

96. Enhancing anaerobic digestion of lignocellulose biomass using a thermophilic microbial consortium pretreatment

Mário Teixeira¹, Ricardo Ferreira¹, Duarte Toubarro¹, Jorge Frias¹, Carla Cabral¹, Fátima Viveiros¹, Lucia Rodriguez¹, Madalena Alves², Nelson Simões¹

¹University of Azores, Ponta Delgada, Portugal. ²University of Minho, Braga, Portugal

In nature, complex microbial communities catalyze cellulose hydrolysis by producing enzymes capable of breaking down cellulose polymers. Hydrolysis is a fundamental step in the carbon cycle of the industrial bioconversion of biomass to biofuels. The production of biofuels from biomass has become necessary, and the discovery of novel pretreatments is mandatory. The study of cellulose hydrolyzing bacterial communities is an essential step in achieving a sustainable future for biofuels.

In this study, we propose the stabilization of an aerobic thermophilic bacterial consortium (BC) with the ability to adapt and hydrolyze cellulose-rich mixed wastes. Cellulosic decaying residues inside the Azorean hot springs were used as inoculum. Samples were enriched through twenty consecutive subcultures on a cellulosic selective medium at 60°C. Enzymatic activity was detected using xylan and carboxymethylcellulose (CMC). Selected BC was able to hydrolyze 50% of cellulose-rich plant mix material in 4 days. Besides hydrolyzing low content lignin material as non-wood plants, newspaper and cardboard, the consortium could hydrolyze high lignin content material with lower efficiency. Cellulase and xylanase were present in BC, volatile fatty acids (VFA) and reducing sugars were higher than control. The pretreatment using BC increased the solubilization of 61% of grass mixtures, enhancing in 77% the production of methane (168 LCH₄/Kg VS added).

The bacterial diversity of BC was accessed by amplifying 16S rRNA hypervariable regions V3/V4, using 454 pyrosequencing and analyzed with QIIME2. The phylogenetic results showed a large diversity in the BC, with *Thermobacillus* representing 59% of the consortia, followed by *Gemmatimonadetes* 9%, *Symbiobacterium* 7%, *Thermobispora* 6%, *Brevibacillus* 4%, *Limnochordaceae* 3.9% and *Geobacillus* 0.6%. Three isolated *Geobacillus* species were identified; *G. stearothermophilus*, *G. thermodenitrificans* and *G. debilis*. Further work will comply with the improvement of this BC to improve the consortia for enhancing the efficiency to solubilize lignocellulose biomass by using the CRISPR/Cas9 system based on omics analysis of the consortium.