CHEMICAL ENGINEERING TRANSACTIONS

VOL. 110, 2024

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Marco Bravi, Antonio Marzocchella, Giuseppe Caputo

ISBN 979-12-81206-10-6; **ISSN** 2283-9216

DOI: 10.3303/CET24110020

Ethanol Production from Soursop Leachate: Nitrogen Sources and Scale-up

Luis J. López-Giraldo*, Hernando Guerrero-Amaya, Johana A. Alvarez, Brayan S. Chacón, Juana V. Robayo

Grupo de Investigación en Ciencia y Tecnología de Alimentos-CICTA, Escuela de Ingeniería Química - Universidad Industrial de Santander, Colombia
ljlopez@uis.edu.co

Soursop (*Anona Muricata Lin*.), a tropical fruit native to Central and South America, is recognized for its rich composition of water, sugars, proteins, vitamins, minerals, and dietary fibre. Despite its potential for food and dietary supplement production, approximately 30% of the Colombian soursop production is discarded due to non-compliance with regulated standards for this highly perishable fruit. This has prompted research into utilizing these discarded residues for ethanol production through fermentation processes.
In this study, the feasibility of ethanol production from soursop leachate was assessed on a laboratory pilot scale. The investigation focused on the use of low-cost inorganic nitrogen sources, namely ammonium sulphate, ammonium chloride, and urea. Additionally, three distinct scale-up methodologies Reynolds number, volumetric power, and impeller tip speed were evaluated in working volumes of 0.5 and 5 L. A comprehensive rheological and hydrodynamic analysis was conducted using native yeasts.
The results revealed that ammonium chloride, at a carbon/nitrogen ratio of 15/1, demonstrated the highest yields, reaching 0.33 g/g. Furthermore, the study indicated that employing volumetric power with increased agitation enhanced biomass production in 4.85 g/L, while impeller tip speed with moderate agitation resulted in higher ethanol production, yielding 0.28 g/g. These findings underscore the versatility of soursop in ethanol production and emphasize the significance of selecting an appropriate scale-up methodology aligned with production objectives. The implications of this choice on the achieved yields are substantial, emphasizing the importance of tailored approaches in optimizing ethanol production from soursop leachate.

1. Introduction

Soursop (*Annona muricata L*.), an Amazonian fruit renowned for its luscious white pulp and sweet-sour flavour, has traditionally been consumed fresh or utilized in the production of various delectable products such as ice cream, creams, sweets, nectars, and juices (Pantoja et al., 2005; Reyner et al., 2017). Despite the historical dispersion of soursop trees in Colombia, recent years have witnessed their escalating significance in both national and international markets, prompting increased interest in commercial cultivation. The agro-industrial production of soursop in Colombia is estimated at approximately 50,000 tons per year (Statistics Agronet Soursop, 2022). However, a substantial portion of this yield is discarded due to non-compliance with the NTC 5208 standard, which regulates parameters like deformities, over-ripeness, impacts, or skin ruptures (Icontec, 2003), rendering it unsuitable for direct commercialization.
To address this challenge, exploring opportunities to add value to these discards has become imperative, with promise identified in the production of alcoholic beverages. Soursop inherently contains about 17.2 degrees Brix of sugars, which, through fermentation processes, can be transformed into ethanol the primary active component (Reyner et al., 2017). However, at an industrial scale, the use of nitrogen sources such as yeast extract incurs high costs, constituting, as per a study by Michalczyk et al. (2021), up to 38% of the total fermentation process costs. Consequently, the investigation into inorganic nitrogen sources, including nitrogenous salts, has gained traction, as they exhibit comparable yields to organic sources but at lower costs (Casey et al., 2013; Raposo et al., 2017).

