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A simulation-based feasibility assessment of malic acid production from molasses using *Rhizopus arrhizus*

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Abstract

Malic acid is a valuable organic acid widely used in food, pharmaceutical, and chemical industries. It can be sustainably produced from underutilized molasses, often classified as waste. This study evaluated the feasibility of malic acid production from molasses, using *Rhizopus arrhizus*. A SuperPro Designer simulation integrated process design, economic analysis, and sensitivity evaluation and the results demonstrated economic viability with a Net Present Value (NPV) of $2,140,000 (7% discount rate), an Internal Rate of Return (IRR) of 15.81%, a Return on Investment (ROI) of 22.70, and a payback period (PP) of 4.40 years for an annual production capacity of 2,830 MT. Sensitivity analysis highlighted the selling price of malic acid as the most important economic factor. This feasibility study provides a novel approach to integrate molasses-based fermentation with simulation tools, offering actionable insights for industrial-scale implementation by quantifying key economic drivers.

Keywords: Malic acid; molasses; Rhizopus arrhizus; techno-economic analysis; SuperPro Designer

1. Introduction

Malic acid is an essential organic compound commonly used in food and beverages, pharmaceuticals, and cosmetics in view of its multifunctional properties as a flavoring agent, pH regulator, and preservative [1,2]. In global market, annual production of malic acid accounts for about 200,000 MT. It is anticipated to grow significantly in the coming years [3,4]. Historically, malic acid has been manufactured via chemical processes, requiring considerable energy and posing environmental issues. There is an increasing interest in microbial fermentation as a more sustainable and cost-effective option considering that it is capable of efficiently converting affordable materials such as cane molasses into valuable goods [5,6].

Molasses is one of the most promising feedstocks for fermentation processes because of its abundance, low cost, and high content of fermentable sugars. Primary and secondary metabolites, such as lactic acid, butyric acid, and various vitamins, have been successfully synthesized with molasses [6,7]. In fact, molasses is frequently not fully utilized and is viewed as a by-product, resulting in problems related to environmental disposal. For this, utilizing molasses for malic acid production enables to tackle any issues related to waste management, which notably enhances the sustainability of the entire production process and aligns with the principles of circular economy and green chemistry [5–10]. However, many of these studies are limited to laboratory-scale experiments and insufficient concern is given to economic feasibility and large-scale process integration.

While microbial fermentation using molasses has been studied for organic acid production, most previous research have focused on laboratory-scale experiments with limited emphasis on comprehensive techno-economic analyses [11,26]. Most of these studies have no robust integration of process design, economic evaluation, and sensitivity analyses necessary to assess industrial-scale feasibility. Moreover, the application of simulation analysis, such as SuperPro Designer, in the production of malic acid has not been fully realized in the literature since most of the available studies did not maximize the simulator capacity for the optimization and prediction of various operational scenarios [2,12].

Rhizopus arrhizus (*R. arrhizus*) is a familiar microorganism

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for malic acid production [11,13,26,38], but its practical application in industrial-scale operations remains underexplored. Critical challenges, including substrate variability, process integration, and cost-effectiveness, remain important for further research to generalize the lab-scale results to industrial applications. Earlier studies did not comprehensively present a framework in which fermentation kinetics, process, economic evaluation, and sensitivity analyses could be incorporated to determine the potential of malic acid production from molasses [2,11].

To fill these gaps, this study used an upgraded simulation created with SuperPro Designer to provide an all-inclusive feasibility analysis of producing malic acid from molasses via *R. arrhizus* fermentation. SuperPro Designer is a comprehensive process simulation and an analysis tool widely used in biotechnology and chemical industries [12]. This work differs from previous research in combining process design, economic assessment, and analysis into one simulation with straightforward suggestions for scale-up. This simulation took into account a number of key economic factors, including net present value (NPV), internal rate of return (IRR), return on investment (ROI), and payback period (PP), purposely to offer a thorough understanding of the bioprocess viability under varied operating conditions [2,12]. This study contributes substantially to the bioprocessing sector by analyzing the financial elements associated with the process. In addition, the findings demonstrated that simulation tools can be utilized efficiently to create cost-effective and sustainable approaches.

2. Materials and Methods

The simulation was performed in batch mode with SuperPro Designer v13 software licensed for academic use at Universitas Indonesia. In the simulation, primary and secondary data were used as the input parameters. Fig. 1 and 2 respectively depict Block Flow Diagram (BFD) and Process Flow Diagram (PFD) for malic acid production.

Malic acid production involved pretreatment, fermentation, and product purification. For the pretreatment, molasses was subjected to sedimentation, which removed any impurities, followed by heating and hydrolysis with sulfuric acid. Neutralizing this resulted mixture was performed using sodium hydroxide with subsequent filtration resulting in a salt solution. Pasteurized salt solution was then mixed with glucose, urea, and calcium carbonate additives through fermentation. Seed fermentation using *R. arrhizus* was carried out in two stages. The fermented product was subsequently bleached with activated carbon, filtered, and spray-dried with malic acid as the final product. The proposed simulation estimated the annual production capacity at 2,830 MT with a total process time of 106.67 hours per batch. This then allows for a maximum of 74 batch cycles per year.

