Optimization of bioethanol production from carbohydrate rich wastes by extreme thermophilic microorganisms

Second-generation bioethanol is produced from residual biomass such as industrial and municipal waste or agricultural and forestry residues. However, Saccharomyces cerevisiae, the microorganism currently used in industrial first-generation bioethanol production, is not capable of converting all of the carbohydrates present in these complex substrates into ethanol. This is in particular true for pentose sugars such as xylose, generally the second major sugar present in lignocellulosic biomass. The transition of second-generation bioethanol production from pilot to industrial scale is hindered by the recalcitrance of the lignocellulosic biomass, and by the lack of a microorganism capable of converting this feedstock to bioethanol with high yield, efficiency and productivity. In this study, a new extreme thermophilic ethanologenic bacterium was isolated from household waste. When assessed for ethanol production from xylose, an ethanol yield of 1.39 mol mol-1 xylose was obtained. This represents 83 % of the theoretical ethanol yield from xylose and is to date the highest reported value for a native, not genetically modified microorganism. The bacterium was identified as a new member of the genus Thermoanaerobacter, named Thermoanaerobacter pentosaceus and was subsequently used to investigate some of the factors that influence second-generation bioethanol production, such as initial substrate concentration and sensitivity to inhibitors. Furthermore, T. pentosaceus was used to develop and optimize bioethanol production from lignocellulosic biomass using a range of different approaches, including combination with other microorganisms and immobilization of the cells. T. pentosaceus could produce ethanol from a wide range of substrates without the addition of nutrients such as yeast extract and vitamins to the medium. It was initially sensitive to concentrations of 10 g l-1 of xylose and 1 % (v/v) ethanol. However, long term repeated batch cultivation showed that the strain was capable of adaptation to higher substrate concentrations, at least up to 20 g l-1 xylose. T. pentosaceus was able to metabolize two typical inhibitors present in lignocellulosic hydrolysate, 5-hydroxymethylfurfural (HMF) and 2-furfural, up to concentrations of 1 and 0.5 g l-1, respectively. Above these levels, xylose consumption was inhibited up to 75 % (at 3.4 g l-1 5-HMF) and 70 % (at 3.4 g l-1 furfural). T. pentosaceus could grow and produce ethanol directly from the liquid fraction of pretreated rapeseed straw, without any dilution or need for additives. When T. pentosaceus was used in combination with S. cerevisiae in a sequential fermentation of pretreated rapeseed straw, it achieved 85 % of the theoretical ethanol yield based on the sugar composition of the pretreated straw. This was 50 % and 14 % higher than the yield obtained with the bacteria or the yeast alone, respectively. When T. pentosaceus was immobilized in rapeseed straw, an improvement of 11 % in ethanol production was observed in batch mode. In continuous mode, it was shown that hydraulic retention time (HRT) affected ethanol yield, and a dramatic shift from ethanol to acetate and lactate production occurred at an HRT of 6 h. The maximum ethanol yield and concentration, 1.50 mol mol-1 consumed sugars and 12.4 g l-1, were obtained with an HRT of 12 h. The latter represented an improvement of 60 % in relation to previously obtained results. The results obtained confirm that the extreme thermophile T. pentosaceus is a promising candidate for bioethanol production from lignocellulosic biomass, and that improvement and optimization of existing processes are possible using different approaches. Further insight into the metabolism of the strain, as well as its improvement by genetic engineering can bring second-generation ethanol production one step closer to its industrial application.

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