Paper Received: 12 February 2024; Revised: 3 April 2024; Accepted: 2 June 2024
Please cite this article as: Lopez-Giraldo L.J., Guerrero-Amaya H., Alvarez J.A., Chacón B.S., Robayo J.V., 2024, Ethanol Production from Soursop Leachate: Nitrogen Sources and Scale-up, Chemical Engineering Transactions, 110, 115-120 DOI:10.3303/CET24110020

Moreover, a critical challenge facing the viability of these bioprocesses on a larger scale is maintaining the yields achieved in laboratory settings. This challenge arises from hydrodynamic changes in bioreactors, resulting in inadequate distribution of essential nutrients for microorganisms (Maluta et al., 2023; Xia et al., 2021). Notably, the literature lacks records on the study of the impact of volume changes on the fermentative yield of soursop leachates or the utilization of inorganic nitrogen sources in the fermentation process. This work seeks to address these gaps by firstly evaluating inorganic nitrogen sources at the laboratory scale, optimizing ethanol yields using leachate from non-commercially viable soursops and native yeasts from Santander. Furthermore, the study aims to elucidate the hydrodynamic behaviour and the influence of fermentation volume changes on ethanol yield.

2. Materials and methods

2.1 Preparation of Soursop and Yeast

Ripe soursop fruits were sourced from Lebrija, Santander, Colombia, selecting fresh fruits that did not meet the technical standard NTC 5208 due to physical defects such as spots, impacts, or deformities. Under aseptic conditions, the soursops were washed to eliminate any adhered residues. The manual opening was performed, separating the pulp from the leachates through manual pressing with canvas-type fabrics. The resulting leachate underwent centrifugation at 7,000 rpm for 7 minutes to remove insoluble fiber and was subsequently sterilized at 121 °C for 15 minutes.

The fermentable sugar content was analyzed using high-performance liquid chromatography (HPLC) with a Thermo Dionex Ultimate 3000 system equipped with an automatic injector, a refractive index detector, a degasser, and a COREGEL 107H column of 7.8 x 300 mm (8µm) placed in an oven at a constant temperature of 30 °C, then the leachate was diluted to 65 g/L with sterile water. Nitrogen quantification was carried out using the Kjeldahl volumetric method. Two native ethanol-producing yeasts designated as 195 and 246, were employed in the fermentation process, they registered under the access agreement to genetic resources and derivatives from Colombia, 303, were obtained from the cocoa fermentation boxes at the Experimental Farm Villa Mónica of Fedecacao. These strains are maintained in the culture collection of the Laboratory of Microbiology and Environmental Mutagenesis at the Industrial University of Santander, Colombia.

2.2 Fermentation conditions and Nitrogen Sources

The study evaluated the use of low-cost inorganic nitrogen sources (ammonium sulfate, ammonium chloride, and urea) and an organic source (peptone), as reference control. An addition, control group without added nitrogen sources was established. All sources operated at a concentration of 10 g/L. The selection of a nitrogen source considered cost and product-substrate yield (Yps) for each yeast. Carbon-nitrogen (C/N) ratios of 5/1, 15/1, and 25/1 were evaluated in terms of Yps performance for each case.

Fermentations were conducted with a working volume of 0.5 L, involving exhaustion inoculations at 30°C for 48 hours, followed by inoculums used in a 1/10 ratio with volumes of 5 and 50 mL, maintained at 30°C with agitation at 150 rpm for 12 hours. Microbial growth was quantified using a Thermo Scientific Multiskan Go spectrophotometer, and quantification of fermentable sugars and ethanol was performed through HPLC as mentioned above in Section 2.1.

