

Production of acetic acid from vine shoots via phosphoric acid-catalyzed steam explosion and fermentation

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1. Introduction

The transition towards a circular bioeconomy requires the efficient valorization of agro-industrial residues into high-value platform chemicals. Volatile fatty acids, particularly acetic acid, have gained attention due to their wide range of industrial applications, prompting the search for sustainable production routes from renewable biomass (Atasoy et al., 2018). Vine shoots represent an abundant and underutilized lignocellulosic feedstock in viticulture regions, possessing a significant carbohydrate content suitable for fermentation processes. However, the recalcitrant nature of this biomass requires effective fractionation strategies. This study evaluates the bioconversion of vine shoots into acetic acid using a phosphoric acid-catalyzed steam explosion pretreatment followed by detoxification and fermentation with *Escherichia coli* and *Staphylococcus epidermidis* strains.

2. Materials and methods

2.1 Raw materials: Vine shoots were air-dried (10.3% moisture), milled (<1 cm), and homogenized. On a dry basis, the biomass contained 37.3% cellulose, 18.5% hemicellulose, and 23.9% lignin (Silva Rabelo et al., 2023).

2.2 Steam Explosion Pretreatment: To increase the production of fermentable sugars, vine shoots were impregnated with phosphoric acid (2.5% w/v, 12 h) and pretreated with steam explosion (190°C, 5 min). Afterwards, the mixture obtained was separated by vacuum filtration and the liquid phase (64.3% oligomers) was subjected to acid hydrolysis to convert the oligomers into monomers (acidic liquor) and used for the subsequent fermentation.

2.3 Combined Detoxification (Activated Carbon Plus Ion Exchange Resins): Prior fermentation, the acidic liquor was detoxified by a sequential process: activated carbon (8% w/v) followed by ion exchange resins (10 % w/v). All steps were carried out at 50 °C and 150 rpm for 30 min, with phase separation performed via vacuum filtration after each stage.

2.4 Microorganisms and inocula: Inocula of *E. coli* SL 100, and *S. epidermidis* were cultivated in a standard liquid medium (1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl). Microorganisms were incubated in 100 mL Erlenmeyer flasks (25 mL working volume) at 37 °C and 150 rpm for 24 h.

2.5 Fermentation of acidic liquors: Fermentation assays were carried out in 100-mL Erlenmeyer flasks. Detoxified liquors were adjusted to pH 6.7 using KOH and H₂SO₄ as needed. A volume of 25 mL of liquor was added to each flask, and experiments were performed in triplicate. A 2.5 mL inoculum (10% v/v) was added to the total volume. The medium was supplemented with yeast extract (10 g/L) and NaHCO₃ (1 g/L). Fermentation was conducted at 37 °C and 150 rpm in a shaking incubator. The total fermentation time was 72 h, with 0.5 mL samples collected at 24, 48, and 72 h.

2.6 Analytical Methods: Sugars and acetic acid were quantified by HPLC using Waters Prostar and Agilent Technologies systems, respectively. All assays were performed in triplicate with a relative standard deviation (RSD) < 3%.

3. Results and discussion

The acidic liquor obtained from the pretreatment of vine shoots with steam explosion was subjected to acid hydrolysis, resulting in a medium containing mainly 14 g/L of glucose and 15 g/L of xylose. Direct fermentation of this medium did not yield any results due to the presence of inhibitors. Therefore, the medium was detoxified before fermentation. During fermentation with *E. coli* and *S. epidermidis*, a preferential consumption of glucose can be observed. Glucose was consumed, with more than 90 % of its initial concentration depleted by 48 h, at which point levels fell below 1.3 g/L. In contrast, xylose assimilation remained limited to an overall range of 24–27 %, a phenomenon attributable to the carbon catabolite repression typical in the presence of glucose (Chiang et al., 2013). Focusing on acetic acid production, it can be observed some difference between final concentration and yield. Although *S. epidermidis* achieved the highest acetic acid concentration (12.7 g/L) (Figure 1a), it provides a yield of 0.43 g/g, as it incorporates a significant amount of pre-existing acetate in the medium (~5.9 g/L). Conversely, *E. coli* SL100 demonstrated superior bioconversion

capability when considering net production; this strain synthesized 9.8 g/L of acetic acid from 17.3 g/L of total metabolized sugars (Figure 2b). In this case, the yield obtained was 0.56 g/g, higher than the one obtained on the fermentation with *S. epidermidis*. These results are comparable to yields reported for recombinant strains on corn hydrolysates (0.5 g/g) (Zhu et al., 2024), highlighting the potential of the SL100 strain for sugar valorization.

