BIOTECHNOLOGICAL POTENTIAL OF SOYBEAN VINASSE IN SOYBEAN BIOREFINERY: A LITERATURE REVIEW AND PATENT SEARCH

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ABSTRACT

Soybean vinasse is a byproduct generated during the distillation process after the fermentation of soybean molasses for ethanol production. As it is a residue rich in oligosaccharides, such as raffinose and stachyose, soybean vinasse has significant potential for application in biotechnological processes, thereby contributing to the operations of a soybean biorefinery. In this study, a technological mapping in patent database and a bibliographic review of research articles were conducted. The patent search resulted in 111 documents while the scientific articles search resulted in 45 documents. After refining by reading the titles and abstracts, 7 patents and 4 articles described the specific application of soybean vinasse in bioprocesses and its potential industrial application. Brazil stands out in the number of documents found, both in patents and research articles, being the only country currently developing technologies for soybean vinasse fermentation. The study concludes that soybean vinasse can be applied in industrial scale biorefineries to produce various products, including organic molecules and enzymes. The research underscores the necessity for continued study of this residue, highlighting its potential for sustainable development and enhanced circular economy practices within the soybean industry.

Keywords: Biorefinery. Soybean vinasse. Patent search.

1 INTRODUCTION

In Brazil, soybeans played a crucial role in introducing the concept of agribusiness and currently have a production volume of more than 120 million tons, generating revenue of 340 billion reais.[1] Brazil is also the second largest oilseed processing hub, focused mainly on the production of concentrated and isolated soybean oil and bran.[2] The process involves transforming grains into soy flakes that undergo cleaning, drying, breaking, dehulling and expansion by extrusion, which makes it more compact, porous and dense, enabling the extraction of its oil and the production of soybean meal. [3] This bran, already defatted, can be washed with a hydroalcoholic solution, in which the sugars and other soluble compounds are removed, and soybean bran concentrate (SPC) and molasses are produced. [4] Soy molasses, a dense syrup rich in sugars like stachyose, raffinose, sucrose, glucose, and fructose, can be converted into bioethanol through fermentation with *Saccharomyces cerevisiae.* [5] This process supports a circular economy by using the distilled product as a solvent in soy protein concentrate manufacturing. However, the process produces soy vinasse, a by-product with significant solids, including non-fermentable sugars like stachyose (11.1%) and raffinose (22.1%), along with proteins (13.3%), lipids (27.8%), and fibers (14.6%). Soy vinasse also has a high heat capacity (3000 kcal/kg at 80ºBrix) and high biochemical oxygen demand (77.2 gO2/L). [3 6] All these characteristics mean that this waste cannot be easily treated as a common industrial effluent, making it necessary to define different strategies for its recovery.

Currently, vinasse is used as a waste fuel for generating steam in boilers. This involves concentrating vinasse to 30-85 ºBrix, heating it to 100 ºC with steam, enabling complete combustion to produce thermal energy that can fuel the production line in which it is produced, promoting self-sufficiency in production. [7] Although this process is highly energy-efficient, this method wastes potential substrates in vinasse that could be used for fermenting high-value products. Currently, Brazil's vinasse production occurs in three manufacturing plants, located in different states: Mato Grosso, Minas Gerais, and Paraná. The expansion of soy biorefineries and new soy alcohol industries will increase vinasse production. This study aims to map technologies and review literature on using soy vinasse in various fermentation processes to evaluate its applications, yields, and the microorganisms involved in biotechnological processing.

2 MATERIAL & METHODS

The patent search was carried out in the Derwent Innovations Index database (https://www.webofscience.com/wos/diidw/advanced-search) using the IPC code C12P* that is associated to fermentation or enzyme-using processes to synthesize a desired chemical compound or composition or to separate optical isomers from a racemic mixture. This IPC code was combined with the keywords ["soy*" or "glycine max" or "g. max") and ("vinasse" or "vine" or "wastewater" or "distillery slop" or "distillery waste" or "stillage "or "distillery residue" or "pot ale" or "distillery bottoms"))] in the field Topic, that includes the title and abstract of the patent documents. The patent search, run on April 17th, 2024, without limiting a time frame, resulted in 111 patent documents, which were individually analyzed to select those that were in fact related to soy vinasse processing. This manual refining, based exclusively on the technical content of the titles and abstracts, resulted in 7 patent families. Data for these patent documents were collected, tabulated and analyzed.