2.3 Scale-Up and Rheological/Hydrodynamic Analysis

Scale-up was conducted in a New Brunswick BioFlo 110 fermenter with nominal capacities varying from 1.3 to 7.5 L, in fermentation volumes of 0.5 and 5 L. The scaling process involved two parts. Firstly, using Reynolds Number (Rei) as the scaling parameter for geometric differences in a 0.1 L Erlenmeyer flask vs. a 1.3 L fermenter with fermentation volumes of 0.05 to 0.5 L respectively, passing from orbital shaking to the bioreactor impeller using equations 1 and 2. Secondly, evaluating impeller tip speed (Vtip) and volumetric power (P/V) as scaling parameters due to geometric similarities between the fermenters with capacity of 1.3 and 7.5 L, using equations 3 and 4, as shown in Table 1 respectively. Fermentation conditions for inoculum preparation were kept constant using yeast and the inorganic nitrogen source with the highest Yps yield as shown above in section 2.2. The configurations and conditions based on the escalation parameter are elaborated in Table 2, where the number of impellers is varied to observe its impact on the process performance (Molnár et al., 2013). Rheological and hydrodynamic parameters were calculated using the equations proposed by Doran (2013) and Mandenius (2016), as detailed in the work of Imamoglu and Sukan (2013). The Anton Paar Physical MCR 302 viscometer was employed to determine viscosity, utilizing flow curves with varied strain rates.

Table 1: Mathematical equations to describe and understand the hydrodynamic and rheological behaviour of the fermentation broth.

Equation	N	Equation	N
$Re_i = \frac{N_i \rho Di^2}{\eta}$	(1)	$\gamma = kN_i$	(8)
$Re_f = \frac{nd_f^2}{v}$	(2)	$\tau = -\eta\gamma$	(9)
$v = \frac{n}{\rho}$	(3)	$\tau_f = \left(\frac{\varepsilon}{v}\right)^{\frac{1}{2}} n$	(10)
$P_o = N_p \rho N_i^3 D_i^5$	(4)	$Fr_0 = \frac{N_i^2 D_i}{g}$	(11)
$P_f = N_p' \rho n^3 d_f^4 V^{\frac{1}{3}}$	(5)	$Fr_f = \frac{(2\pi n)^2 d_o}{2g} > 0{,}4$	(12)
$N_p' = 70Re_f^{-1} + 25Re_f^{-0,6} + 1{,}5Re_f^{-0,2}$	(6)	$\varepsilon = \frac{P}{\rho V}$	(13)
$V_{tip} = N_i D_i \pi$	(7)		

Table 2: Proposed configurations and scaling parameters for each fermentation volume.

Volume scale-up, L	Methodology used	Impeller diameter, m	Stirring speed, rpm	Number of impellers
0.5	Rei	0.052	197	2
5	Vtip	0.058	178	2
5*	P/V	0.058	357	3

**Was used to identify the fermentation that was carried out using the parameter P/V in the volume of 5 L.*

3. Results and discussion

3.1 Nitrogen sources

The results of fermentations for yeast strains 195 and 246, in addition to the control group, using different nitrogen sources, are illustrated in Figure 1 (a) and (b). The nitrogen concentration in the leachate averaged 1.24%, evaluated in grams of nitrogen per 100 grams of sample, falling within the range reported by León Méndez et al., (2016), indicating that fruits typically contain between 0.1 and 1.5% nitrogenous compounds. Approximately 50% of these compounds are free amino acids available for fermentative processes (León Méndez et al., 2016). The Yps yield, reflecting the efficiency of the fermentative process, was the lowest for both yeasts without adding nitrogen source. Notably, peptone yielded the best results, with values of 0.326 and 0.384 g/g for each yeast. However, when evaluating inorganic nitrogen sources studied, the yields achieved were close to those obtained with peptone, from 0.268 to 0.294 g/g for yeast 195, and from 0.307 g/g to 0.313 g/g for yeast 246. Based on these results, it could be feasible to replace peptone, a source of organic nitrogen commonly used in small-scale fermentation processes, with an inorganic nitrogen source, which would reduce production costs.