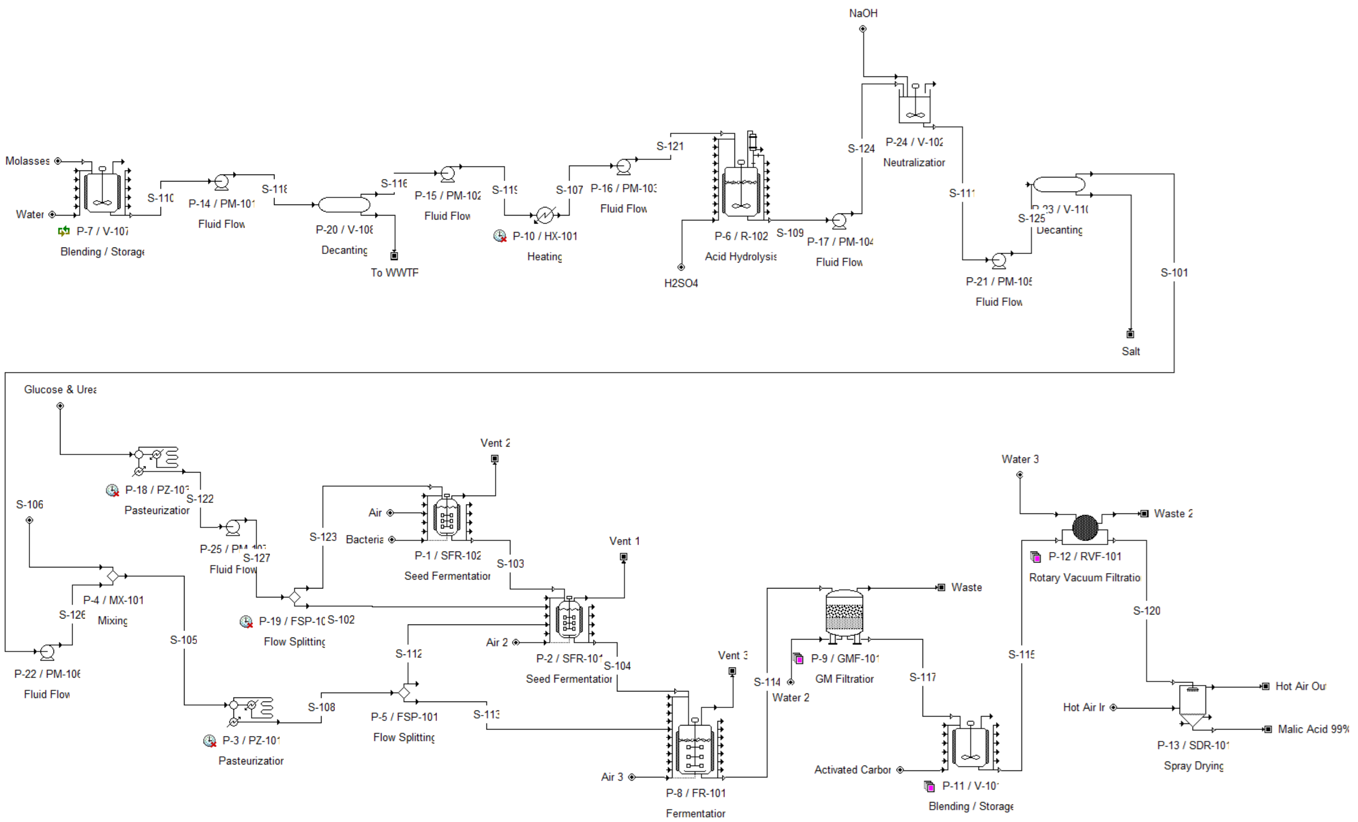

Fig. 1. Block flow diagram (BFD) for malic acid production

2.1. Process design and simulation

2.1.1. Pretreatment process

In the pretreatment stage, the primary unprocessed substrate, sugarcane molasses, contained 10.5% ash, 1.06% calcium hydroxide, 4.2% carbon, 0.41% fats, 6.96% fructose, 8.86% glucose, 3.68% proteins, 5% sorbitol, 40.33% sucrose, and 19% water. This molasses was diluted with water in a blending tank (V-107) at 690.625 kg/h within a batch storage system. This process facilitated solid dissolution or suspension. Afterwards, the resulted mixture underwent sedimentation (V-108), effectively separating solids from the liquid and yielding a solution containing fructose, glucose, sucrose, and water for subsequent processing.

The concentrated solution as a substrate was then heated up to 50°C to maximize the hydrolysis rate. Hydrolysis was carried out in the presence of sulfuric acid (H_2SO_4) in a stirred-tank reactor (STR, R-102) with the addition of acid at a rate of 2.78 kg/h and a residence time of 1.17 hours at 60°C. Table 1 presents the hydrolysis results with a rate constant (k) of 0.00697.

