Bioresource Technology 176 (2015) 267–272

Contents lists available at ScienceDirect

Bioresource Technology

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

Concurrent calcium peroxide pretreatment and wet storage of water hyacinth for fermentable sugar production

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HIGHLIGHTS

- A novel process was developed to pretreat and store biomass concurrently.
- CaO_2 loading and moisture content were critical to process performance.
- 10% CaO_2 loading and 80% moisture content were require to maintain alkaline pH.
- The reducing sugar yield of water hyacinth was enhanced by ~2.5-fold.
- The process could be used to consolidate the pretreatment and storage of biomass.

ARTICLE INFO

Article history:
Received 21 September 2014
Received in revised form 4 November 2014
Accepted 5 November 2014
Available online 13 November 2014

Keywords:
Pretreatment
Wet storage
Water hyacinth
Delignification
Enzymatic saccharification

ABSTRACT

In the present study, a novel concurrent process of pretreatment and wet storage was developed and investigated by applying calcium peroxide for preservation and conversion of fresh water hyacinth biomass to fermentable sugars. The effects of CaO_2 loading concentration and moisture content on the lignin reduction, carbohydrate preservation and enzymatic saccharification of water hyacinth biomass were evaluated by experimental design using a response surface methodology. The data showed that the concurrent process could conserve 70% carbohydrates and remove 40% lignin from biomass of water hyacinth at the best condition in this study. The enzymatic digestibility and reducing sugar yield from the best condition of concurrent process were around 93% and 325 mg/g (dry weight) of fresh biomass, respectively. The result suggested that the concurrent process developed in this work could be a potential alternative to consolidate the pretreatment and storage of aquatic plant biomass for fermentable sugar production.

1. Introduction

Fermentable sugar generated from plant biomass is the key issue to sustain the production of bio-products such as bioethanol and lactic acid. Presently, fermentable sugars used for bio-productions are majorly generated from starch crops. However, using starch crops as feedstock will cause the land competition between food production and bioproductions (Valentine et al., 2012). Therefore, there is a growing demand of cheap and sustainable source of feedstock for production of fermentable sugars. Many research efforts have been devoted to utilizing lignocellulosic biomass as feedstock for fermentable sugar production (Mood et al., 2013). In order to liberate the monosaccharides from the cell wall complex, pretreatments are usually applied to increase enzymatic digestibility of lignocellulosic biomass. Among several pretreatment methods, alkali pretreatment possesses many favorable advantages including low operation cost, less degradation of cellulose and low formation of inhibitors for enzymatic hydrolysis and fermentation (Galbe and Zacchi, 2012). The main mechanisms of alkali pretreatment are degradation of ester bonds and cleavage of glycosidic linkages in the cell wall matrix, which causes the depolymerization of lignin structure, and therefore reduces the interaction between lignin and hemicellulose and increases the accessibility of cellulose to enzymatic attack (Cheng et al., 2010). Additionally, alkali pretreatment can also cause partial de-crystallization of cellulose fibers and therefore increases the efficiency of cellulase hydrolysis.

Aquatic plants have been proposed as future feedstock for production of third generation biofuels and could also be a potential feedstock for other bioproductions. In comparison with terrestrial plants, aquatic plants have many advantages to be utilized as feedstock for fermentable sugar production including fast growth rate, less lignin content and no competition for food production (Wilkie

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http://dx.doi.org/10.1016/j.biortech.2014.11.016
0960-8524/

and Evans, 2010). Moreover, the cultivation of aquatic plants can be integrated into wastewater treatment which offers an extra benefit for process development. Water hyacinth is a floating aquatic plant which can be found globally in subtropical and tropical regions because of their abilities of rapid nutrient uptake and promote biomass growth. The annual productivity of water hyacinth could reach 200 ton dry matter in eutrophic waters in the tropical areas (Hasan and Chakrabarti, 2009). However, owing to its prolific growth characteristic, the over growing water hyacinth can block the water surface and cause the degradation of water quality especially in a eutrophic water system; thus, water hyacinth is recognized as an invasive species by many countries. Regardless of its infamous reputation as an invasive aquatic plant, nonetheless, water hyacinth has also been utilized for bioremediation of pollutants in fresh water system including heavy metals and organic matters (Ismail et al., 2014).

However, the high moisture content of fresh aquatic biomass makes the storage a challenge. Fresh harvested aquatic biomass contains more than 90% water. If the fresh harvested aquatic biomass is not stored appropriately, it will spoil quickly. Additionally, storage is a key issue to ensure year round supply of biomass for bioproductions (Kenney et al., 2012; Tian, 2013). Biomass storage can be categorized into dry storage and wet storage based on the moisture content of biomass. For dry storage, the moisture content of biomass is generally lower than 25%. Therefore, biomass drying usually takes extensive processing period depending on the surrounding environment conditions (e.g., humidity and temperature). Wet storage of biomass typically holds more than 50% moisture content during the storage period. Wet storage has several merits over dry storage including lower loss of energy content during storage period, reduced fire risk and better enzymatic digestibility (Liu et al., 2013). Wet storage can be achieved with the aid of microorganisms or chemicals to drop or increase the environmental pH to inhibit the growth of unfavorable microorganism.