As shown Figure 1 (b), using ammonium chloride as a nitrogen source in a 15:1 ratio achieved high Yps values for both yeasts, considering that the carbon source is the content of fermentable sugars obtained from the soursop. This aligns with studies such as that of Shankar et al., (2013), where the use of ammonium chloride with *Saccharomyces cerevisiae* MK yeast achieved greater enzyme production with a range of 0.48 IU/mL. This phenomenon can be attributed to the easy dissociation of the ammonium ion from chlorine in an aqueous medium, making them directly assimilable by yeasts (Oiv, 2022). Also is possible, if required, to take the relation 25:1, due to the fact that the yield losses are minor to 15% whit respect relation 15:1, decreased production costs.

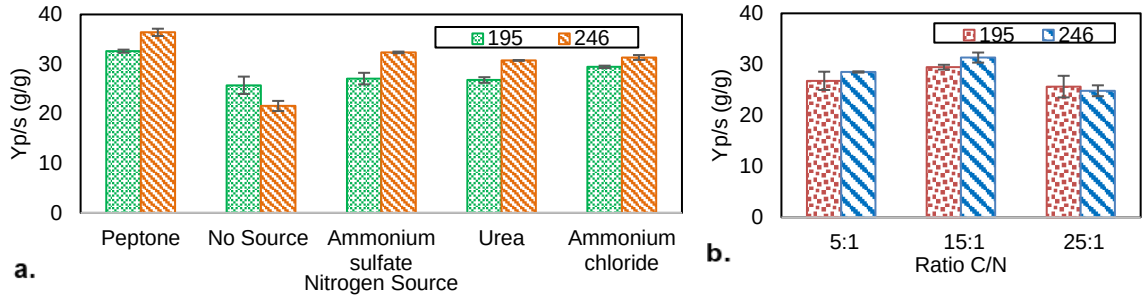

Figure 1: Yps yields for yeasts 195 and 246, a. Variation in nitrogen sources and b. Variation of C/N ratio.

3.2 Scale-up and Rheological/Hydrodynamic Analysis

Figure 2 presents microbial growth and ethanol production using different scaling parameters with yeast 246. Agitation speed varied from 150 rpm to 197 rpm for volumes from 0.05 to 0.5 L, using the Reynolds Number as the parameter. For the 5 L volume, the agitation speed ranged from 178 rpm to 357 rpm, changing the scaling parameter from Vtip to P/V. A higher overall growth is observed when using the Re parameter for the 0.5 L volume, followed by Vtip, and finally P/V for 5 L volumes, with respective Yps yields of 0.339, 0.285, and 0.236 g/g. The results of Yps performance and rheological and hydrodynamic parameters for each evaluated fermentation volume are detailed in Table 3.

In terms of ethanol and biomass production, Vtip parameter achieved values of 21.932 g/L and 10.292 g/L, respectively. Conversely, for P/V, a lower quantity of ethanol was obtained (17.208 g/L), but with higher biomass production (15.145 g/L) for the same fermentation volume. This suggests shifts in the microorganism's metabolic pathway, favoring ethanol production under more anaerobic conditions (lower agitation) and biomass production under more aerobic conditions (higher agitation). Depending on the production goal, conditions and scaling parameters may vary in a bioprocess. These findings align with Zhou et al. (2018), who investigated the effects of temperature, agitation, and aeration on the production of glycoprotein GP-1 by *Streptomyces kanasenisi* ZX01 (Zhou et al., 2018).

