Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

Use of treated effluent water in ethanol production from cellulose

Divya Ramchandran [a], *N. Rajagopalan* [b], *Timothy J. Strathmann* [c], *Vijay Singh* [a,*]

[a] *Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, 1304 W. Pennyslavania Avenue, Urbana, IL 61801, USA*

[b] *Illinois Sustainable Technology Center, One Hazelwood Dr., Champaign, IL 61820, USA*

[c] *Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 205 N. Mathews Avenue, Urbana, IL 61801, USA*

ARTICLE INFO

Article history:
Received 6 November 2012
Received in revised form
9 April 2013
Accepted 10 April 2013
Available online 29 May 2013

Keywords:
Bioethanol
Cellulose
Effluent water use
Enzymatic hydrolysis
Fermentation

ABSTRACT

The bioethanol industry exerts a significant demand on water supplies. Current water consumption rate in corn dry grind ethanol plants is (11–15) $dm^3\ m^{-3}$ of ethanol produced and (23–38) $dm^3\ m^{-3}$ for cellulosic ethanol plants. The main goal of this study was to examine the feasibility of use of treated wastewater effluent in place of potable freshwater for cellulosic ethanol production. The effects of using two different types of filtered treated effluent; Bloomington- Normal, IL (Residential type) and Decatur, IL (Industrial/Residential Mix type); on the rate of fermentation and final ethanol yield from a pure cellulosic substrate were evaluated. Characterization analysis of both effluent water samples indicated low concentration of toxic elements. Final ethanol concentrations obtained with Bloomington- Normal and Decatur effluent and with a control treatment using de-ionized water were similar, resulting in 360 g kg^{-1} (0.36 g g^{-1}), 370 g kg^{-1} (0.37 g g^{-1}) and 360 g kg^{-1} (0.36 g g^{-1}), respectively. These findings suggest that with proper characterization studies and under appropriate conditions, the use of treated effluent water in cellulosic ethanol production is feasible.

1. Introduction

The United States (US) fuel ethanol industry is a leading example of renewable fuels manufacture with an annual production of 52.6 hm^3 of ethanol in 2011 [1]. At present, corn based dry grind and wet milling facilities account for 95% of fuel ethanol produced in the US [2]. The successful growth of corn based ethanol industry has laid a foundation for the use of cellulosic feedstocks for ethanol production. One of the major concerns in ethanol plants is the amount of water consumed in the process of ethanol production. Dry grind ethanol plants currently consume water at the rate of (11–15) $dm^3\ m^{-3}$ of ethanol produced whereas cellulosic ethanol plants are estimated to consume water at the rate of (23–38) $dm^3\ m^{-3}$ [3]. At present, the average water requirement for a 189.3 dam^3 ethanol plant is (567.8–946.4) dam^3 per year. One third of the water requirements are used directly in the ethanol production process and two thirds is used in utility systems [4]. The amount of water usage in bioethanol production questions the feasibility and sustainability of use

* *Corresponding author*. Tel.: +1 217 419 6303; fax: +1 217 244 0323.
E-mail addresses: nrajagop@illinois.edu (N. Rajagopalan), strthmnn@illinois.edu (T.J. Strathmann), vsingh@illinois.edu (V. Singh).
0961-9534/$ – see front matter
http://dx.doi.org/10.1016/j.biombioe.2013.04.003

of energy crops for ethanol production in the long run [5]. Thus, water management technologies are critical for the successful operation of an ethanol plant.

