Thermotoga maritima and *Caldicellulosiruptor sacharolyticus* co-culture for biohydrogen production

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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 70ºC) 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 (PH_2) (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 (75ºC).

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 * OD_{620} - 4.106 for *T. maritima* and CDW (g L^{-1}) = 16.327 * OD_{620} - 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 infurther 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.

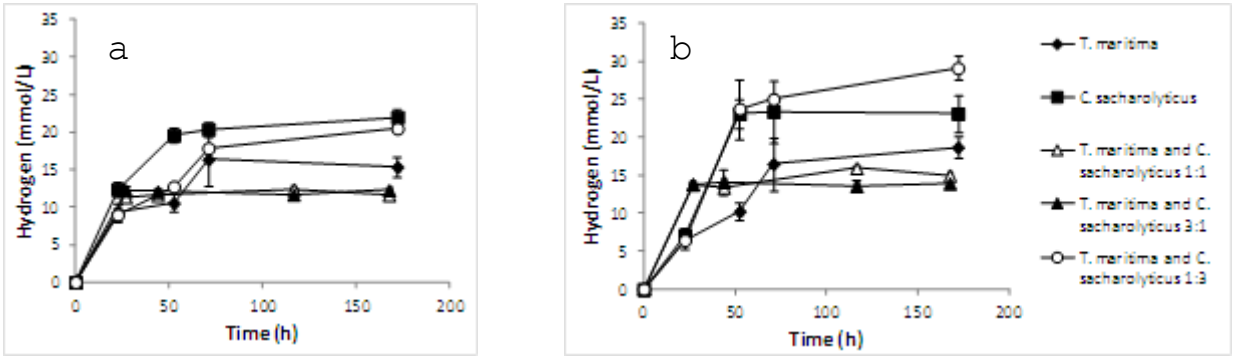

Figure 1. Hydrogen production from cellobiose (a) and a mixture of glucose and xylose (b) using different ratios of *T. maritima* and *C. saccharolyticus*.

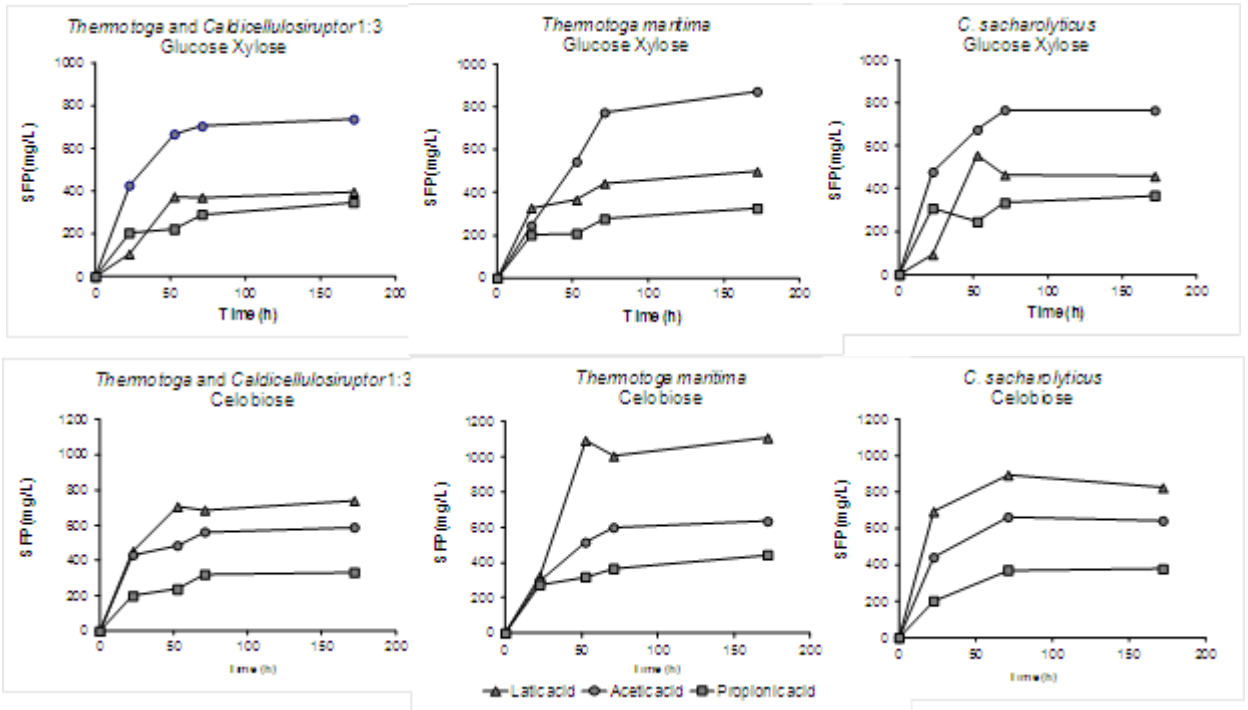

Figure 2. Soluble fermentation products produced from glucose/xylose and cellobiose using *T. maritima* and *C. saccharolyticus* in co-culture and monocultures.

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