PHENOLICS COMPOUNDS PRODUCTION BY *Micrococcus luteus* USING SUGARCANE BAGASSE PRETREATED WITH OZONOLYSIS AS SUBSTRATE

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ABSTRACT

Sugarcane cultivation in Brazil for sugar and alcohol production yields significant agricultural waste. The challenge lies in utilizing lignocellulosic residues in biotechnology due to their complex structures, necessitating specific treatments to break down the resistant lignin in bagasse fibers. Chemical treatments like acid or alkaline hydrolysis are common, but pretreatment methods enhance hydrolysis efficiency. Among oxidizing agents, ozone is highly reactive towards substances with electron-dense functional groups, notably the alkene bonds in lignin. Its benefits include effective conversion of lignin into derivatives, absence of toxic residues, and operation at ambient conditions. This study explores ozonolysis as a pretreatment method for lignocellulosic material, aiming to produce vanillin through microbial biotransformation. *Micrococcus luteus* exhibited significant vanillin production (145 mg/L) after 10 hours of cultivation with hydrothermally treated compounds. This highlights the potential for economically valuable compounds such as vanillin and acetovanillone from hydrolyzed sugarcane bagasse using microbial culture. Overall, this approach demonstrates a sustainable strategy for converting agricultural waste into valuable compounds through biotechnological processes.

Keywords: Ozonolysis. Ferulic acid. Bioconversion. Vanillin. Sugarcane bagasse.

1 INTRODUCTION

Ozone is the most reactive oxidant for substances with high electron density functional groups [1, 2], primarily reacting with alkenes via the Criegee mechanism [3]. Its selectivity towards lignin over carbohydrates allows for effective degradation in lignocellulosic materials like sugarcane bagasse, making it advantageous for separating lignin from other components [4]. Ozone offers efficient bioconversion of lignin without toxic residue generation, albeit at a higher cost due to ozone quantity requirements [5]. Pre-treatment mechanisms with ozone are commonly employed in wood bleaching and water treatment industries [6, 7, 8]. The biotechnological utilization of lignocellulosic biomass involves subsequent chemical or biological transformation processes to obtain value-added products. Biological processes, such as microbial cultivation or enzyme utilization, offer cost-effective and pure product alternatives. Ferulic acid, a significant lignocellulosic derivative, can be biotransformed into vanillin, a compound widely used in food and pharmaceutical industries [9, 10, 11]. With Brazil being a leading producer of sugarcane and its derivatives, there's immense potential for developing technologies to harness these materials for value-added products. This study aimed to test ozone pre-treatment in lignocellulosic material to produce vanillin through microbial biotransformation.

2 MATERIAL & METHODS

We took 20g samples of sugarcane bagasse, which were washed, dried, crushed, and sieved to sizes ranging between 1.41 and 2.83mm. These samples then underwent ozonolysis at a flow rate of 0.624 mg/min^{-1} for 10 minutes, employing a rotation scheme to ensure uniform ozone exposure. Following ozonolysis, the pre-treated samples were subjected to hydrothermal hydrolysis using ultrapure water at a ratio of 95mL per 5g of ozonated bagasse. The hydrolysis took place in 250mL Erlenmeyer flasks, sealed with aluminum foil, and placed in an autoclave at 1 atm and 120 °C for one hour. After completion, the samples were filtered, retaining the liquid while discarding the solid residue.

To assess the ability of *Micrococcus luteus* to convert lignin-derived compounds, primarily ferulic acid, into vanillin and related substances like acetovanillone, we utilized the hydrolysates obtained from the previous step for fermentation. Each fermentation trial involved adding 10 or 50% of bagasse hydrolysates to 15mL of LB culture medium, supplemented with a 10µL bacterial inoculum (OD 600 nm = 0.8). The mixture was then incubated at 32°C and 150 rpm for ten hours. Every two hours, we collected 200µL aliquots from the fermentation medium for absorbance-based growth determination and removed 2mL aliquots for chromatographic analysis and pH measurement.

Analysis of the collected aliquots during fermentation, as well as the initial hydrolysates, was performed using high-performance liquid chromatography (HPLC). Solvent "A" comprised 5 mM ammonium formate + 0.1% formic acid in water, while solvent "B" consisted of 5 mM ammonium formate + 0.1% formic acid in methanol.

3 RESULTS & DISCUSSION

We assessed the effectiveness of the treatment and its capacity to generate derivative compounds suitable for bacterial metabolism, leading to vanillin production. Specifically, *Micrococcus luteus* yielded 145 mg/L of vanillin after a 10-hour cultivation period using hydrothermally treated compounds (Figure 1 and 2), indicating the promising potential of microbial culture in acquiring economically valuable compounds such as vanillin and acetovanillone from hydrolyzed sugarcane bagasse. Additionally, the ozone pre-treatment itself produced substances like coumaric acid (32 mg/L) and acetovanillone (21 mg/L), which can either serve as end-products or act as precursors for other compounds within the industry (Figure 3).

