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Anaerobic Fermentation Process for Production of Biohydrogen from Pretreated Fruit Wastes

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Abstract : Fruit waste was used as a feedstock to produce biohydrogen in this study. Fruit waste used in this study was collected from several fruit juice stalls in Singapore. Based on our observation, the fruit waste contained 35-40% orange, 10-20% watermelon, 10-15% apple, 10-15% pineapple, 1-5% mango. They were mixed with water (1:1 ratio based on wet biomass) and blended to attain homogenous mixtures. Later, fruit waste was subjected to one of the following pretreatments: autoclave (121 °C for 20min), microwave (20min) or both. After pretreatment, the total sugar concentration in the hydrolysate was high (>12g/l) when both autoclave and microwave were applied. In contrast, samples without pretreatment measured only less than 2g/l of sugar. While using these hydrolysates as carbon sources, Clostridium strain BOH3 produces 2526-3126 ml/l of hydrogen after 72h of anaerobic fermentation. The hydrogen yield was 295-300 ml/g of sugar which is close to the hydrogen yields from glucose (338 ml/gm) and xylose (330 ml/gm). Our HPLC analysis showed that fruit waste hydrolysate contained oligosugars (25-27%), sucrose (18-23%), fructose (25-30%), glucose (10-15%) and mannose (2-5%). Additionally, pretreatment led to the release of free amino acids (160-512 mg/l), calcium (7.8-12.9 ppm), magnesium (4.32-6.55 ppm), potassium (5.4-65.1 ppm) and sodium (0.4-0.5 ppm) into the hydrolysate. These nutrients were able to support strain-BOH3 to grow and produce high level of hydrogen. Notably, unlike other pretreatment methods (with strong acids and bases), these pretreatment techniques did not generate any inhibitors (e.g. furfural and phenolic acids) to suppress the hydrogen production. Interestingly, strain BOH3 can also ferment pretreated fruit waste slurry and produce hydrogen with a high yield (156-343 ml/gm fruit waste). While fermenting pretreated fruit waste slurry, strain-BOH3 excreted several saccharolytic enzymes majorly xylanase (1.84U/ml), amylase (1.10U/ml), pectinase (0.36U/ml) and cellulase (0.43U/ml). Due to expressions of these enzymes, strain BOH3 was able to directly utilize pretreated fruit waste hydrolysate and produces high-level of hydrogen.

Keywords : autoclave pretreatment, biohydrogen production, clostridial fermentation, fruit waste, and microwave pretreatment
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