The articles search was carried out through the Web of Science Core Collection (https://www-webofscience.ez22.periodicos.capes.gov.br/wos/woscc/advanced-search) with the combined keywords: "soy* vinasse" or "soy * vine" or "soy wastewater*" or "soy distillery residue*" or "soy distillery residue*" or "soy vinasse*" or "soy distillery residue*" or "soy distillery residue*" or "soy distillery residue*", in the topic field, which comprises title, summary, plus keyword and author keywords. The articles search, carried out on April 18, 2024, with no time limit, resulted in 45 article documents, which were analyzed individually to select those that were indeed related to soy vinasse processing. This manual refinement, based exclusively on the technical content of the titles and abstracts, resulted in 4 article documents that were used for data collection, tabulation and analysis.

3 PATENT SEARCH

After manually screening the patent search, 7 documents were identified as related to soy vinasse processing technologies. Of the 111 patents found, 49 referred to fermentative processes utilizing other types of waste, such as soy or soybean wastewater, which includes residues from the tofu industry. Additionally, 15 patents documented the use of waste from various sources but supplemented the culture medium or final product with soy peptone, soybean meal, and other soy derivatives products.

Among the documents that discuss the application of soy vinasse for producing new products, the results extend beyond just the biotechnological use of this residue and encompass the production of vinasse from plant-based materials, in which soybean hulls are added to a mixture containing hydrolyzed carbohydrate-rich raw material. After ethanolic fermentation, the alcohol is separated from the vinasse, so that this process is useful for producing ethanol and whole stillage, with the whole stillage being applicable in nutritional, nutraceutical, flavor-enhancing, cosmetic, and pharmaceutical products. [8 9]

Vinasse, once produced, can be concentrated into syrup form, so that the condensed vinasse can be treated in an anaerobic digester to produce biogas, which can be used as fuel or upgraded to higher quality biomethane. This process not only produces biogas but also treats the effluent. [10] Among the two results found in the search for patents to produce biogas from soy vinasse, the technology to produce microbial oil from vinasse and, sequentially, the treatment of the effluent from this process for the production of biogas stand out. This technology encompasses an integrated process for producing oil and biogas from vinasse. Lipids are produced from vinasse by the oleaginous yeast *Yarrowia lipolytica* under aerobic conditions, while biogas is obtained anaerobically from the effluent of the lipid production process. These lipids, in turn, are extracted and used to produce biodiesel and antifoam. Furthermore, the biogas produced from the effluent of the lipid production process serves as a method for reducing the organic load. [11]

In terms of producing products with high added value, from soy vinasse can be used as a substrate for producing alpha-galactosidase (EC 3.2.1.22) through a submerged fermentation process. Alpha-galactosidase is an enzyme capable of catalyzing the hydrolysis of alpha-(1,6)-galactosidic bonds, found in oligosaccharides such as stachyose, melibiose and raffinose, causing the release of alpha-D-galactose. These oligosaccharides can be found in residues such as vinasse itself and soy molasses, and the application of this enzyme is an alternative for converting these into fermentable sugars. In this process, the microorganism used is *Leuconostoc mesenteroides* INRA 23, so that the proposed process allows us to obtain values of 5.0 to 12.0 U/mL of enzymatic activity for alpha-galactosidase. [12]

4 LITERATURE REVIEW

The manual refinement of the literature search resulted in 4 documents where soybean vinasse is used as a carbon source in culture media. On the other hand, 41 documents presented in the search do not refer to the application of soybean wastewater but rather to other residues produced during soybean processing, either to produce soybean oil or soy dairies. The syntax used for the articles search restricted the application to soybean processing wastes and did not extend to residues originating from other matrices.