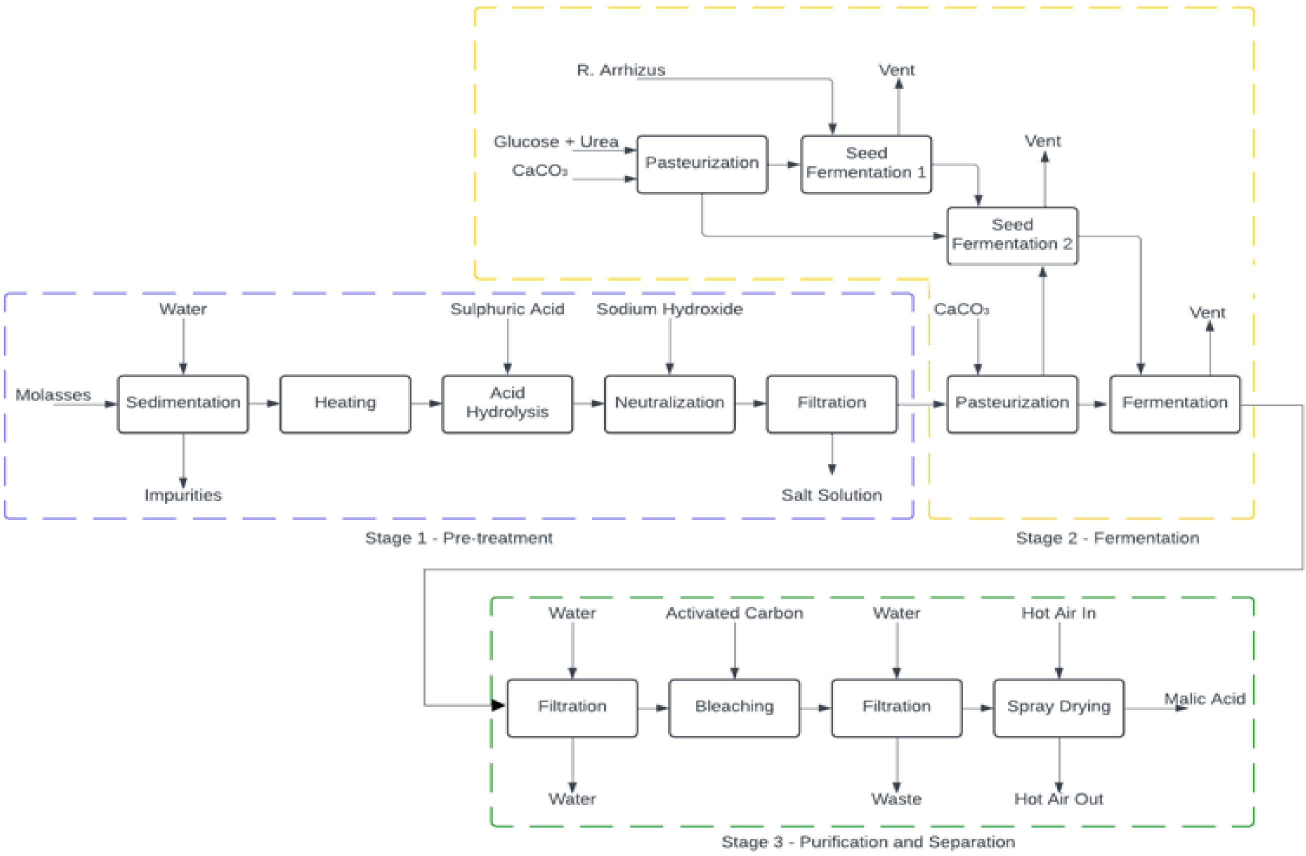

Fig. 2. Process flow diagram (PFD) for malic acid production

Table 1. Sucrose conversion rate via sulfuric acid hydrolysis in a kinetic STR

Sucrose inlet (kg/h)	Sucrose outlet (kg/h)	Conversion (%)
278.53	9.2	96.7

The hydrolysate was then neutralized (V-102) with sodium hydroxide (NaOH), stabilizing the pH at approximately 7.0 for compatibility with the fermentation process. Lastly, the neutralized substrate went through a desalination (V-110) step to eliminate any excess salts and residual minerals that might inhibit fermentation. The resulted light phase was then enriched with urea and essential minerals and fully prepared for fermentation.

2.1.2. Fermentation process

Pasteurization at 121°C eliminated any unwanted microorganisms in malic acid production via fermentation with *R. arrhizus* (PZ-101 and 103); here two pasteurization pathways were used. The first one sterilized the glucose and urea solution, while the second one sterilized the hydrolysis products (glucose, fructose, urea, water, and activated carbon) to prevent any contamination during fermentation.

