Thermotoga maritima and Caldicellulosiruptor saccharolyticus co-culture for biohydrogen production

A. A. Abreu¹, M. Mota¹ and M.M. Alves¹

¹IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal. (E-mail: angela_abreu@deb.uminho.pt; mmota@deb.uminho.pt; madalena.alves@deb.uminho.pt)

HIGHLIGHTS
T. maritima and C. saccharolyticus co-culture (1:3) is advantageous for hydrogen production from mixture of glucose and xylose. The higher hydrogen production observed in co-culture was associated to lower lactic acid formation.

Keywords
Thermotoga maritima; Caldicellulosiruptor saccharolyticus; biohydrogen; co-culture

INTRODUCTION
Biohydrogen fermentation at extreme-thermophilic temperatures (over 70°C) has been recognized as thermodynamically advantageous over mesophilic fermentation. Extremely thermophilic bacteria such as, Thermotoga maritima and Caldicellulosiruptor saccharolyticus have high polysaccharide-hydrolysing capacity and are able to use most of the reducing equivalents formed during glycolysis for the production of hydrogen. T. maritima and C. saccharolyticus are capable to utilize a wide range of substrates, from simple sugars to complex carbohydrates such as, cellulose, hemicellulose, starch and pectin. Furthermore, C. saccharolyticus is relatively insensitive to high hydrogen partial pressure (PH₂) (Verhaart et al, 2010). The choice to co-culture these two microorganisms is based on the growing evidence that co-cultures can synergistically be more effective in substrates conversion than monocultures (Liu et al. 2008). In the present study, the potential of co-culturing T. maritima and C. saccharolyticus for the conversion of a mixture of glucose and xylose, as well as, cellobiose to hydrogen was evaluated under extreme thermophilic conditions (75°C).

METHODS
C. saccharolyticus DSM 8903 and T. maritima DSM 3109 were individually cultured in modified DSM 640 medium containing 1gL⁻¹ glucose. After reaching an optical density (OD) at 620 nm of 0.2-0.3, the cultures were used as inoculum for the subsequent batch assays. Inoculums’ dry cell mass concentration was estimated using the pre-determined relation CDW (g L⁻¹) = 25.458 * OD₆₂₀⁻ 4.106 for T. maritima and CDW (g L⁻¹) = 16.327 * OD₆₂₀⁻ 2.803 for C. saccharolyticus. Since T. maritima is a slightly halophilic bacteria, different NaCl concentrations (0.9, 2.7, 3.5 and 5 g L⁻¹) were tested in batch experiments for both cultures separately, in order to find the compromise NaCl concentration for the co-culturing. Afterwards, ratios of 1:3, 1:1 and 3:1 (w/w), as well as, individual culture of T. maritima and C. saccharolyticus were tested for biohydrogen production. Glucose and xylose 1:1 (w/w) and cellobiose at final concentration of 2g L⁻¹ were used as substrates. Additionally, soluble fermentation products were followed during all experiment.

RESULTS AND DISCUSSION
Hydrogen production by C. saccharolyticus decreased significantly for NaCl concentrations higher than 2.7g L⁻¹ (data not shown). Consequently, this concentration was used in further co-culture experiments. Hydrogen production from cellobiose was slightly higher when C. saccharolyticus was used individually (Figure 1). The utilization of cellobiose was associated to the production of high
concentrations of lactic acid, independently of the inoculum used (Figure 2). This fact contributed to the lower hydrogen production observed, comparatively to the utilization of glucose and xylose. The co-culture composed by *T. maritima* and *C. saccharolyticus* in the proportion 1:3 showed the best hydrogen production from a mixture of glucose and xylose (30mmol H$_2$ L$^{-1}$) corresponding to hydrogen yield of 2.7 mol mol$^{-1}$ hexose (Figure 1). Higher hydrogen production in co-culture was associated to lower lactic acid formation. The results suggest that the utilization of *T. maritima* and *C. saccharolyticus* in co-culture (1:3) is advantageous for hydrogen production from a mixture of glucose and xylose.

![Figure 1](image1.png)

*Figure 1.* Hydrogen production from cellobiose (a) and a mixture of glucose and xylose (b) using different ratios of *T. maritima* and *C. saccharolyticus.*

![Figure 2](image2.png)

*Figure 2.* Soluble fermentation products produced from glucose/xylose and cellobiose using *T. maritima* and *C. saccharolyticus* in co-culture and monocultures.

**ACKNOWLEDGEMENTS**

The financial support from FCT through the post doctoral grant SFRH/BPD/82000/2011 is gratefully acknowledged.

**REFERENCES**
