, it is crucial to acknowledge that such generalizations do not always hold true, as evidenced by a study on amylase production by *Aspergillus awamori* through SSF using biowaste [20]. The authors observed that the net unitary production cost of enzymes was considerably influenced by the purchase price (±15%) of raw babassu cake.

On the other hand, the cost of the inoculum medium emerged as a significant processing cost affecting the ROI. The adoption of a more cost-effective medium than the one used during the experimental phase, such as PDA, has the potential to boost the ROI by as much as 50%, particularly as batch size increases. Nevertheless, it is imperative to ensure that altering the culture medium does not compromise the growth of microorganisms. If there is a perceived risk of such an impact, it is advisable to explore adjustments in the inoculation process conditions to maintain optimal microbial growth and kinetics despite the change in medium composition. This careful consideration of medium selection and its consequences on the bioprocess is critical to achieving both economic efficiency and the desired production outcomes.

As depicted in Figure 9, the sensitivity analysis of enzyme prices reveals a significant level of uncertainty. This uncertainty is primarily attributed to the wide-ranging costs of commercially available enzymes. As previously discussed, this variability in prices can significantly impact the overall cost of the process. As reviewed by Ferreira et al. [9], the cost of producing lignocellulose-degrading enzymes exhibits significant variation, ranging from approximately USD 1 per kg to over USD 90 per kg.

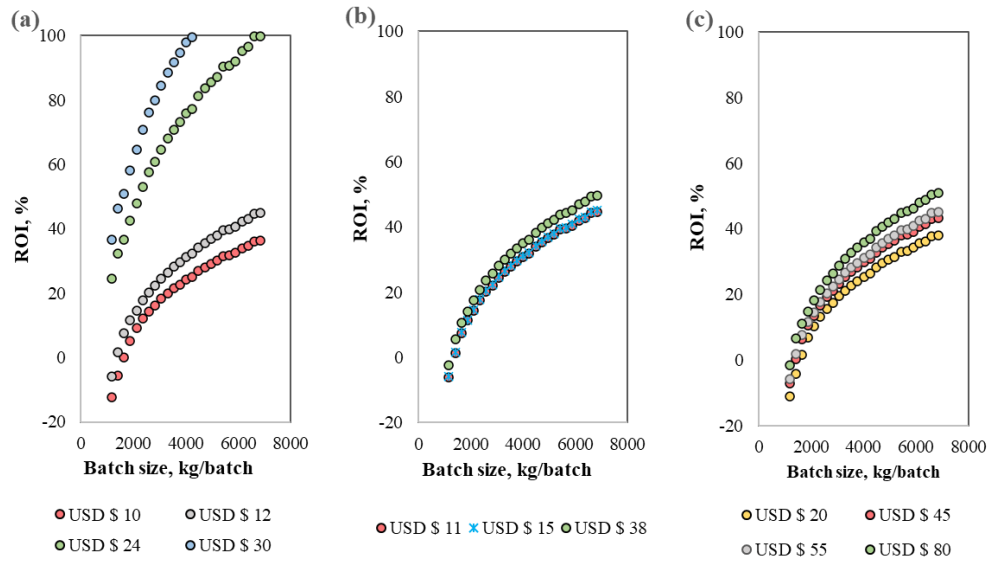


Figure 9. Sensitivity analysis of selling prices' ((**a**) xylanase; (**b**) pectinase; (**c**) cellulase) effect on the return of investment (ROI, %). Prices are USD per 1000 enzymatic units.

The baseline prices in this study were determined based on the available experimental data regarding enzymatic units. Therefore, the final prices in USD/kg (previously listed in Table 1) are dependent on the enzymatic units that are retained in the enzymatic extract after the recovery process. It is evident that the return on investment can be maximized if the price of xylanase is increased. The price of pectinase had the least impact, followed by cellulase. However, it is crucial to acknowledge that the enzymatic extract primarily comprises xylanase activity. A techno-economic study for the production of recombinant cellulase by *Escherichia coli* using oil palm empty fruit bunch showed that the cellulase

selling price has the highest influence on process profitability [14]. In this sense, this analysis highlights the higher risk associated with the variability in enzymatic extract prices.

4. Conclusions

The techno-economic analysis allowed us to select the most profitable enzymatic extract, which was the one obtained in scenario 1 (ECEX, primarily focusing on xylanase production). It exhibited a break-even point at 2000 kg/batch. Meanwhile, scenario 3 (ECEC), focusing on cellulase production, could also be promising and cost-effective when processing above 6000 kg/batch.

Scenario number 1 offered a cost-effective process at the base case of 5000 kg/batch, with an ROI of 37.59%, payback time of 2.66 years, IRR of 26.8%, and net present value of USD 7,325,537. The resulting unit production cost of 2.25 USD/kg is economically competitive. Such economic indexes could be improved by increasing batch plant capacity and reducing processing costs.

For instance, the breakdown of annual operating costs revealed that labor expenses, raw materials, and consumables constitute the major cost components. We noted the importance of considering alternative culture media and optimizing water usage to enhance process efficiency and sustainability.

The sensitivity analysis highlighted the risks associated with fluctuating raw material costs and the significant impact of enzyme prices on process profitability.

The raw material costs related to the agroindustrial residues and food processing by-products (BSG and AP) did not have an effect on process profitability by increasing or decreasing the cost by ± 30. Additionally, the analysis explored the influence of inoculum medium costs, demonstrating that selecting a more cost-effective medium, such as potato dextrose agar (PDA), could substantially enhance ROI, especially with increasing batch size. However, it is crucial to ensure that replacing the culture medium will not compromise microorganism growth, requiring careful evaluation of the inoculum preparation steps. On the other hand, it was found that changes in enzyme prices, particularly xylanase, significantly affected the process's profitability. This underscores the importance of price stability and competitive pricing in the enzyme market.

Overall, the sensitivity analysis provided valuable insights into how changes in raw material costs and product prices can impact the economic performance of the process. This offers a more comprehensive understanding of the potential financial variability in the chosen scenario.

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