

**TITLE:** EFFECT OF HYDRHOTERMAL PRETREATMENT SEVERITY FROM BREWERY SPENT GRAIN ON METHANE YIELD AND BACTERIA AND ARCHAEA COMMUNITIES

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**ABSTRACT:**

In this study were evaluated the effects of hydrothermal pretreatment temperature and time from brewery spent grain (BSG) on methane recovery. Anaerobic batch reactors were inoculated with granular sludge and conducted using raw BSG (R) and BSG hydrothermal pretreated under the conditions: at 150°C for 10 minutes (B1), at 210°C for 20 minutes (B2), at 150°C for 20 minutes (B3), at 210°C for 10 minutes (B4), at 180°C for 15 minutes (B5) and control (C). Bacteria and Archaea communities were analysed using polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). Hydrothermal pretreatment time increase from 10 to 20 minutes, at 150°C and at 210°C, affected bacterial populations, since was observed 64% between B1-B3 and 36% between B2-B4, respectively, of similarity coefficient. However, carbohydrates removal efficiency was more discrepant at 210°C (B2 = 63.70 % and B4 = 90.45%) than at 150°C (B1 = 67.85% and B3 = 75.40%). Concerning to diversity index (Shannon-Wiener), there was decrease (B1 = 2.80 and B3 = 2.55; B4 = 2.70 and B2 = 2.55) while there was time increase of BSG pretreatment. BSG pretreated for 20 minutes, considering the same temperature, released high concentrations of soluble sugars and derived compounds from lignocellulosic degradation, as verified, contributing to the selection of populations which have adapted to these environments. With respect to Domain Archaea, it was observed for essays with BSG pretreated at 150°C (B1 and B3) similarity of 81% and for essays with BSG pretreated at 210 °C (B2 and B4), similarity of 64%. It was possible to infer the higher pretreatment temperature and longer pretreatment time determined a more significant alteration of archaeal population. The highest diversity indexes were observed in B4 (2.26) and in B5 (2.33), where were verified the highest methane yield (411 and 326 mL.g<sup>-1</sup>SV, respectively). Probably, the high substrates availability for methanogenesis, proved by the carbohydrate removal efficiencies and organic acid production favored methanogenic archaeal diversity.

**Keywords:** Anaerobic digestion, waste brewery, pretreatment, batch reactors, microbial diversity, PCR/DGGE.

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