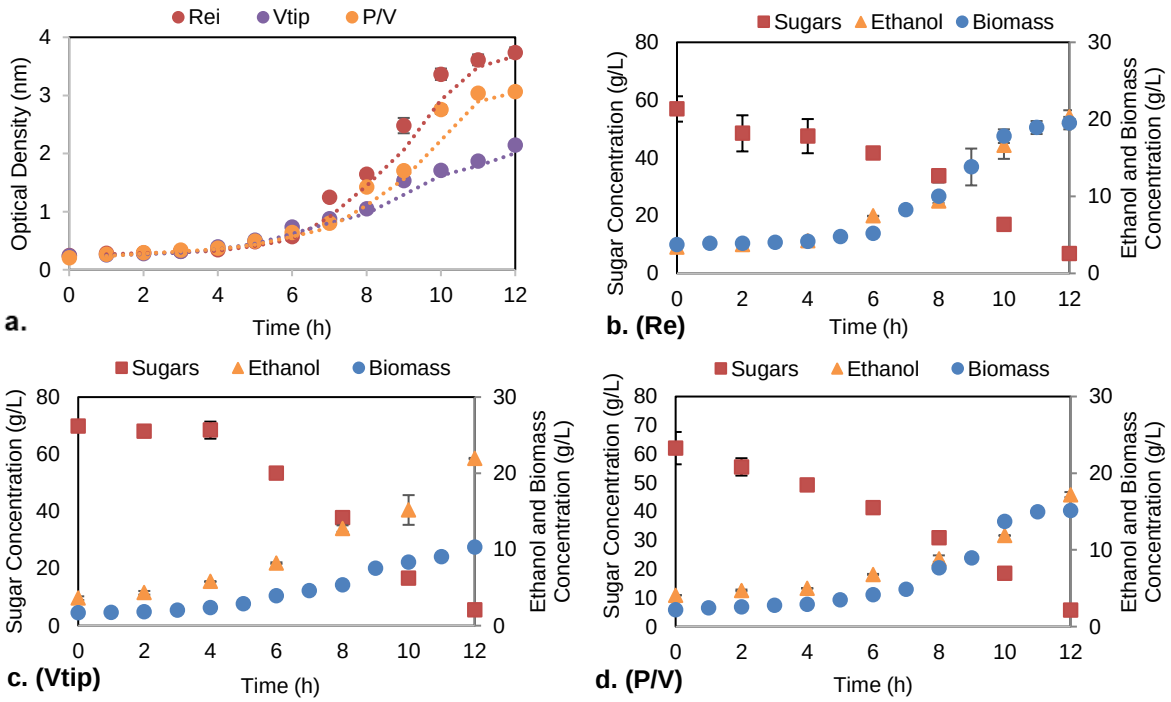

Figure 2. (a) Optical density behavior for the scale-up parameters evaluated; sugars consumption, ethanol, and biomass production for fermentations carried out with (b) Re for 0.5 L; (c) Vtip for 5 L and (d) P/V for 5 L.

The rheological parameters for the three evaluated fermentations displayed a flow transition phase based on Reynolds Number (Re), aligning with typical observations in stirred tanks. The viscosity measured at 58.66 mPa*s indicated that the sterile soursop leachate behaved as a Newtonian fluid, maintaining constant viscosity at all shear rates applied to the fluid, as principle by Doran (2013). Energy consumption, calculated as P/V in Erlenmeyer flasks and bioreactors, exhibited variations across different volumes. The 0.05 L volume with orbital agitation showed the highest energy consumption, followed by the 0.5 and 5 L volumes. Notably, the 5 L volume, utilizing Vtip as the scaling parameter, demonstrated lower energy consumption, in line with the observed increase in ethanol production during scale-up, as shown in Figure 2 (c). This emphasizes the importance of selecting appropriate scaling parameters.
The dissipation rate, shear rate, shear stress, and Froude number presented higher values for the 5 L volume, indicating that the use of P/V as a scaling methodology possibly provided efficient distribution of nutrients and oxygen. This contributed to an increase in biomass, though with higher energy consumption, underscoring the interplay between scale-up parameters, nutrient distribution, and energy efficiency. Considerations of energy efficiency, nutrient distribution, and overall process objectives should guide the selection of scaling parameters, highlighting in this process, Vtip for the ethanol production from soursop leachate.

Table 3: Yields, rheological and hydrodynamic parameters for each case evaluated.