In this study, a novel process of concurrent pretreatment and wet storage of water hyacinth with calcium peroxide was proposed and invested. Calcium peroxide is a white to yellowish powder which has been extensively applied in water treatment, seed disinfection and food processing (Pohanish, 2008; Zhai and Jiang, 2014). When reacted with water at pH lower than 12, calcium peroxide decomposed into hydrogen peroxide, hydroxide ions and carbonate, and the generated hydrogen peroxide will further decompose into highly reactive superoxide and hydroxyl radical. The mechanism is briefly described by following equations (Novotortsev et al., 2012):

Decomposition of calcium peroxide

$$CaO_2 + 2H_2O \rightarrow H_2O_2 + Ca(OH)_2 \quad (1)$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \quad (2)$$

Generation of superoxide and hydroxyl radical

$$H_2O_2 \leftrightarrow HO_2^- + H^+ \quad (3)$$

$$HO_2^- + H_2O_2 \rightarrow OH^{\cdot} + O_2 + H_2O \quad (4)$$

Calcium peroxide possesses many distinctive properties in comparison with other peroxides when it is used as disinfectant and oxygen generator, including better thermal stability, environmental harmless end products, extended release period of hydrogen peroxide and reasonable cost (Chevalier and McCann, 2008; Olyaie et al., 2012). Moreover, the presentation of calcium hydroxide together with hydrogen peroxide during the decomposition of calcium peroxide might be able to increase the efficiency of delignification and enzymatic saccharification of biomass (Cabrera et al., 2014; Yu et al., 2013). Based on the described mechanism and properties, applying calcium peroxide to wet storage of aquatic biomass might be able to simultaneously disinfect the biomass to reduce the loss of energy content and depolymerize the lignin structure to increase the enzymatic digestibility. Moreover, the alkaline in the process effluent could be partially recovered through carbonation to form precipitated calcium carbonate. The calcium carbonate is then thermally converted to calcium oxide which can be further converted to calcium peroxide by reacting with hydrogen peroxide. Because the decomposition of calcium peroxide is related to moisture content in its surrounding environment, the aims of this study is to develop the process and evaluate the effects of calcium peroxide loading concentration and biomass moisture content on the carbohydrate preservation, lignin removal and enzymatic convertibility of water hyacinth after the concurrent pretreatment and storage period.

2. Methods

2.1. Chemicals and enzymes

All chemicals used in this study were either analytical grade or HPLC grade purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Alpha-amylase (product# A0447) and glucoamylase (product# M0035) were also obtained from Tokyo Chemical Industry Co., Ltd. Cellulase enzyme complex ACCELLERASE® 1500 and beta-glucosidase ACCELLERASE® BG were provided by DuPont™ Genencor® Science (Rochester, NY, USA) as gifts. All enzymes were stored at 4 °C to maintain the activities.

2.2. Biomass preparation

The whole plants of water hyacinth were harvested from an artificial pond in Tai Yi Ecological Leisure Farm at Nantou, Taiwan (23.978N, 121.006E) in September 2013. Upon arrival in the laboratory, the fresh harvested biomass were washed extensively with tap water to remove the mud. The biomass were then sun dried to a moisture content less than 75% and chopped through a 12.5 mm sieve by using an automatic vegetable chopper (Dachuan Precision Industry Co., Ltd., Taoyuan, Taiwan). The chopped biomass was washed again with tap water and then stored in Ziploc® bags at −20 °C until used.

2.3. Design of experiment

A response surface methodology (RSM) was applied to investigate the effects of CaO_2 loading and moisture content on the lignin removal, carbohydrate preservation and enzymatic saccharification of water hyacinth after a 90 day wet storage. A two factor, three level, central composite rotatable design (CCRD) was employed to generate experimental combinations by using a statistical software JMP 10. Table 1 summarizes the coding of factor levels and their corresponding values and responses. A triplicates of nine base experimental combinations plus three extra central point were generated and carried out in random order.

2.4. Concurrent pretreatment and wet storage

Pretreatment and storage of biomass was performed in a 200 ml capped glass bottle. Precisely weighed biomass was loaded directly into each reactor and then blended thoroughly with the designed amount of distilled water and calcium peroxide in the reactor. The reactors were capped and incubated at 30 °C in an incubator with specified timing based on the experimental designs. After storage, the biomass were washed with distilled water and vacuum