The spores of *R. arrhizus* were cultivated on agar plates in medium A for 5 days at 28°C [14]. Following sporulation, spores were harvested from fungal mycelium using glycerol and 50% saline solution. The final suspension, which contained approximately 30 x 10^7 spores/mL, was stored at -80°C until being used as the initial inoculum for all experiments [14]. The inoculum was prepared by incubating at 34°C with agitation at 200 rpm using 100 mL non-baffled Erlenmeyer flasks containing 20 mL of culture medium covered with cellulose. The initial spore concentration in the inoculum was 109 spores/L. After 112 hours under these conditions, the inoculum was transferred to the production medium at a 10% v/v ratio. This inoculum process was designed to yield *R. arrhizus* biomass with optimal morphology and metabolic state. The preculture results were transferred to a seed fermenter (SFR-101 and 102), producing inoculum for larger-scale fermentation. At the same time, slower-growing strains required larger inocula to minimize fermentation duration and cost. Spores are sometimes directly introduced into the fermentation vessel via an air stream [15]. During seed fermentation, *R. arrhizus* was cultured with glucose and urea, and once exponential growth was achieved, the plant was ready for malic acid production.

The reaction in the fermenter (FR-101) consisted of the interaction between urea, glucose, *R. arrhizus* from seed fermentation, and oxygen with the product of the reaction as carbon dioxide, water, and the exponential growth of *R. arrhizus*. Parallel fermentations were repeated as *R. arrhizus* metabolized glucose and fructose into malic acid. Although *R. arrhizus* primarily produces fumaric acid, malic acid can still be obtained as a by-product through the hydration of fumaric acid.

Fermentation kinetics, including the maximum specific growth rate (μ_{max}), substrate consumption rate, and product formation rate were derived from the literature. These parameters were used to estimate fermentation time, substrate-to-product yield, and biomass formation in the SuperPro Designer Simulation. The batch cycle time was modified in accordance to the time required to achieve 90-95% of the theoretical malic acid yield under these specific kinetic conditions. Additionally, downstream equipment sizing utility loads were scaled based upon the reaction times, ensuring consistency between the biological performance and process engineering assumptions. The data for the growth of *R. arrhizus* and glucose conversion are shown in Tables 2 and 3, respectively.

Table 2. Fermentation parameters

Parameter	Value
Ks (Monod model)	96.7 mg/L
μ_{max}	0.0696 h^{-1}
α	0.3853
β	0.0032

Table 3. The glucose conversion rate in a fermenter

Reactor	Glucose conversion rate to inoculum growth (%)
Seed fermenter 1	41.74
Seed fermenter 2	41.74
Main fermenter	51.33

The results showed that *R. arrhizus* reached its optimal growth during the growth phase. Superior performance was observed in the main fermenter, a condition attributed to the higher availability of glucose and urea than that of in the seed fermenters. In this nutrient-rich environment, *R. arrhizus* successfully grew and efficiently converted fructose and glucose to malic acid. The conversion of substrate to malic acid is listed in Table 4.

Table 4. Substrate conversion rate to malic acid

Substrate	Conversion (%)	Malic acid produced (kg/h)
Glucose	99.97[a]	204.30
Fructose	100[b]	189.84

[a]Kinetic reaction at k = 0.00229 at 46°C

[b]Stoichiometric reaction

Although the simulation assumed nearly 100% of the conversion of glucose and fructose into malic acid, experimental data from the literature indicated that actual biological yields were significantly lower at only 58% [14]. Simulation models had inherent limitations for not accounting for biomass formation, by-product production, and unique metabolic constraints on microbial systems; as a consequence, these biological factors fundamentally reduced the proportion of substrate directed solely to product formation. As a direct result, in real situations the actual production rates frequently do not reach the highest theoretical potential as suggested by simulations. This situation highlights a need to include these biological limits in upcoming model development for a more accurate feasibility assessment.

2.1.3. Purification process

After fermentation, the process stream still contained unconverted sugars, microbial biomass, catalyst, and unused medium; it then required further filtration. This initial solid-liquid separation was performed through screw press filtration (GMF-101) with the primary goal of producing an aqueous malic acid solution through efficient impurity separation. Following this, a granular media filtration unit was used, effectively separating both cells and discarded components from the malic acid and water mixture.

Since molasses, the main raw material for malic acid production, tended to give a dark color to the resulting solution, the decolorization process was deemed essential to make the product marketable. This was achieved through bleaching with activated carbon, effectively adsorbing colorants. This process was carried out in a mixing storage unit (V-101) to increase adsorption efficiency. The dark pigments were adsorbed as the solution passed over the activated carbon, producing a more straightforward, marketable malic acid solution.

After bleaching, the solution still contained malic acid and activated carbon granules, necessitating a further filtration step. For this, a rotary vacuum filter optimized for separating components based on particle size (RVF-101) was used and specifically designed to differentiate components according to their particle size. This filter accurately separated the activated carbon granules by a suspicious adjustment, resulting in a clear aqueous malic acid solution. In the end, spray drying converted the malic acid solution into a solid form (SDR-101). This economical technique utilized hot air to dry the mixture. The purification process eliminated any impurities and crystallized malic acid, producing the main product at 358.56 kg/h with 99.1% malic acid and 0.9% water.