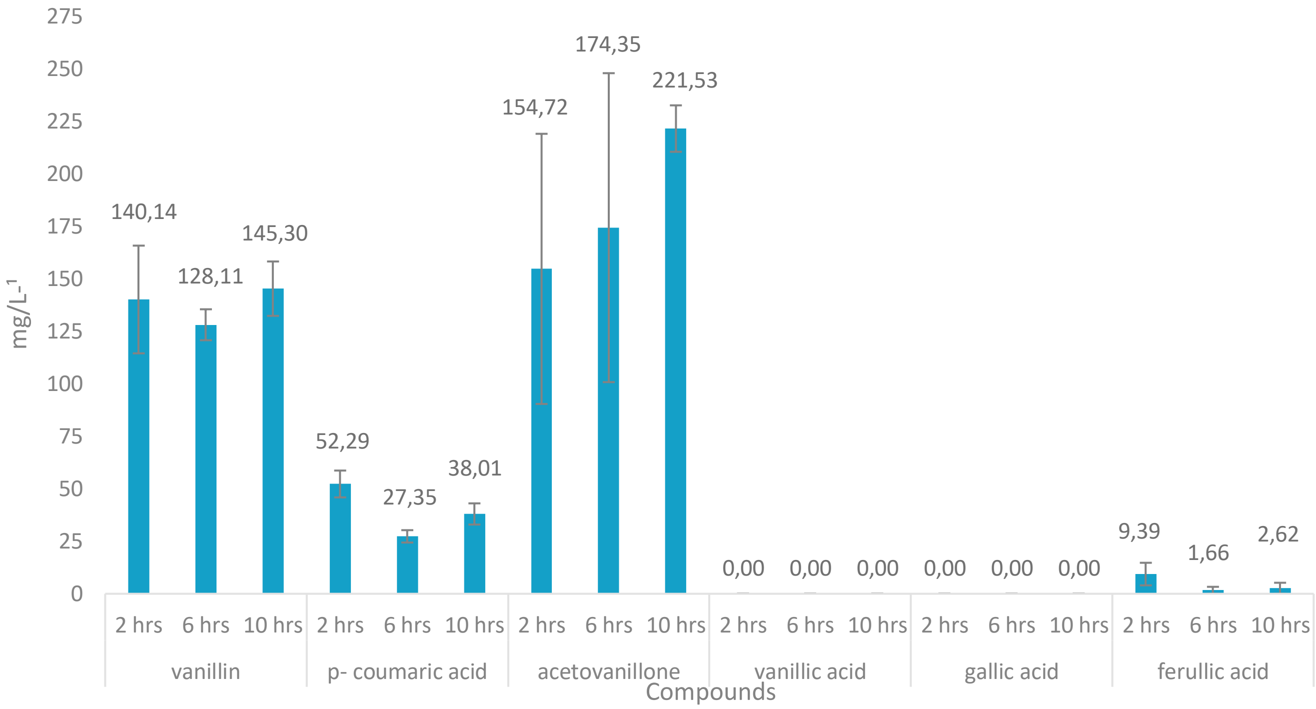

Figure 1 Overview of compound concentration for *M. luteus* cultures with liqueurs hydrothermally extracted from bagasse subjected to ozonolysis for 10 minutes. Cultures had 10% of the liquor concentration. Values expressed in mg/L^{-1}.

