Bioresource Technology 134 (2013) 117–126

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

High concentrations of cellulosic ethanol achieved by fed batch semi simultaneous saccharification and fermentation of waste-paper

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HIGHLIGHTS

- Batch addition of paper waste in SSSF results in up to 11.6% (v/v) ethanol.
- Low overall enzyme loadings (3.7 FPU/g substrate).
- High cumulative substrate loadings (65% w/v).
- High ethanol concentrations will improve distillation efficiencies.

ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form 14 January 2013
Accepted 16 January 2013
Available online 9 February 2013

Keywords:
Simultaneous saccharification and fermentation (SSF)
Cellulosic ethanol
Waste paper
Cellulase
Fermentation

ABSTRACT

A fundamental goal of second generation ethanol production is to increase the ethanol concentration to 10% (v/v) or more to optimise distillation costs. Semi simultaneous saccharification and fermentations (SSSF) were conducted at small pilot scale (5 L) utilising fed-batch additions of solid shredded copier paper substrate. Early addition of Accellerase® 1500 at 16 FPU/g substrate and 30 U/g β-glucosidase followed by substrate only batch addition allowed low final equivalent enzyme concentrations to be achieved (3.7 FPU/g substrate) whilst maintaining digestion. Batch addition resulted in a cumulative substrate concentration equivalent to 65% (w/v). This in turn resulted in the production of high concentrations of ethanol (11.6% v/v). The success of this strategy relied on the capacity of the bioreactor to perform high shear mixing as required. Further research into the timing and number of substrate additions could lead to further improvement in overall yields from the 65.5% attained.

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1. Introduction

Most ethanol for transportation fuel is produced from starch or sucrose (first generation). These substrates can be employed in batch processes at relatively high concentrations facilitating high yields of ethanol at over 11% (v/v). This minimises the costs of distillation (Katzen et al., 2003). However, to enhance the sustainability of biofuel production, there is a desire to move away from crops relevant to human food, and there has been an international effort to enhance the efficiency of ethanol production from lignocellulosic waste streams from the agrifood chain (Waldron, 2010). Exploitation of such wastes has the potential to add value to food production and minimise the overall carbon footprint. Several demonstration plants have been recently constructed (Bacovsky and Worgetter, 2010). However, ethanol production from lignocellulose is not yet economically viable. There are a number of factors that make second generation approaches very expensive. These include the high cost of energy used in pretreatments, the difficulty of achieving sufficiently high substrate loadings, the cost and diversity of enzymes required for acceptable hydrolysis, the difficulty of effectively fermenting both hexose and pentose sugars, and the high energy costs associated with distillation of the low alcohol concentrations (Black and Veatch Limited, 2008).

The balance of these challenges is often waste-stream dependent. For example, waste paper and paper sludge from pulping do not require the energy-intense thermophysical pretreatments used to enhance enzymolysis of lignocellulose substrates. This is because they have already been "pretreated" by the pulping process which effectively de-lignifies the biomass and removes a significant amount of the poorly fermentable hemicellulose (Roberts, 1996). Very large quantities of waste paper and card are present in municipal waste streams. In the UK, for example, 12.3 M tonnes of paper waste was generated in 2008 (Defra, 2011), hence, a number

Abbreviations: SSF, simultaneous saccharification and fermentation; SSSF, semi-simultaneous saccharification and fermentation; βG, beta-glucosidase (cellobiase).

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0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.084

of studies have been performed to evaluate the potential of ethanol production from these sources (Ballesteros et al., 2002; Chen et al., 2011; Dwiarti et al., 2012; Kang et al., 2011; Prasetyo et al., 2010). There have been continual improvements in the yield of ethanol from paper and sludge. Nevertheless, final ethanol concentrations achieved have been generally less than 1–2% by weight. This is mainly due to difficulties in achieving high substrate loadings. Above about 15% (w/v) the absorption of water by the paper results in a solid which requires very high forces for agitation and mixing as compared with the gelatinised starch or soluble sugar in first generation biorefineries. Furthermore, high lignocellulosic substrate concentrations are subject to the "solids effect" (Kristensen et al., 2009) in which expected glucose yields become reduced as substrate concentration is increased. Since paper waste contains cellulose at about 50% dry weight, a 15% (w/v) loading could not be expected to yield more than 3.75% (w/v) ethanol. One approach to addressing this problem involves the use of fed batch additions of substrate in combination with simultaneous saccharification and fermentation (SSF) or variations thereof. As saccharification proceeds, the cellulosic biomass is degraded. This will liberate more free water, reducing the viscosity or stiffness of the substrate suspension. The liquefaction could thereby facilitate further substrate addition, increasing the sugars available for fermentation. This was first demonstrated for paper wastes by Ballesteros et al. (2002) who achieved 1.8% (w/w) ethanol as did Kuhad et al. (2010). More recently however, Kang et al. (2011) achieved 7.6% (v/v) / 6% (w/v) ethanol from fed-batch SSF of paper mill sludges, although the process required an energy-intensive pre-de-ashing process. Nevertheless, the study demonstrated the potential to increase concentrations of ethanol derived from an insoluble cellulosic feedstock.