The yield of malic acid production from molasses per batch was calculated using the following formula:

$$Yield = \left(\frac{malic\ acid\ produced}{molasses\ processed}\right) x100\% \quad (1)$$

For the current process:

$$Yield = \left(\frac{37.903}{73.667}\right) x100\% \quad (2)$$

From the total molasses input, the malic acid yield achieved was 51.5%. This low yield was directly related to the composition of molasses, consisting of only 70% fermentable sugars with other 30% being non-fermentable material.

Therefore, the conversion efficiency from the fermentable sugar was much higher, approximately 73.6%. This clearly showed that the total yield of raw molasses was more limited by the non-fermentable fraction in the raw material rather than by the fermentable substrate's low bioconversion efficiency.

2.2. Process design and simulation

Economic performance depends on several aspects: raw materials, equipment, labor, construction, and utility. The unit cost of malic acid production was calculated by dividing the total annual production cost by the amount of malic acid produced. The raw materials for this study were molasses (0.13 $/kg), *R. arrhizus* (16.16 $/kg), sodium hydroxide (33.73 $/kg), sulfuric acid (21.67 $/kg), and urea (0.65 $/kg). The operating cost, fixed cost, and revenue were calculated and economic indicators, such as IRR, ROI, NPV, and PP were analyzed as well.

2.3. Sensitivity analysis

Sensitivity analysis was conducted to evaluate how changes in various parameters affected the estimated cost of malic acid production. This study examined the impact of ±10% fluctuation in key cost components such as raw materials, product prices, and labor costs on economic indicators of ROI, IRR, PP, and NPV.

3. Results and Discussion

3.1. Techno-economics of malic acid production

Techno-economy analysis is crucial in providing the comprehensive financial management insights for the entire malic acid production, including total plant investment, annual operating costs, revenue, net profit, ROI, PP, IRR, and NPV. There are two types of costs: capital expenditure and operational expenditure (OPEX). Table 5 shows the capital expenditure (CAPEX) and operational expenditure (OPEX) for malic acid production.

Table 5. CAPEX and OPEX for malic acid production

Cost item	Final cost ($)
Capital investment	
Direct fixed capital investment	2,894,000
Working capital	373,000
Start-up cost	145,000
Total capital investment	**3,412,000**
Annual operating costs	
Raw materials	3,503,000
Labor-dependent	343,000
Facility-dependent	552,000
Laboratory/QC/QA	52,000
Waste treatment/disposal	9,000
Utilities	238,000
Total annual operating costs	**4,697,000**

The CAPEX includes direct fixed capital, working capital, and start-up costs. The direct fixed costs for malic production were estimated, as shown in Table 6. Process equipment consisting of a seed fermenter, a production fermenter, sterilization equipment, separation equipment, and a heating element was estimated (the equipment costs can be found in the supplementary data, Table S1). Equipment costs were calculated by SuperPro using process-specific parameters, scaling laws such as the six-tenths rule, and cost indices, such as the Chemical Engineering Plant Cost Index (CEPCI). As commonly applied in economic calculations, material balances, construction, and installation costs are considered in determining direct fixed capital investment costs [16,17]. Direct fixed capital investments include total plant costs, indirect costs, and contingency to be calculated. SuperPro estimates all of the contributing costs for the direct cost as a percentage of the total equipment purchase cost.

Table 6. Direct fixed capital investment breakdown

Direct fixed capital investment	Cost ($)
Plant direct cost	
Equipment purchased cost	478,000
Installation	195,000
Process piping	167,000
Instrumentation	191,000
Insulation	14,000
Electrical	48,000
Buildings	215,000
Yard improvement	72,000
Auxiliary facilities	191,000
Plant indirect cost	
Engineering	393,000
Construction	550,000
Contractor fees and contingency	
Contractor's fee	126,000
Contingency	252,000
Total Direct fixed capital investment	**2,894,000**

The cost of raw materials was derived from their respective market values. The raw material cost breakdown is provided in the supplementary data in Table S2. Labor costs were calculated based on the total expenses for plant operators, adjusted with the minimum wage in South Lampung, Indonesia, the plant's location, which is 2,5 $/hour. Cost adjustments were also made to account for the number of work shifts people working at the production facility.

Table 7 summarizes the results of the techno-economic analysis for the malic acid production from various feedstocks. As shown by the data in Table 9, the estimated unit production cost of malic acid was $1.66/kg, whereas the selling price was $1.91/kg. The batch fermentation process presented a significant potential for large-scale industrial malic acid production from molasses with a payback period of approximately 4.40 years. The findings showed the IRR of 15.81%, an ROI of 22.70%, and an NPV of $ 2.14 million at a discount rate of 7% over the years, indicating that this process's economic viability and practical application are feasible on a large scale.

