Separate and Simultaneous Enzymatic Hydrolysis and Fermentation of Wheat Hemicellulose With Recombinant Xylose Utilizing Saccharomyces cerevisiae

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Abstract

Fermentations with three different xylose-utilizing recombinant Saccharomyces cerevisiae strains (F12, CR4, and CB4) were performed using two different wheat hemicellulose substrates, unfermented starch free fibers, and an industrial ethanol fermentation residue, vinasse. With CR4 and F12, the maximum ethanol concentrations obtained were 4.3 and 4 g/L, respectively, but F12 converted xylose 15% faster than CR4 during the first 24 h. The comparison of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) with F12 showed that the highest, maximum ethanol concentrations were obtained with SSF. In general, the volumetric ethanol productivity was initially, highest in the SHF, but the overall volumetric ethanol productivity ended up being maximal in the SSF, at 0.013 and 0.010 g/Lh, with starch free fibers and vinasse, respectively.

Index Entries: Xylose conversion; ethanol; starch free fibers; vinasse.

Introduction

In Europe, wheat is one of the major feedstocks employed in the industrial production of ethanol for potable spirits, technical alcohol, and fuel ethanol. This production of ethanol is based on enzyme-catalyzed conversion of wheat endosperm starch to glucose with subsequent fermentation of glucose to ethanol by the yeast Saccharomyces cerevisiae (1). The wheat endosperm cell wall material is currently left behind as an unhydrolyzed, unfermented residue.

Wheat endosperm cell walls comprise various nonstarch polysaccharides notably (1→3)(1→4)-β-D-glucans, glucomannans, cellulose, and arabinoxylans as well as some protein. Arabinoxylans make up approx 70% by

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weight of the wheat endosperm polysaccharides (2). Arabinoxylans are pentose polymers that consist of a backbone of \((1\rightarrow4)\)-linked \(\beta\)-d-xylopyranosyl residues with single \(\alpha\)-arabinofuranosyl substituents attached to the C(\(\bar{O}\))-2, C(\(\bar{O}\))-3 or to both C(\(\bar{O}\))-2,3 of the xylose residues with the A : X ratio typically being approx 0.5 : 1 (3). The xylan backbone may additionally be substituted with \(\alpha\)-d-glucopyranosyl uronic acid, its 4-O-methyl derivative, and/or acetyl groups (4). The arabinofuranosyl units may be esterified with ferulic acid and/or \(p\)-coumaric acid (5).

Utilization of the wheat endosperm cell wall polysaccharides in ethanol production will require both, (1) generation of a fermentable hydrolysate from the cell wall polysaccharides, notably from the arabinoxylan because of its dominance in these cell walls, (2) a microorganism able to utilize the resulting pentoses for ethanol production. Arabinoxylans are readily hydrolyzed to monosaccharides by acid treatment. However, acid hydrolysis unavoidably generates byproducts that inhibit the subsequent microbial fermentation (6). For this reason enzymatic hydrolysis is preferable. Furthermore, this method is considered to be a more economically viable in future bioethanol processes (7,8). Hence, production of ethanol from wheat endosperm cell walls using biological conversion includes both degradation of the polysaccharides by enzymes and microbial conversion of the monomeric sugars (hexoses and pentoses) to ethanol.

As a result of the complexity and heterogeneity of the arabinoxylan structure, complete enzymatic degradation into monosaccharides requires both side-group cleaving and depolymerizing enzyme activities (9). A synergistic interaction during degradation of soluble arabinoxylan using enzyme side-activities in a cellulase preparation from Trichoderma reesei (Celluclast 1.5 L) and a hemicellulosic enzyme preparation produced by Humicola insolens (Ultrapro L) has recently been identified (10,11). Depending on the degree of entanglement among the polysaccharide structures in the substrate, endoglucanases (EC 3.2.1.4) and \(\beta\)-glucanases (EC 3.2.1.6) may also be required in enzymatic degradation of wheat endosperm cell walls.

Traditionally, yeast, \(S.\ cerevisiae\) is widely used for ethanol production, however, it cannot ferment pentoses naturally. A number of natural pentose fermenting microorganisms have been identified and many efforts have been made to genetically engineer different microorganisms, for example, \(S.\ cerevisiae\), Zymomonas mobilis and Escherichia coli to efficiently produce ethanol from both hexoses and pentoses (for recent reviews see refs. [12–15]). Because xylose is the dominant pentose monosaccharide in most lignocellulosic and hemicellulosic hydrolysates, identification and development of efficient xylose fermenting microorganisms have received most attention. Several recombinant, mutated, and evolved xylose-fermenting \(S.\ cerevisiae\) strains have thus been developed during the past 10–15 yr. In a recent report, \(S.\ cerevisiae\) F12 was identified as being attractive