In a typical dry grind ethanol plant, water is used for liquefaction, fermentation, separation, and drying processes. The major water consuming steps in biochemical cellulosic ethanol production process are pretreatment of cellulosic feedstock, washing of pretreated biomass, enzyme hydrolysis and fermentation. Various strategies have been employed in dry grind ethanol plants to reduce water consumption such as recycling within the process, use of heat exchangers to reduce cooling tower loads and use of treated waters in cooling towers [3]. Introducing modifications in a fully functional existing plant can be cumbersome and expensive; thus one of the strategies being considered recently is the use of alternative sources of water [6]. Previous study has indicated the feasibility of using cooling tower blow down water in dry grind ethanol fermentation process [3]. Water reuse in ethanol plants results in increased ion concentration which can be stressful to yeast [7]. The major cations found in water are sodium (Na^{+}), potassium (K^{+}), magnesium (Mg^{2+}) and calcium (Ca^{2+}). Potassium is known to be involved in osmoregulation and charge balancing. Low potassium levels are toxic to yeast cells. Competitive inhibition of sodium and potassium leads to low potassium levels and high sodium levels in the yeast cells and is the reason for sodium toxicity for *Saccharomyces cerevisiae*. Sodium is generally used as caustic soda (NaOH) in fuel alcohol plants for cleaning purposes. Sodium levels greater than 500 mg L^{-1} results in yeast stress and inhibits their growth and fermentation activity [8]. The inhibitory effect of sodium depends on the ratio of sodium and potassium and the pH of the medium. At pH 5, with a Na:K ratio 20, potassium uptake is reduced by 70% whereas at pH 4, the effect of sodium toxicity is negligible [7]. Effect of calcium ions on the fermentation of sucrose by *S. cerevisiae* has been previously studied [9]. It was observed that sucrose fermentation was inhibited with increased calcium ion concentration which was explained on the basis that calcium can inhibit the activity of invertase enzymes required for the breakdown of sucrose to glucose and fructose. Magnesium is essential for yeast growth as it helps to maintain cell structure and plays an important role in cell division, growth and enzymatic activity [3]. Calcium interferes with magnesium uptake and is considered to be toxic to yeast cells [3]. Trace elements are also vital for yeast growth but excess of the same can be deleterious to the organism. The toxic effects result from blockage of functional groups and enzyme sites, denaturation and inactivation of essential enzymes and disturbance in membrane functionality [10]. Copper (Cu) is an important cofactor for many enzymes such as lactase, cytochrome-c oxidase and Cu–Zn superoxide dismutase [11]. It helps in the detoxification of yeasts and enhances their respiration activity [11]. The optimal concentration of Cu is known to be 10^{-6} mol L^{-1} (0.06 mg L^{-1}) and is toxic to yeast in excess amounts [3]. Manganese is required at a concentration of 2×10^{-6} mol L^{-1} to 10×10^{-6} mol L^{-1} (0.11 mg L^{-1}–0.55 mg L^{-1}) as it plays an important role in the glycolytic pathway being a part of pyruvate carboxylase and enhances bud growth [11].

The presence of ions also affects the enzymatic activity of cellulases. Effects of various metal ions have been studied previously on the enzymatic activity of cellulase using Avicel as the substrate [12,13]. Magnesium (Mg^{+2}), calcium (Ca^{+2}) and barium (Ba^{+2}) ions were observed to have a stimulatory effect on hydrolysis (increase in total reducing sugars) at a concentration of 0.01 mol L^{-1} [12]. Cations such as ferrous (Fe^{+2}) and cupric (Cu^{+2}) ions were shown to have an inhibitory effect on cellulase hydrolyzing reactions of cellobiohydrolase, endo-β-glucanase and β-glucosidase causing a 70% loss in hydrolysis whereas ferric (Fe^{+3}) ions resulted in a 90% loss of initial hydrolysis rate at a concentration of 0.01 mol L^{-1} which was explained on the basis of detrimental binding to cellulases causing a conformational change, replacement of important cofactors and redox mechanisms [13]. Mercury (Hg^{+2}) ion at 0.01 mol L^{-1} level have been observed to have a pronounced inhibitory effect on cellulases, most likely due to its interaction with sulfate containing amino acid residues and cobalt (Co^{+2}), zinc (Zn^{+2}), manganese (Mn^{+2}) and nickel (Ni^{+2}) had a slight inhibitory effect at the same level of concentration [13].

The main goal of this study was to reduce the amount of fresh process water used in cellulosic ethanol production and maximize the use of treated effluent water, i.e. the wastewater released from nearby wastewater treatment plants (WWTP's) during hydrolysis and fermentation steps in the cellulosic ethanol production process. Specifically, glucose production during hydrolysis of a pure cellulosic substrate and final ethanol production during fermentation were evaluated using two different types of effluent water – Bloomington-Normal, IL (a residential wastewater source) and Decatur, IL (a mixed residential/industrial wastewater source) – and compared with processing in a deionized water control.

2. Materials and methods

Avicel PH101 (Sigma Aldrich, St. Louis, MO), a microcrystalline cellulose was used as the substrate for this study. The moisture content of Avicel was determined by standard convection oven method - NREL LAP-001 protocol (drying at 105 °C for 4 h) [14]. The two different types of treated effluent water samples used in this study were collected from Bloomington-Normal Water Reclamation District, Southeast Wastewater Treatment Plant (BNWRD Se WWTP), Bloomington, IL and Sanitary District of Decatur, Decatur, IL and transported to the Illinois Sustainability Technology Center, Champaign, IL, USA. Bloomington effluent was residential wastewater and the temperature, pH and conductivity of water sample at the time of measurement were 21.2 °C, 7.2 and 818 μS cm^{-1} respectively. Decatur effluent water sample comprised of 75% industrial and 25% residential wastewater and the temperature, pH and conductivity of water sample at the time of measurement were 27.2 °C, 7.58 and 2.8 mS cm^{-1} respectively. Water samples were stored at 4 °C prior to analysis. Both the effluent water samples were filtered through 2.7 μm (Grade GF/D) glass microfiber filter papers (Whatman, Buckinghamshire, UK) prior to use in experiments.

Enzyme used for hydrolysis was Accellerase Duet (Dupont Industrial Biosciences, Palo Alto, CA). Accellerase Duet enzyme is derived from a genetically modified strain of *Trichoderma reesei* and is a single product containing all major enzyme activities. It has an endoglucanase activity of