Parameters scale-up	Volume 0.05 L	Volume 0.5 L	Volume 5 L	Volume 5 L*
Yps. g/g	0.313 ± 1.979	0.339 ± 0.016	0.285 ± 0.015	0.236 ± 0.032
Re	164.304	164.304	182.587	366.653
Vtip. m/s	--	0.544	0.544	1.093
Np	2.138	3.500	3.500	3.500
P/V. W/m^3	346.196	106.605	13.165	106.605
Fr	0.252	0.058	0.052	0.211
ε. W/kg	0.328	0.101	0.012	0.101
γ. 1/s	60.000	32.895	29.601	59.441
τ. N/m^2	2.748	1.930	1.736	3.487

**Used to identify the fermentation that was carried out using the parameter P/V in the volume of 5 L.*

4. Conclusions

Ammonium chloride was the best inorganic nitrogen source, maximizing Yps yield at 0.313 g/g in fermentations with soursop leachate. This is realized at a fermentable sugar concentration of 65 g/L and a C:N ratio of 15:1. Implementation of ammonium chloride offers the potential for substantial cost reductions, when compared to the use of an organic nitrogen source. This could be cost-effective alternative enhances the economic feasibility of ethanol production from soursop leachate. Moreover, main the pivotal role of scaling methodology in influencing ethanol production, biomass, and energy consumption during soursop leachate fermentation.
The volumetric power as a scaling method favoured biomass production, promoting homogeneity in the fermentation broth. Though, with higher energy consumption and a lower ethanol production. On the other hand, the impeller tip speed stimulated ethanol production, with superior energy efficiency. This approach resulted in a lower biomass concentration, potentially indicative of alterations in the yeast's metabolic pathway. In conclusion, the selection of the scaling method should be tailored to the specific objectives of the fermentation process, considering both yield optimization and energy efficiency. In this study, impeller tip speed was identified as the appropriate scaling parameter, aligning with the goal of achieving maximum ethanol yields from soursop leachate.

Nomenclature

Yps – Substrate product yield. g/g
Re_i – Impeller Reynolds number, dimensionless
Ref – Reynolds number for shaken flask, dimensionless
Vtip – Impeller tip speed. m/s
P/V – Power consumption per unit volume of liquid. W/m^3
Fr – Froude number, dimensionless
ε – Energy dissipation rate per unit mass of fluid. W/kg
γ – Shear rate. 1/s
τ – Shear stress. N/m^2
η – Dynamic viscosity of fluid. kg/(m.s)
υ – Kinematic viscosity of fluid. m^2/s
ρ – Fluid density. kg/m^3
Di - Impeller diameter. m
Df – largest inner diameter of shaken flask. m
Do – Shaking diameter. m
k – constant with magnitude dependent on the geometry of the impeller, dimensionless.
Np – Power number for stirred tank, dimensionless.
Ni – Impeller rotation speed, rpm

Acknowledgments

The authors express them sincerely to Ciencia y Tecnología de Alimentos (CICTA) for technical support in chemical analyses. Likewise, for the farmers of Vereda Puyana, Lebrija, Santander, Colombia, for providing the fruits used.

References

Ammonium chloride International Oenological CODEX. 2022. International Organisation of Vine and Wine OIV. <www.oiv.int/standards/international-oenological-codex/part-i-monographs/monographs/ammonium-chloride> accessed 10.12.2023.

Casey, E., Mosier, N. S., Adamec, J., Stockdale, Z., Ho, N., & Sedlak, M. 2013. Effect of salts on the Co-fermentation of glucose and xylose by a genetically engineered strain of Saccharomyces cerevisiae. Biotechnology for Biofuels 6(1), 1–10. doi.org/10.1186/1754-6834-6-83/FIGURES/8.

Doran, P. 2013. Cap 8.1 Functions of Mixing. Bioprocess Engineering Principles, 255–332. doi.org/10.1016/B978-0-12-220851-5.00008-3.

