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Enzymatic pre-treatment of biomass for improvement of biogas production

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Abstract

Production of biogas from biomasses and organic residues by anaerobic digestion using methanogenic bacteria is an important biotechnological process for sustainable production of biofuel. One of the limiting factors of this process is the poor conversion rate into biogas of the energy contained in the biomass. This is mainly due to the difficult metabolism of the plant cell wall components by the microbial consortium present in the digestor, mainly due to the complexity of cellulose, hemicellulose and lignin. Cellulose is very abundant and its full conversion into methane would increase the efficiency of the process. Biogas production from polysaccharides and other biopolymers occurs through four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. It is evident the importance of a more efficient hydrolysis to get more biogas produced.

We developed three heterologous expression systems for production of the following enzymes:

(i) – endocellulase (endo-glucanase) from Bacillus pumilus

(ii) - cellobiohydrolase from Xanthomonas sp.

(iii) - beta-glucosidase from Bacillus amyloliquefaciens

These three enzymes are known to participate in the depolymerization of cellulose that occurs in three steps: (i) cellulose polymer cleavage and oligomers formation; (ii) removal of dimers (cellobiose) from the cellulose oligomers; (iii) release of glucose from cellobiose dimers.

The three genes encoding the above-mentioned enzymes were amplified by PCR, cloned in pTOPO, sequenced to verify the correct amplification, then cloned in pQE, an expression vector giving 6xHis tagged proteins. E. coli M15 was the expression system. The three enzymes were then purified by a single step-affinity chromatography, thanks to the six-histidine tag, and used in the experiments of cellulose digestion. Considering that two enzymes were not soluble when expressed in E. coli (cellobiohydrolase and beta-glucosidase formed inclusion bodies), an alternative heterologous expression system was taken into consideration for the production of the enzymes, the yeast Pichia pastoris.

The final goal of the project is the development of a pre-treatment method to be used for the conversion of biomasses

and agro-industrial organic residues containing cellulose into a substrate to be fermented by methanogenic bacteria for production of biogas.

While the heterologous expression in Pichia is still under development, we already have an efficient system for production of the recombinant bacterial endo-glucanase. The optimal conditions for the use of this enzyme have been determined: the optimal pH is 6.0 and the optimal temperature is 40 C. In these conditions, pH 6.0 and temperature of 40ºC, the enzyme maintained up to 50% of its activity after one week.

The enzyme was tested on some substrates and was found to be able to depolymerize microfibril cellulose (Sigma), residual short fiber cellulose from paper industry, corn cob powder and corn stalk powder with a specific activity of 251, 142, 75 and 70 IU/mg, respectively.

We are currently measuring the methanogenic potential of different cellulose-containing organic residues with and without pre-treatment with the cellulolytic enzyme. Following this experiment, the economic sustainability of this process will be calculated, comparing the cost of pre-treatment and the benefit achieved in term of increased biogas production.

Biography:

Giuliano Degrassi has completed his PhD in 2000 from the Open University of London. He is the Coordinator of the ICGEB Outstation of Buenos Aires and Leader of the Industrial Biotechnology Group. He has published more than 40 papers in peer reviewed international journals and has been the organizer of more than 20 ICGEB courses on different aspects of biotechnology.

Speaker Publications:

1. de Almeida Lopes KB, Carpentieri-Pipolo V, Fira D, Balatti PA, López SMY, Oro TH, Stefani Pagliosa E, Degrassi G (2018) Screening of bacterial endophytes as potential biocontrol agents against soybean diseases.
2. de Almeida Lopes KB, Carpentieri-Pipolo V, Oro TH, Stefani Pagliosa E, Degrassi G (2016) Culturable endophytic bacterial communities associated with field grown soybean. Journal of Applied Microbiology 120: 740-55.
3. Degrassi G, Polverino De Laureto P, Bruschi CV (1995) Purification and characterization of ferulate and p-coumarate decarboxylase from Bacillus pumilus. Applied and Environmental Microbiology 61: 326-332.

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