The economic feasibility of malic acid production is substantially determined by the selection of raw materials, microbial strains, and process parameters, as summarized in Table 9. The total capital investment (TCI) of $3.412 million was lower than that of alternative feedstocks such as soybean molasses ($18,552 million) [18] and liquefied corn starch ($21.438 million) [19], making sugarcane molasses a cost-effective choice. The lower TCI was attributed to the simplicity of molasses-based fermentation's pretreatment and downstream processes. This was contrast to the higher TCI, often due to complex feedstock handling and purification steps, especially for substrates such as corn starch. The annual operating cost of $4.697 million/year was found higher than that of the crude glycerol-based process [3], suggesting a need for further optimization.

Conversely, the cost of producing one kilogram was $1.66/kg, greater than the cost of crude glycerol at 0.43/kg [3], but remained in a similar range to other processes using molasses [18,20]. These factors emphasize a significant opportunity for reducing costs by enhancing strain efficiency and improving the fermentation process. With an annual production capacity of 2,830 MT, the revenues are $5.357 million. However, this is still lower than some alternative feedstocks, such as soybean molasses ($8.75 million), highlighting a need for increased production scale-up or development of higher value-added applications.

Table 7. Comparison in the techno-economic analysis of malic acid production from various feedstocks.

Parameter	Unit	This study	[3]	[18]	[19]	[20]
Raw material		Sugarcane molasses	Crude glycerol	Soybean molasses	Liquefied corn starch	Sugarcane juice
Microbe		*R.arrhizus*	*Aspergillus niger*	*Aureobasidium pullulans*	*Aureobasidium pullulans*	*Aureobasidium pullulans*
Total Capital Investment	$	3,412,000	7,067,279	18,522,000	21,438,000	10,698,000
Operating Cost	$/year	4,691,000	1,179,787	5,515,000	10,323,000	3,318,000
Revenues	$/year	5,357,000		8,750,000	15,000,000	7,500,000
Batch Size	Kg	38,247.46	10,000			
Annual production	MT	2,830		5,000	5,000	2,500
Unit Production Cost	$/kg	1.66	0.43	1.10	2.046	1.33
Unit Production Revenue	$/kg	1.89	2.56		3.0	3.0
Gross Margin	%	12.44	45.16	59.09%	30,3	81.9
Return On Investment	%	22.70	12.85	17.46	14,5	25.4
Payback Time	years	4.40	7.78	5.7	6,9	3.94
IRR (After Taxes)	%	15.81	11.17			
NPV (at 7.0% Interest)	$	2,140,000	2,246,000			
Selling price	$/kg	1.91		1.75	1.75	1.75

Economic indicators showed the promising results. The ROI of 22.56% and payback time of 4.43 years were competitive, especially compared to more costly feedstocks. However, the gross margin of 12.32% was relatively low compared with sugarcane juice (81.9%) and crude glycerol (45.16%). Therefore, urgent improvements, including strain enhancement, are deemed critical to increase malic acid yields and minimize by-product formation [3]. In this study, the NPV of $2.16 and IRR of 15.69% indicated solid financial performance although it was slightly less competitive than the value reported for crude glycerol. It should be noted that higher IRR values often result from the use of substrates requiring minimal pretreatment and result in higher product yields [3].

3.2. Sensitivity analysis

A sensitivity analysis was performed to identify which of these elements exerted the most significant influence on the economic viability of the production process. Also, the analysis assessed the feasibility of the production plant under potential fluctuations in these cost components in the foreseeable future. This study examined the impact of varying key cost components, raw material costs, product prices, and labor costs on the economic indicators of ROI, IRR, PP, and NPV. In addition, a tolerance of 10% was investigated. Figure 3 presents the sensitivity analysis results.

It was identified from the sensitivity analysis (Fig. 3) that a selling price became the most sensitive variable of concern on ROI, IRR, PP, and NPV; a 10% increased value thereof increased the ROI by 34.48%, the IRR by 25.45%, and the NPV to $5.006 million, whereas such reduced the payback to 2.9 years. Similar results were observed in research on bio-succinic acid production; this study found that an increase in the product's price affected the project's financial sustainability [21]. A 10% decrease in the selling price without a corresponding price reduction would lead to substantial economic losses, such as an NPV of -$699,000, making a competitive price essential through improved product quality or strategic market positioning.

A strategic approach can be suggested to address this weakness. This may include increasing the value of molasses by-products, such as converting the non-fermentable solids into products of value (e.g. ethanol, animal feed, and biogas). This can create additional income and strengthen the price stability

[21]. Product diversification, such as co-producing fumaric acid or leveraging malic acid in higher-value niche markets (e.g. pharmaceuticals, biodegradable polymers), may further buffer market volatility [22]. In addition, forward contracts, regional market targeting, and quality-driven positioning can improve market competitiveness and pricing power [23].

