

Green version

**n-Butanol production from mixtures of sugarcane molasses and lignocellulosic hydrolysate**

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In the context of 2G ethanol production from sugarcane bagasse integrated into existing plants, an important fraction of pentose (C5) sugars from the lignocellulosic hydrolysate material cannot be metabolized by *Saccharomyces cerevisiae*, traditionally used in 1G plants. Hence, the use of microorganisms that naturally metabolize pentose sugars is highly desirable. Among the possibilities of using the C5 as feedstock, the n-butanol production is attracting interest due to its significant advantages over existing biofuels. It is considered a superior biofuel with energy content of 29.2 MJ/L and similarity properties with gasoline. n-Butanol can be produced naturally by Acetone-Butanol-Ethanol fermentation using solventogenic *Clostridium*, capable to metabolize naturally C5 sugars and others wide variety of substrates. However, biobased n-butanol production faces challenges such as low yield and high product inhibition, resulting in low productivity to address an inefficiency process in terms of energy as a result of product recovery from a dilute stream. Furthermore, the ABE fermentation is also sensitive to inhibitors generated in the biomass pretreatment. Facing these challenges, this work proposes to evaluate different ratios 0, 25, 50, 75 and 100% of sugars from sugarcane molasses and lignocellulosic hydrolysate of sugarcane bagasse, totaling about 30 g/L by *C. saccharoperbutylacetonicum* DSMZ 1492 in order to find the best combination where occurs the highest butanol production. According to the results, when sugars came from mixtures of molasses (75%) combined with 25% of concentrated and unconcentrated lignocellulosic hydrolysate were obtained the highest butanol production 7.8 and 6.1 g/L, and  $Y_{p/s}$  of 0.27 and 0.24, respectively, after 30 h of fermentation. Consequently, this study demonstrated that C6 and C5 substrate mixtures could become an alternative strategy to achieve higher butanol productivity.

Pink version

**Butanol production from sugarcane bagasse hemicellulose hydrolysate mixed with molasses**

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In the context of 2G ethanol production from sugarcane bagasse integrated into existing mills, an important fraction of the sugars from the bagasse, the pentose sugars (C5), cannot be metabolized by the traditional *Saccharomyces cerevisiae*. Among several possibilities of using C5 as feedstock, the conversion to n-butanol is attracting interest because it is a chemical and an advanced biofuel with superior properties. Butanol is produced naturally in the Acetone-Butanol-Ethanol (ABE) fermentation by solventogenic *Clostridium* species. This microorganism is capable of metabolizing C5 sugars and other types of substrates. However, solventogenic clostridia are sensitive to inhibitors generated in the biomass pretreatment. As such, we hypothesized that the addition of sugarcane molasses to the bagasse hemicellulose hydrolysate may be an efficient strategy to circumvent the inhibitors challenge (by diluting the inhibitors) and avoid the prohibitive costs of detoxification steps. To test this hypothesis, fermentations with *C. saccharoperbutylacetonicum* DSMZ 1492 were conducted having different amounts of molasses added to the hemicellulose hydrolysate (from hydrothermally pretreated bagasse) and nutrients supplementation. Total initial sugar concentration was 30 g/L and sugars ratios (C12:C5) varied from 0% to 25, 50, 75, and 100%. After 30 h of fermentation, the best results in terms of butanol concentration (6.1 g/L) and yield (0.24) were obtained when 75% of the sugars were from the molasses. When the hydrolysate was concentrated by evaporation (from 15 to 52 g/L) previously to mixing, butanol concentration and yield increased to 7.8 g/L and 0.27, respectively, because of the removal of volatile inhibitors such as acetic acid and furfural. The non-concentrated hydrolysate was able to be fermented reaching 5.85 g/L of butanol and yield 0.22 with enrichment of pure xylose to get the optimum initial sugar concentration. In this manner, this study indicates that the addition of molasses must be at high ratios to make possible the conversion of concentrated C5 liquors into biobutanol at higher yield and productivity levels.