In the current study we have investigated approaches for fed-batch "saccharification and semi-simultaneous saccharification and fermentation" (SSSF) of shredded copier paper. The aim of the research has been to successfully achieve ethanol concentrations at levels comparable to those produced during first generation approaches whilst using minimal quantities of commercial cellulases. This provides a basis for reducing the costs of distillation (Hengstebeck, 1961; Katzen et al., 2003).

2. Methods

2.1. Materials

Commercially available cellulase Accellerase® 1500 (Genencor, Rochester, N.Y., USA); *Trichoderma reesei* and accessory enzyme β-glucosidase (βG) – Novozyme 188 (Novozyme Corp, Bagsvaerd, Denmark), were chosen for their high activities. These enzyme preparations were used "as provided" without any desalting or other purification steps. The substrate was M-Real Evolve Office 80 g/m^2 paper (The Premier Group, Birmingham, UK); digestions and fermentations were carried out in 0.1 mol/L sodium acetate Buffer (Sigma Aldrich, Gillingham, UK).

2.2. Substrate preparation

M-Real Evolve paper was shredded using a PS-67Cs cross shredder (Fellowes, Doncaster, UK) to 3.9 × 50 mm particle size (Din Security Level 3), portioned into 125 g aliquots and sterilised by autoclaving in dry sealed bags (121 °C for 15 min).

2.3. Yeast preparation

Yeast (*Saccharomyces cerevisiae,* strain number NCYC 2826; National Collection of Yeast Cultures, Norwich, UK) was grown from a slope culture by inoculation into 1 L of Difco, Yeast and Mould (YM) broth (Fisher Scientific UK Ltd., Loughborough, UK): and allowed to grow over the period of ⩾3 days at 25 °C. The temperature was then reduced to 4 °C and the yeast was allowed to settle. YM media was decanted and the yeast cells reconstituted to 500 mL using yeast nitrogen base (Formedium, Hunstanton, UK). Prior to inoculation into hydrolysate the total viable count was measured using a NucleoCounter® YC-100™ (ChemoMetec, Denmark).

2.4. 2 L reaction vessel

Initial studies were carried out using a 2 L fermenter (1.5 L working volume) equipped with a 502D agitator (LH Fermentation, Maidenhead, UK), an LH temperature regulator (LH Fermentation, Maidenhead, UK), a GFM17 mass flow meter (Aalborg®, US) and attached to an MX3 Bio sampler autosampler (New Brunswick Scientific, USA). Data were logged using Orchestrator software (Measurement Systems Ltd. (MSL), Newbury, UK). An additional condenser was installed in advance of the mass flow meter in order to prevent the expulsion of water vapour which would both decrease the sample volume and negatively affect the mass flow meter's performance.

2.5. 10 L reaction vessel

A tailored 10 L (5 L working volume) reaction vessel (Limitech A/S, Aabybro, Denmark) with additional computer control systems was used for additional study. It was equipped with a high speed mixer and a slow speed agitator (Fig. 1) and was temperature regulated using a Haake C35 (Thermo Scientific, Basingstoke, UK) circulator attached to a water jacket on the vessel. A GFM17 mass flow meter (Aalborg®, US) was attached to the gaseous vent at the top of the vessel and data logged using Orchestrator software (Measurement Systems Ltd. (MSL), Newbury, UK). Samples (10–15 mL) were taken during incubation from a tapped sampling point at the bottom of the vessel.

2.6. Initial vessel set-up

Shredded paper substrate was added to the vessel which was then brought to desired volume (1.5 or 5 L) with 0.1 mol/L NaOAc buffer (pH 5.0). The 2 L vessel was then autoclaved. This was not possible for the 10 L vessel which, instead, was heated to 90 °C for 10 min to sufficiently sterilise the initial buffer and paper substrate. The vessels were then equilibrated to 50 °C, the working temperature of Accellerase® 1500. Accellerase® 1500 (16 FPU/g of substrate) and βG (30 U/g of substrate) were added and stirred continuously.

2.7. HPLC – carbohydrate analysis

Samples (2 mL) were placed into sealed tubes and heated at 100 °C for 10 min to denature the enzymes and stop any further fermentation. Residual solids were then removed by centrifugation at 13,000 rpm for 5 min. Finally the supernatant was filtered using 0.2 μm syringe filters (Fisher Scientific UK Ltd., Loughborough, UK) into 300 μL glass vials (Essex Scientific Laboratory Supplies Ltd., Hadleigh, UK). Analyses of ethanol, glucose, xylose and cellobiose were carried out by HPLC using a Series 200 LC instrument (Perkin Elmer, Seer Green, UK) equipped with a refractive index detector. An Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) with matching guard columns was used, operating at 65 °C with ultrapure water as mobile phase at a flow rate of 0.6 mL/min.

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