Fig. 3. Sensitivity analysis of percentages with ± 10% variation for (a) return on investment (ROI), (b) internal rate of return (IRR), (c) payback period (PP), and (d) net present value (NPV)

The variation of raw material costs could also have a considerable impact where a 10% increase reduced the ROI to 14.89% and extended the PP to 6.72 years. This result agrees with previous studies in which feedstock cost was considered one of the most determining factors in fermentation processes from an economic point of view [18,19]. On the other hand, labor costs had a relatively small effect on all variables due to their low proportion of the total cost. Similar findings were reported with the variation of labor costs having the least effect due to their relatively minor share of overall operating expenses [24].

3.3. Critical discussion: operational challenges, study limitations, and future perspectives

Following an evaluation of the techno-economic feasibility and sensitivity related to malic acid production using molasses, this section critically discusses about the operational challenges associated with the process implementation on an industrial scale. This addresses the limitations of the simulation studies conducted and positions our findings in the context of current scientific research. This objective is to comprehensively comprehend the practical consequences and potential growth pathways necessary to facilitate the commercialization of this process. Some critical issues are presented as follows:

- Challenges of fermentation and microbial physiology in the context of the literature

Acid tolerance of *R. arrhizus* is an essential factor affecting malic acid fermentation, given that organic production inherently causes the acidification of the medium. Although *R. arrhizus* is relatively good compared to other microorganisms, it still shows decreased enzymatic activity at pH < 4, which may limit product accumulation in long-term fermentation [14,25–27]. Unstable pH levels can lower product formation rates and impact yields and cell survival [28]. Achieving a molasses production target of 51.5% largely relies on maintaining an ideal pH level. Maintaining precise pH levels in large-scale fermentation vessels seems to be difficult in industrial manufacturing processes. Local acidification may result, causing either a decline in cellular activity or reduced cellular viability [29]. Future studies or optimized process designs may investigate ways to enhance acid resistance in *R. arrhizus* through strain modification. Research into future pH control methods may involve customizing existing techniques, such as implementing in-situ neutralization to preserve conversion efficiency.

In addition, molasses as a raw material brings challenges, namely inhibition by carbon sources and impurities. Its nature as a complex by-product has made it contain both fermentable sugars and various non-sugar compounds, including inorganic salts, pigments, phenolic compounds, and other components that can be inhibitors for microbial growth or fermentation activity [30–32]. Although *R. arrhizus* is known to have relatively good tolerance to several inhibitors, at high substrate concentrations or in the presence of the accumulation of certain compounds in molasses, carbon source inhibition may occur, reducing the fermentation rate and overall yield [33]. The optimization of the initial molasses concentration and consideration of molasses pretreatment (e.g., dichlorination or removal of toxic components) are crucial mitigation steps that must be further explored to maximize process performance.

Regarding fermentation time reduction strategies, this study referred to a batch fermentation time of 106 hours per batch. Strategies included fed-batch fermentation to maintain substrate concentration, and avoid inhibition, or even continuous fermentation. Fermentation for steady-state production has been shown to reduce process times significantly in other bioprocesses, and it is worth exploring for malic acid [27]. Recent literature suggests approaches such as fed-batch fermentation or genetically engineered strains that successfully reduced fermentation times into less than 48 hours without compromising yields [27,34]. Implementing these technologies in molasses-based processes could increase volumetric productivity and lower fixed costs per ton of product.

- Downstream processing (DSP) challenges and scale-up risks

The DSP poses distinct challenges that can significantly affect the economic viability and technical feasibility of producing malic acid. One prevalent issue in the bioprocessing sector is the formation of biofilms or the accumulation of biomass on the surface of DSP equipment, including filtration membranes, heat exchangers, and bioreactor walls [35,36]. Biofouling can significantly decrease mass and heat transfer efficiencies, increase the pressure drops across the filtration system, and shorten the equipment life. The simulation relied on standard data to assess the efficiency of the assumed DSP unit. It is crucial to consider the implementation of cleaning-in-place (CIP) procedures and practical maintenance schedules when assessing operational expenditures and planning manufacturing, which should include replacing or sanitizing filters/membranes. Long-term operational sustainability hinges on thoughtful planning to mitigate significant drops in productivity [37].