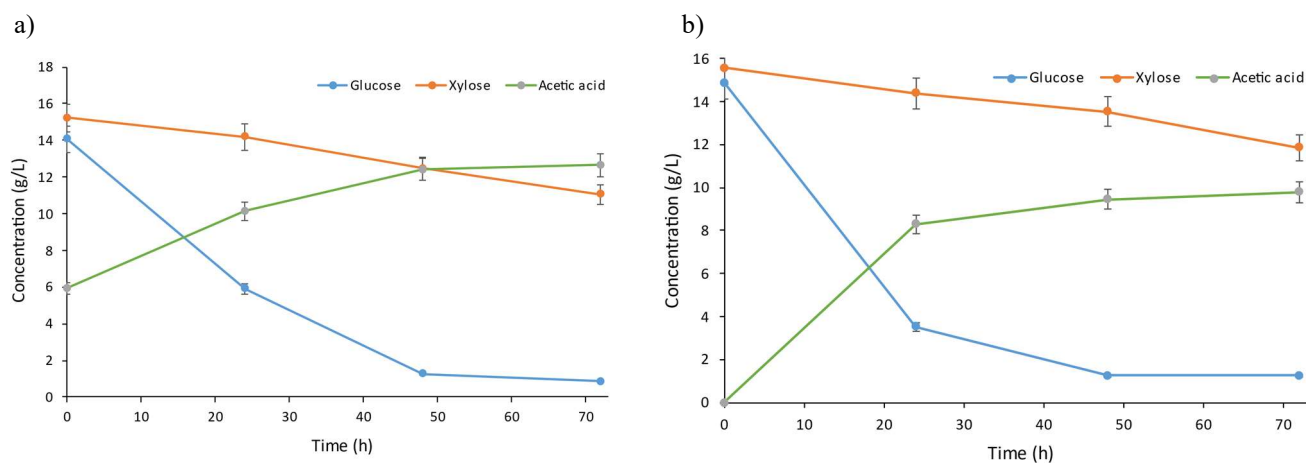


Figure 1. Acetic acid production through fermentation with *S. epidermidis* (a) and *E. coli* SL100 (b).

4. Conclusion

This study shows that phosphoric acid-catalyzed steam explosion is an effective pretreatment to overcome the recalcitrance of vine shoots, producing a hydrolysate suitable for bacterial fermentation. A key finding is the metabolic trade-off observed between the strains: while *S. epidermidis* shows robustness in final product accumulation, *E. coli* SL100 stands out as the superior candidate for industrial upscaling due to its higher conversion efficiency per unit of sugar. Ultimately, this work establishes a validated pathway to valorize viticulture waste, offering a competitive alternative to fossil-based acetic acid production.

5. References

Atasoy, M., Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresource Technology*, 268, 773–786. <https://doi.org/10.1016/j.biortech.2018.07.042>

Chiang, C., Lee, H. M., Guo, H. J., Wang, Z. W., Lin, L., & Chao, Y. (2013). Systematic approach to engineer *Escherichia coli* pathways for co-utilization of a glucose–xylose mixture. *Journal of Agricultural and Food Chemistry*, 61(31), 7583–7590. <https://doi.org/10.1021/jf401230r>

Silva Rabelo, C. A. B., Vidal, A. M., Casanova-González, R., Romero-García, J. M., Varesche, M. B. A., Romero, I., & Castro, E. (2023). Vine shoots pre-treatment strategies for improved hydrogen production and metabolites redistribution in *Clostridium butyricum*. *Industrial Crops and Products*, 204, 117317. <https://doi.org/10.1016/j.indcrop.2023.117317>

Zhu, J., Liu, W., Guo, L., Tan, X., Sun, W., Zhang, H., Zhang, H., Tian, W., Jiang, T., Meng, W., Liu, Y., Kang, Z., Gao, C., Lü, C., Xu, P., & Ma, C. (2024). Acetate production from corn stover hydrolysate using recombinant *Escherichia coli* BL21 (DE3) with an EP-bifido pathway. *Microbial Cell Factories*, 23(1), 300. <https://doi.org/10.1186/s12934-024-02575-y>

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