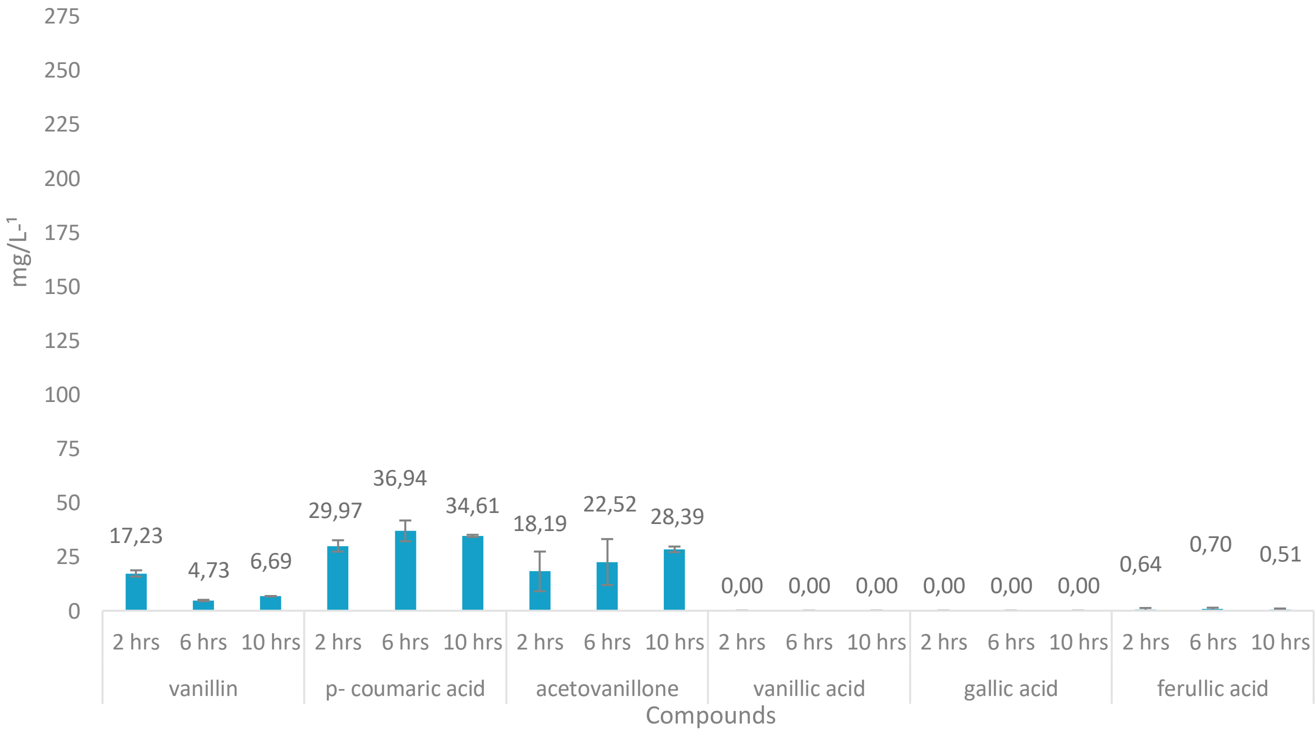

Figure 2. Overview of compound concentration for M. luteus cultures with liqueurs hydrothermally extracted from bagasse subjected to ozonolysis for 10 minutes. Cultures had 50% of the liquor concentration. Values expressed in mg/L-1.

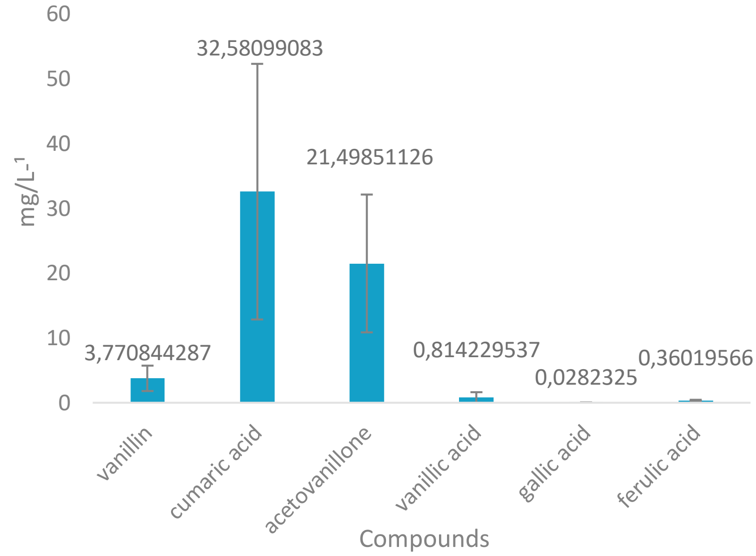

Figure 3 Quantification of compounds in sugarcane bagasse extracts treated with ozonolysis for 10 minutes and extracted with water. Values expressed in mg/L^{-1}.

4 CONCLUSION

The results obtained in this study allowed us to conclude that the bacterium *M. luteus* was able to tolerate and transform compounds present in ozonated and hydrothermally treated sugarcane bagasse hydrolysates. Chromatographic data revealed that compounds such as vanillin and acetovanillone are products of this biotransformation, and the decrease in ferulic acid concentrations during the production of these compounds suggests it as the precursor substrate for this biotransformation process. Although coumaric acid was detected in all samples, no correlation was observed between this compound and vanillin production. *M. luteus* was able to produced the highest amount of vanillin when cultivated in a medium containing only 10% of the hydrothermal extraction liquor, indicating that the highest production occurred in assays with the lowest extract concentration, making this economically and environmentally the best extract. Overall, the results demonstrate the feasibility of obtaining economically important compounds such as vanillin and acetovanillone from hydrolyzed sugarcane bagasse using microbial cultivation.

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