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ScienceDirect
Bioresource Technology 98 (2007) 2942–2948
BIORESOURCE TECHNOLOGY

Contaminant occurrence, identification and control in a pilot-scale corn fiber to ethanol conversion process

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Received 3 March 2006; received in revised form 20 September 2006; accepted 3 October 2006
Available online 15 November 2006

Abstract

While interest in bioethanol production from lignocellulosic feedstocks is increasing, there is still relatively little pilot-plant data and operating experience available for this emerging industry. A series of batch and continuous fermentation runs were performed in a pilot-plant, some lasting up to six weeks, in which corn fiber-derived sugars were fermented to ethanol using glucose-fermenting and recombinant glucose/xylose-fermenting yeasts. However, contamination by *Lactobacillus* bacteria was a common occurrence during these runs. These contaminating microorganisms were found to readily consume arabinose, a sugar not utilized by the yeast, producing acetic and lactic acids that had a detrimental effect on fermentation performance. The infections were ultimately controlled with the antibiotic virginiamycin, but routine use of antibiotics is cost prohibitive. The severity of the problem encountered during this work is probably due to use of a highly contaminated feedstock. Lignocellulosic conversion facilities will not employ aseptic designs. Instead, techniques similar to those employed in the corn-based fuel ethanol industry to control infections will be used. Effective control may also be possible by using fermentative microorganisms that consume all biomass-derived sugars.

Keywords: Corn fiber; Pilot plant; Fermentation; Contamination; Antibiotics

1. Introduction

There is currently a high level of interest in ethanol as transportation fuel because of its value as a fuel extender and oxygenate. This fact has driven the current corn-based fuel ethanol industry in the US to record production levels that have more than doubled from 2000 to 2004 (Renewable Fuels Association). Nevertheless, research also continues on conversion of lignocellulosic biomass to ethanol because of its potential for producing a low cost fuel. Such a large supply of lignocellulose is available that conversion of this material to ethanol could displace a large fraction of our gasoline consumption (Perlack et al., 2005). One such lignocellulosic feedstock that is readily available in the near term is corn fiber. It has received considerable attention from various researchers (Dale et al., 1996; Grohmann and Bothast, 1997; Gulati et al., 1996; Saha et al., 1998) and in a previous paper (Schell et al., 2004) we documented our experiences converting corn fiber to ethanol in a 900 kg dry biomass/day process development unit (PDU).

The work reported here occurred in 1995 and 1996 as part of a Cooperative Research and Development Agreement between Amoco Corporation (Naperville, IL) and later the SWAN Biomass Co., the US Department of Energy (DOE), and the National Renewable Energy Laboratory (NREL). One purpose of the project was to generate pilot-scale performance data on conversion of corn fiber to ethanol using a pilot-plant that employed unit operations for feedstock handling, biomass pretreatment, seed/inoculum production, fermentation and solids recovery. Corn fiber was pretreated by a high-temperature dilute sulfuric acid

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doi:10.1016/j.biortech.2006.10.002

process that converts both the starch and hemicellulosic fraction of the fiber to soluble sugars. This stream containing soluble sugars and the remaining unconverted solids consisting primarily of cellulose was converted to ethanol using a simultaneous saccharification and fermentation (SSF) process. This process enzymatically hydrolyzes cellulose to glucose, which together with the soluble sugars produced during pretreatment is then fermented to ethanol by yeast. Cellulase was purchased from a commercial supplier and yeast was grown in the pilot-plant's seed fermentors.

During continuous plant operation for periods of up to six weeks, the main process fermentors often became highly contaminated with bacteria. Understanding the impact of contamination on process performance is an often-neglected issue for the emerging biorefining industry. While the corn-based fuel ethanol industry has developed techniques to minimize this problem, the compositional differences and different processing requirements for lignocellulosic biomass conversion pose unique challenges. The focus of this paper is on identification and control of contamination so performance data will not be presented.

We previously discussed contamination problems that were observed during initial operation of the PDU (Schell et al., 2004). During these runs and in the work reported here, bacterial contaminants were observed to consume arabinose producing lactic and acetic acid as by-products. This paper elaborates on these results and presents information on subsequent runs not included in the previous paper. We also report on the use of a liquid culturing technique to rapidly detect the presence of microbial contaminants.

Contamination sources were categorized as either direct or indirect. Potential direct sources were materials added to the fermentors, which included pretreated corn fiber, inoculum, enzyme, corn steep liquor (CSL), and air (a small amount was added to the headspace to maintain positive fermentor pressure). Potential indirect sources of contamination included dirty transfer lines or water used for pump and agitator seals. All vessels and transfer lines were steam sterilized prior to use, however, sterilization may have been inadequate. Although microorganisms were detected in many of these sources, our goal was to identify the primary source of the contaminating microorganisms present in the main process fermentors.

2. Methods

2.1. Corn fiber feedstock

Corn fiber was obtained from a corn wet mill located in Canada (Casco, Ont., Canada) and stored in a refrigerated trailer at 10 °C for no longer than a month before use. The fiber moisture content was 55–60% (w/w) as received.

2.2. Biocides

Three different biocides were used to combat contamination including the antibiotics penicillin (Allpen™, Alltech, Nicholasville, KY) and virginiamycin (Lactrol™, Pfizer, Richmond, VA), the latter is an antibiotic effective against *Lactobacillus* (Hynes et al., 1997). We also used the antimicrobial agent nisin (Nisalpin™, Alpin & Barrett, Tarrytown, NY). The actual amount of each compound used is presented in the results section, since the dose was varied over the course of the work.

2.3. Microorganisms

The glucose-fermenting microorganism used in this work was *Saccharomyces cerevisiae* strain L1400, which was also the host strain for the glucose/xylose-fermenting recombinant strain LNH-ST (Toon et al., 1997). Cells were grown on 1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose and mixed with 20% (w/w) glycerol and stored in cyrovials at −70 °C prior to use.

2.4. Pilot plant operation

All work was performed in the PDU that was specifically designed to convert lignocellulosic biomass to ethanol. The equipment, process configuration, and procedures used during this work are extensively described in Schell et al. (2004). Corn fiber was pretreated by dilute sulfuric acid hydrolysis using a proprietary pretreatment device and fed directly to a 9000-L fermentor. This process hydrolyzed both starch and hemicellulose to monomeric and oligomeric sugars. Early work used glucose-fermenting yeasts to produce ethanol using a SSF process configuration. In later runs, recombinant yeasts were used to produce ethanol from both glucose and xylose. Continuous fermentations were conducted in three 9000-L fermentors connected in series. Pretreated corn fiber, inoculum, cellulase enzyme, and corn steep liquor (nutrient source) were added to the first 9000-L fermentor only. Two 160-L and two 1500-L fermentors were used to produce inoculum, but generally, the 9000-L fermentation train was only inoculated once at the beginning of the run. Yeast grew at a rate sufficient to maintain a stable population.

A number of runs listed in chronological order in Table 1 were performed over a period of approximately eight months. The time of inoculation of the first fermentor is defined as time zero. Throughout this paper, the first, second, and third fermentor will be used to identify the location in the continuous fermentation train where

Table 1
Key operational parameters for the five fermentation runs

Run #	Yeast stain	Operating mode	Approximate duration (weeks)
1	L1400	Continuous	3
2	L1400	Continuous	1
3	LNH-ST	Batch	variable[a]
4	LNH-ST	Continuous	6
5	LNH-ST	Continuous	6

[a] One five-day and one six-day run in a 1500-L fermentor and one 4.5-day run in a 9000-L fermentor.