Furthermore, this study has certain limitations as a simulation-based analysis for accurately forecasting challenges related to scaling up. Many of the parameters used in the simulation were idealized and derived from laboratory or literature sources, which might not accurately represent the actual operational conditions [29,38]. For instance, the simulation assumed consistent process efficiency, homogeneity, and the absence of unforeseen operational issues, such as unplanned downtime, which could impact productivity and cost at scale. Uncertainties related to market fluctuations for molasses and malic acid are also difficult to model in these static simulations [16,39]. Scale-up risk is another significant challenge in bioprocessing. Various constraints arise when fermentation volumes are significantly increased, such as decreased oxygen transfer efficiency, pH imbalance, and management of issues such as sterility [36,37,40]. In addition, the physiological behavior of *R. arrhizus* can change under different scale-up conditions (e.g. different nutrient gradients or shear stress), affecting growth kinetics and actual product formation rates [41]. Studies have shown that scale-up without a systematic approach often decreases yield. For the downstream processing side, purification units, such as filtration, precipitation, and liquid-liquid separation on a large scale, face the problem of biofouling, high viscosity, and product loss during processing. The efficiency of malic acid separation in industry can be lower than that of simulations assuming optimal conditions [37]. Moreover, molasses might result in process variation across batches, making maintaining long-term manufacturing stability and quality control challenging[32]. Therefore, the economic viability estimates should be interpreted as indicative potential, and more experimental validation should be carried out at a pilot scale to find and mitigate unforeseen risks related to scaling up [42]. This study offers a strong foundation for preliminary decision-making and conceptual design. Nevertheless, additional process development and optimization through more comprehensive empirical studies and risk assessments are required before commercial implementation.

- Study contributions, implications, and future research directions

This study contributes to the existing literature by delivering an in-depth techno-economic evaluation of malic acid production from molasses using *R. arrhizus.* Compared to other feedstock options, this pathway has been relatively overlooked. The assessment highlights significant economic factors and focuses on critical areas of vulnerability, such as product market price and feedstock cost, which are highly relevant to the industrial biotechnology sector's financial challenges. This study identifies some challenges and proposes mitigation strategies to improve price resilience. This framework is able to guide industry development through product diversification and continuous cost optimization.

The research offers a reliable basis for the initial assessment of investments and strategic planning, particularly for individuals looking to use molasses as a raw material in the bioeconomy. Future research focus should be directed at (1) experimental validation of yield and productivity at larger bioreactor scales, with particular attention to pH control and inhibitor mitigation strategies in molasses; (2) investigation of more intensive fermentation methods (such as fed-batch or continuous) to reduce cycle duration and increase efficiency and (3) formulation of more effective and sustainable DSP strategies to combat biofouling and minimize waste. Last, more comprehensive risk analysis and studies of diverse market scenarios will be essential in guiding future investment choices.

3.4. Practical recommendations

Taking into account the technical-economic feasibility analysis, as well as identifying various operational constraints, a series of practical recommendations have been developed for the improvement and implementation of industrial-scale malic acid production from molasses:

1. Strategy for raw material sourcing and quality control. Considering the critical role that raw material variability and molasses composition play in economic sustainability, establishing a robust sourcing strategy is strongly recommended [16]. This involves fostering long-term partnerships with local sugar mills or agro-industrial suppliers to stabilize prices and ensure the quality of raw materials. Strict quality control upon receipt of molasses (including the analysis of sugar and impurity content) and investment in simple pretreatment steps to remove inhibitors or standardize sugar composition can substantially improve fermentation performance and reduce process risks [31,32].

2. Fermentation reactor configuration and operation. The selection and design of the bioreactor configuration are

essential to achieve optimal fermentation conditions on an industrial scale. Especially for *R. arrrhizus,* which tends to form filamentous biomass, the design of a stirred tank (STR) bioreactor needs to consider the factors, including impeller design and agitation speed, ensuring the homogeneous mixing and efficient oxygen transfer, and minimizing shear stress on the cells [15,38]. In addition, the transition from batch to fed-batch or even continuous modes needs to be evaluated in detail. Fed-batch modes can help to overcome any substrate inhibition problems and increase volumetric productivity. At the same time, continuous fermentation offers the potential for a higher throughput [43]. Precise pH control in large bioreactors should also be a top priority.

3. Product purification priorities. The DSP strategy should prioritize cost efficiency and the end product purity as required by the target market [44]. For malic acid production, separating biomass, unsummed sugars, by-products, and salts requires an effective combination of technologies. Initial steps, such as membrane filtration, can efficiently separate biomass. However, biofouling management must be integrated through modular design and effective CIP schedules [37]. In addition, a focus on minimizing liquid waste from the DSP stage and potential recovery of by-products can improve overall economic viability.

4. Conclusion

A comprehensive techno-economic analysis was conducted for a batch fermentation process utilizing *Rhizopus arrhizus* to assess the feasibility of malic acid production. The production process involved three main stages: pretreatment, fermentation, and purification. The simulation model estimates that it will produce 2,830 metric tons per year. With a unit production cost of $1.66 per kilogram, this capacity covers 1.4% of global malic acid demand. The economic analysis showed a promising potential for commercial-scale implementation, characterized by a return on investment (ROI) of 22.70%, an internal rate of return (IRR) of 15.81%, net present value (NPV) of $2.14 million, and a payback period of 4.4 years. Furthermore, a sensitivity analysis highlighted those fluctuations in malic acid prices substantially affected the key economic indicators, including ROI, IRR, NPV, and payback period. However, it is crucial to acknowledge that this analysis relies on idealized simulation parameters, necessitating future pilot-scale validation and further optimization of purification methods for practical implementation.

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