TITLE: BACTERIAL AND ARCHAEAL COMMUNITY INVOLVED IN THE CO-PRODUCTION OF HYDROGEN AND METHANE USING A THREE-STAGE ANAEROBIC DIGESTION PROCESS

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ABSTRACT:

Agricultural biomasses are important platforms for the biofuels production from renewable raw materials. Since the fermentation for hydrogen production from organic substrates is partial, the combination with methane production can benefit energy recovery Sugarcane bagasse (SCB) was used as a lignocellulosic substrate and glucose as control, combining the co-production of H_2 (stage I) and CH_4 (stages II and III) by a three-stages anaerobic fermentation process in batch reactors. In addition, 2-bromoethanesulfonic acid (BES) was used in the first stage to optimize H_2 production by inhibiting the methanogenic archaeas, as well as a hydrothermal (200°C, 20 bar, 10 min) associated with SCB enzymatic pretreatment by *Aspergillus niger*. Two fermentative inocula (In 1 and In 2) were used in stage I and a methanogenic inoculum in stages II and III (In 3). Three experimental series were performed: A (In 1), B (In 1 plus In 2) and C (In 2). The final metabolites from stage I were transferred to a second reactor for the subsequent methane production stage (stage II). Finally, the final metabolites from Stage II were transferred to a third reactor for the next phase of methane production (stage III). Stages II and III were inoculated with In 3. The higher H_2 yield was in C stage I (4.3 and 38.5 mmol H_2/g substrate for SCB and glucose, respectively). Moreover, the higher CH_4 yield was obtained in the stage III for C (6.3 and 18.2 mmol CH_4/g substrate for SCB and glucose, respectively). For all conditions the H_2 production occurred primarily via acetic acid. Predominance of *Enterococcus* and *Clostridium* that produce cellulolytic enzymes may favored the H_2 production from SCB and subsequent CH_4 production mainly from members of *Methanoregulaceae* and *Methanosaetaceae* families. Furthermore, the development of homoacetogenic bacterias (*Acetobacterium, Clostridium, Eubacterium, Holophaga*) was observed in the reactors. These microbial groups acted in synergism for the hydrolysis of SCB and hydrogen and methane production.

Keywords: batch reactors, Illumina Miseq, mesophilic condition, pretreatment

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