SYSTEMIC ANALYSIS, METABOLIC ENGINEERING AND ECONOMIC EVALUATION OF HEMICELLULOSIC HYDROLYSATE UTILIZATION IN BIOPROCESSES

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Figure 1. Biostat B bioreactor performing bacterial cultivation to collect data in the project

Among the sugars of lignocellulose, xylose, present in the hemicellulose, is recovered more easily and in better yields than glucose. Our objective is to increase the efficiency (yield and productivity) of bioprocesses using the biomass hemicellulosic fraction studying the ethalonogenic *Escherichia coli* KO11 and PHA-producing *Burkholderia* species. Polyhydroxyalkanoates (PHA) are polymeric bacterial products that can be used as biodegradable plastics. This research focuses on fundamental understanding of those cellular factors that we consider still the most essential in improvement of xylose utilization by bacteria and of process consolidation aspects to reach the level required in commercial production of biofuels and bioplastics from the hemicellulosic hydrolysates. The following strategies are proposed: (i) construction of genome based *in silico* models of xylose metabolism, (ii) through analysis of those in silico models a metabolic engineering strategy will be proposed to increase yield and productivity of the bioproduct. (ii) ecobiotechnology techniques will be also applied, such as evolutionary engineering to obtain strains with better performance. (iv) high cell density fed-batch cultivations with the best strains will be performed using hemicellulosic hydrolysate as carbon source. (v) From yield and productivity values obtained in this project, a preliminary economic evaluation will be performed to verify the economic potential of this technology.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Multiple copies of the catabolism and transport genes of xylose were introduced in *Escherichia coli* KO11 and *Burkholderia sacchari* LFM 101. Expression of xylA on *E. coli* reduced the rate of xylose consumption and increased final ethanol production by 30% . Results in mixtures of glucose and xylose indicate that the low level of xylAB expression also contributes to the inefficient xylose consumption, in addition to catabolite repression. A faster rate of xylose consumption in the presence of glucose was observed on *B. sacchari* when compared to cultures only in glucose, suggesting that this carbohydrate might have an opposite effect to catabolite repression in this strain (Freire, 2012).

Also to improve aspects on polymer productivity from sugarcane bagasse derivatives, a *B. sacchari* mutant transporting hexoses and pentoses by a non-PTS system uptake system was obtained and presented a released glucose catabolite repression over the pentoses. In mixtures of sugars usually generated on sugarcane bagasse hydrolysate, specific growth rates and specific sugar consumption were 10% and 23% times higher, resulting in a reduced time to exhaust all sugars in the medium. Effects of elimination of PTS components over carbon flux distribution and PHA biosynthesis were evaluated (Lopes et al., 2011 a, *see figure*).

A similar approach was applied to a newly isolated *Bacillus sp*, by deleting the ccpA gene, resulting on a partial release on catabolic repression of glucose over pentoses and a faster consumption of carbohydrates (Lopes et al., 2011b).

Economic assessment of biopolymer production in a sugarcane-based biorefinery context was performed, focusing on a PHA production from xylose in the context of a standard sugar and ethanol plant, cogenerating steam and electrical energy from sugarcane bagasse and agricultural residues. PHA production from xylose, discarded in most mills nowadays, may enable profitability of 2nd generation bioethanol. Productivity varied from 0,28 to 1,11 g/L.h, and PHB price ranged from R$ 4,50 to R$ 9,00/kg. Bioreactor cost was studied in 3 scenarios from US$ 475 to 3.013 thousand and the production capacity was analyzed in ten different scenarios, from 1,000 to 35,000 thousand tons/year. Result reviews offer contribution margin, net operational profit, as well as breakeven point analysis. Recommended is to redirect part of the research efforts from improving PHB yields to process productivity improvement, which turned out to be the key factor to economic feasibility (Raicher, 2011).

MAIN PUBLICATIONS

Lopes MSG, Gosset G, Rocha RCS, Gomez JGC, Silva LF. 2011. PHB biosynthesis in catabolite repression mutant of *B. sacchari*. *Curr Microbiol.* **63**: 319-326.

Lopes MSG, Steinert N, Rojas JD, Hillen W, Gomez JGC, Silva LF. 2011. Role of *CcpA* in polyhydroxybutyrate biosynthesis in a newly isolated *Bacillus sp* MA 3.3 *J. Mol. Microbiol. Biotechnol.* **20**: 63-69.

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Gomez JGC, Méndez BS, Nikel PI, Pettinari MJ, Prieto MA, Silva LF. 2012. Making green polymers even greener: towards sustainable production of PHA. In: *Adv. Appl Biotechnol.* Petre M (editor) p. 41-61, ISBN 978-953-307-820-5.

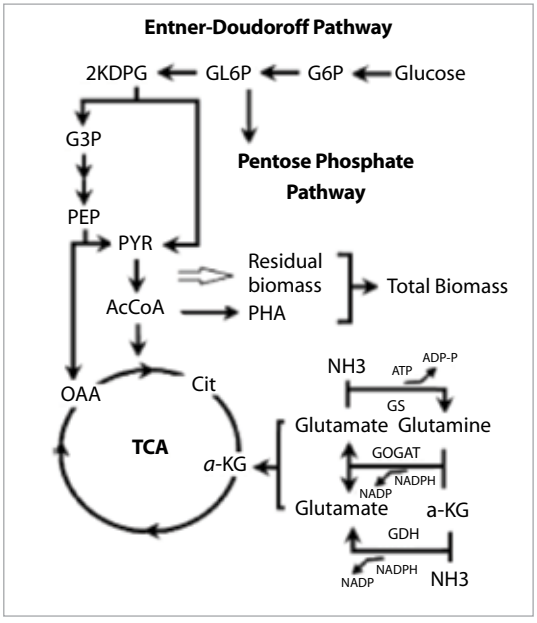

Figure 2. Schematic representation of the polyhydroxybutyrate (PHB) metabolism in B. sacchari. *Glutamate is metabolized to a-KG and then converted to oxaloacetate in the tricarboxylic acid cycle (TCA). Oxaloacetate (OAA) can yield pyruvate (PYR) after decarboxylation and then acetyl-CoA (AcCoA) can be used to produce PHB (Lopes et al., 2011a).*

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