Statistics Agronet Soursop (in Spanish). 2022. Agronet Gov <agronet.gov.co/estadistica/Paginas/home.aspx?cod=1> accessed 10.12.2023

Fresh fruits. Soursop Specifications NTC 5208 (in Spanish). Icontec CO <tienda.icontec.org/gp-frutas-frescas-guanabana-especificaciones-ntc5208-2003.html> accessed 10.12.2023.

Imamoglu, E., & Sukan, F. V. 2013. Scale-up and kinetic modeling for bioethanol production. Bioresource Technology 144, 311–320. https://doi.org/10.1016/j.biortech.2013.06.118

León Méndez, G., Granados Conde, C., Rosario, D., & Fortich, O. 2016. Characterization of the pulp of Annona Muricata L. grown in the north of Bolívar Department in Colombia (in Spanish). Revista Cubana de Plantas Medicinales, 21(4). CU.

Maluta, F., Alberini, F., Paglianti, A., & Montante, G. 2023. Hydrodynamics and Scale-up of Anaerobic Stirred Digesters. Chemical Engineering Transactions, 105, 289-294. https://doi.org/10.3303/CET23105049

Mandenius, C.-F. 2016. Bioreactors Design, Operation and Novel Applications. WILEY-VCH. SW. 323-347

Michalczyk, A. K., Garbaczewska, S., Morytz, B., Białek, A., & Zakrzewski, J. 2021. Influence of Nitrogen Sources on D-Lactic Acid Biosynthesis by Sporolactobacillus laevolacticus DSM 442 Strain. Fermentation 2021, Vol. 7(2), 78-79. https://doi.org/10.3390/FERMENTATION7020078

Molnar, B., Egedy, A., & Varga, T. 2013. CFD Model Based Comparison of Mixing Efficiency of Different Impeller Geometries. Chemical Engineering Transactions, 32, 1453-1458. https://doi.org/10.3303/CET1332243

Pantoja, L., Maeda, R., Carvalho, S. M. S., Aguiar, J., Monteiro, F. M., & Lima, Q. A. 2005. Biotechnological use of soursop in the production of fermented alcoholic beverages using yeast immobilized in calcium alginate (in Spanish). Brazilian Journal of Food Technology. Special Issue V Sipal, 96–102. BR.

Raposo, S., Constantino, A., Rodrigues, F., Rodrigues, B., & Lima-Costa, M. E. 2017. Nitrogen Sources Screening for Ethanol Production Using Carob Industrial Wastes. Applied Biochemistry and Biotechnology, 181(2), 827–843. doi.org/10.1007/S12010-016-2252-Z.

Reyner, M., Huaman, C., Alfredo, M., Lopez, G., Bernardo, A., Caballero, G., Sandro, J., Romero, S., Manuel, O., & Saavedra, M. 2017. Preparation and commercialization of Soursop Cocktail. <hdl.handle.net/20.500.14005/2920> accessed 10.12.2023.

Shankar, T., Thangamathi, P., & T. Sivakumar, R. R. 2013. Optimization of Invertase Production Using Saccharomyces Cerevisiae MK under Varying Cultural Conditions. International Journal of Biochemistry and Biophysics, 1(3). doi.org/10.13189/ijbb.2013.010301.

Xia, J., Wang, G., Fan, M., Chen, M., Wang, Z., & Zhuang, Y. 2021. Understanding the scale-up of fermentation processes from the viewpoint of the flow field in bioreactors and the physiological response of strains. Chinese Journal of Chemical Engineering, 30, 178–184. doi.org/10.1016/J.CJCHE.2020.12.004

Zhou, Y., Han, L. R., He, H. W., Sang, B., Yu, D. L., Feng, J. T., & Zhang, X. 2018. Effects of Agitation, Aeration and Temperature on Production of a Novel Glycoprotein GP-1 by Streptomyces kanasenisi ZX01 and Scale-Up Based on Volumetric Oxygen Transfer Coefficient. Molecules 2018, Vol. 23, Page 125, 23(1), 125. doi.org/10.3390/MOLECULES23010125