The production of α-galactosidase through submerged fermentation using soybean vinasse as a substrate was also proposed in the literature. *Lactobacillus agilis* LPB 56 was selected for its highest enzymatic activity among nine strains. The optimal conditions for α-galactosidase production were found to be 30% soluble solids, a C/N ratio of 9, and a 25% (v/v) inoculum size, resulting in the highest enzyme activity (11.07 U/mL) achieved after 144 hours of fermentation. [13]

Soybean vinasse was also applied as a substrate for producing L-lactic acid from soybean vinasse on both laboratory and pilot scales. The use of vinasse enriched with soybean molasses resulted in a higher lactic acid concentration (138 g/L). The soybean vinasse and molasses provided the necessary nutrients for bacterial growth and lactic acid production, eliminating the need for supplementation with inorganic nitrogen sources and yeast extract. *Lactobacillus agilis* LPB 56 was selected from among 10 strains, achieving an 85% yield in converting stachyose and raffinose into lactic acid at the pilot scale. [6]

Studies have been conducted on the field of applying soybean vinasse as a low-cost medium to replace commercial media for producing steam sterilization biological indicators. Various recovery media were created using soybean or sugarcane molasses and vinasse to prepare a self-contained BI based on *G. stearothermophilus* ATCC 7953 growth. A medium with a soybean vinasse 1.4% w/v yielded the best results, with heat resistance ($D_{121\,°C}$ value) of 2.9 ± 0.5 min and Spearman–Kaber heating time/dosage estimate (Usk) of 12.7 ± 2.1 min. Therefore, this study showed that the viability of *G. stearothermophilus* spores can be assessed equally well by a standard broth culture and soybean vinasse medium. [14]

Soybean molasses and nutrient-supplemented vinasse were also evaluated for their effectiveness as media for *Bacillus atrophaeus* spores, which are used as biological indicators to monitor sterilization processes. Results showed that vinasse medium were capable of promoting the microorganism growth, even adding no supplements, achieving 4.3×10^7 CFU mL^{-1}. The addition of complementary carbon and nitrogen sources to soybean vinasses did not increase biomass production. [15]

5 BIOTECHNOLOGICAL APPLICATIONS

Encompassing both patent search and literature review, a total of six biotechnological applications for soybean vinasse were found. These applications involve using vinasse as a component of the cultivation medium (Table 1). Of the six applications, two are patents filed in Brazil, and four are research articles developed in Brazil. This is related to Brazil being the second-largest producer of soybean oil in the world. It also demonstrates that the country is actively engaged in research on soybean biorefineries and circularity, besides of being pioneer in soybean ethanol production globally.

Table 1 Biotechnological products from soybean vinasses

Reference	Type of document	Product	Microorganism
(SOCCOL; IAREMA; VANDENBERGHE, 2015)	Patent	Alpha-galactosidase	*Leuconostoc mesenteroides* INRA 23
(VILELA, 2015)	Patent	Oil/Biogas	*Yarrowia lipolitica*
(DLUGOKENSKI et al., 2011)	Article	Sterilization biological indicator	*Geobacillus stearothermophilus* ATCC 7953
(KARP et al., 2011)	Article	Ácido L-lático	*Lactobacillus agilis* LPB 56
(SANADA et al., 2009).	Article	Alpha-galactosidase	*Lactobacillus agilis* LPB 56
(SELLA et al., 2008)	Article	Sterilization biological indicator	*Bacillus atrophaeus*

6 CONCLUSION

Patent searches and literature reviews demonstrate that soy vinasse can be industrially applied to produce various bioproducts, such as enzymes and organic molecules. However, the limited number of technologies developed for using soybean vinasse as a fermentation substrate underscores a field with significant potential for innovation and development. Given the increasing number of soybean biorefineries in Brazil implementing ethanol production from soy molasses, the importance of harnessing the potential of vinasse becomes evident, particularly when considering its rich composition in carbohydrates, such as oligosaccharides. Therefore, investing in research on the use of soy vinasse can significantly contribute to sustainable development and circularity in the industry.

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