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Short Communication

Green biorefinery of fresh cattail for microalgal culture and ethanol production

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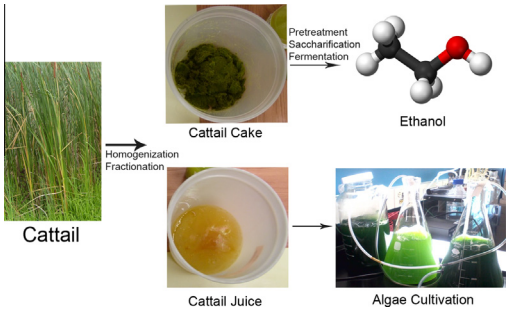
HIGHLIGHTS

- Green biorefinery represents an appropriate approach to utilize aquatic biomass.
- Cattail juice could be a highly nutritious source for microalgal culture.
- Sugars released from the cattail cake can be efficiently fermented to ethanol.
- A more intensive pretreatment is required to reach a high ethanol yield.

GRAPHICAL ABSTRACT



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ABSTRACT

Green biorefinery represents an appropriate approach to utilize the fresh aquatic biomass, eliminating the drying process of conventional bioenergy-converting system. In this study, fresh cattails were homogenized and then filtered, whereby cattails were separated into a fiber-rich cake and a nutrient-rich juice. The juice was used to cultivate microalgae *Chlorella* spp. in different media. In addition, the solid cake was pretreated with the sonication, and used as the feedstock for ethanol production. The results showed that the cattail juice could be a highly nutritious source for microalgae that are a promising feedstock for biofuels. Sugars released from the cattail cake were efficiently fermented to ethanol using *Escherichia coli* KO11, with 8.6–12.3% of the theoretical yield. The ultrasonic pretreatment was not sufficient for pretreating cattails. If a dilute acid pretreatment was applied, the conversion ratio of sugars from cattails has the potential to be over 85% of the theoretical value.

1. Introduction

The quick growth of the world population and rapid progress of a number of emerging economies have both directed to a sharp increase in global energy consumption. However, the increasing cost of fossil fuels as well as the escalating social and industrial awareness of the environmental impacts associated with the use of fossil fuels has created the need for more sustainable fuel options which are both more economic and environmentally friendly. Considerable attention has been given to lignocellulosic biomass such as agricultural residues and energy crops for biofuel production (Harun et al., 2010; Ho et al., 2013). One kind of viable feedstock is aquatic plants, such as cattails and duckweed. Aquatic plants have been widely used for phytoremediation, and had been considered as an energy crop with high potential (Wilkie and Evans, 2010). In addition to their superior productivity, the use of aquatic plants for biofuel production will add value to land and reduce emissions of greenhouse gases by replacing petroleum products.

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One drawback of utilizing aquatic plants is their high water contents, which can be as high as 75–85% for the stem, or about 90% for fractions submerged in water. Water present in green biomass has a negative effect on the conventional bioenergy-converting approach, as it requires a high heat of vaporization (Xiu et al., 2014; Zhang et al., 2011a). In general, drying the "nature-wet" raw material is needed, and limits the options for green biomass as feedstock and overall process economy.

The green biorefinery represents an appropriate approach to utilize the whole fresh aquatic biomass, eliminating the drying process of conventional bioenergy-converting system (O'Keeffe et al., 2011). In a green biorefinery, the wet-fractionation technology is used as the first step to isolate the green biomass substances in their natural form (Richter et al., 2009). Thus, green biomass is separated into a fiber-rich press cake and a nutrient-rich press juice (Xiu and Shahbazi, 2015). In addition to cellulose and starch, the press cake contains valuable dyes and pigments, crude drugs, and other organics. The green juice contains proteins, free amino acids, organic acids, dyes, enzymes, hormones, other organic substances, and minerals (Kamm and Kamm, 2007). Both fractions have an economic value.

This paper is to investigate the green biorefinery options (Fig. 1) for an aquatic plant: cattail. Freshly harvested cattails from the North Carolina A&T State University (NC A&T) farm were homogenized, and then separated into a fiber-rich cake and a nutrient-rich juice. The resulting solid cakes were pretreated with an ultrasonic homogenizer and used for ethanol production. Both the simultaneous saccharification and fermentation process, and the separate hydrolysis and fermentation were carried out using the microbe *Escherichia coli* KO11. Growth of microalgae *Chlorella* spp. with the juice was also investigated.

2. Methods

2.1. Cattail harvest and processing

The fresh cattails, *Typha latifolia*, were collected from the constructed wetland at a NC A&T's farm in summer 2013, chopped with pruning shears, cut into small pieces, and stored at −20 °C. A laboratory blender was used to homogenize the cattail with addition of water. Cattail to added de-ionized water ratio was 1:5. The cattail slurry was filtered using the whatman grade 595 filter paper, separating the liquid from the solid biomass. In this study, subsamples of the liquid and filtered biomass were called the cattail juice and the cattail cake, respectively. Both fractions were used freshly or kept in a freezer at −20 °C for downstream processing.

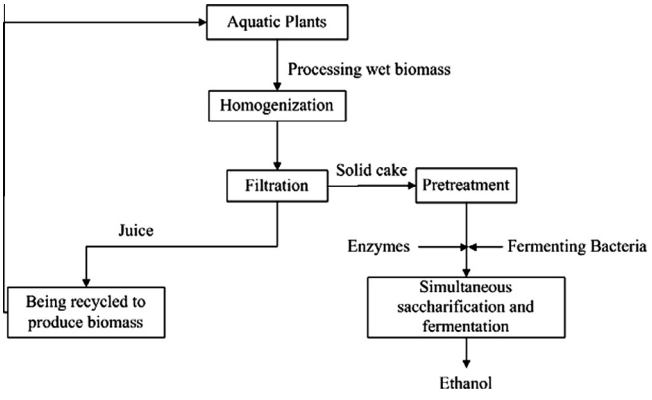

Fig. 1. Green biorefinery of aquatic plants.

2.2. Biomass analytical procedures

The fresh cattail, the solid cake, and the cattail juice were analyzed for the ash content, the solid content, and carbohydrates (cellulose, hemicellulose, and lignin) using the laboratory analytical procedures developed by the National Renewable Energy Laboratory (NREL). Elemental analyses for carbon (C), hydrogen (H), and nitrogen (N) contents were determined using a Perkin–Elmer 2400 CHN/S analyzer (Waltham, MA). The contents of C, H, and N were calculated on a dry basis. Mineral analysis was performed for elements (K, Mg, CA, P, Al, Ba, Mn, Fe, Cu, Sr and Zn) using an Agilent ICP 7500 (Santa Clara, CA). Calibration curves were generated using standard solutions that have been serially diluted.

The content of the cattail juice was also examined by using an Agilent Gas chromatography/mass spectrometry (GC/MS) (Santa Clara, CA), and a Waters High-performance liquid chromatography (HPLC) (Milford, MA), and a Waters LC/MS system (Acuity UPLC/Synapt Q-TOF MS, Milford, MA). All the experiments and analyses were performed in duplicate or triplicate.

2.3. Pretreatment of cattail cake

The cattail cake was diluted with the 0.05 M citrate buffer to 1–3 g dry biomass/100 mL solution. The pretreatment was done by using a Branson Sonifier S-250D Digital Ultrasonic Cell Disruptor/Homogenizer (Danbury, CT), which was operated with a frequency of 20 kHz, supplied power of 200 W, and a platinum probe with the 13 mm diameter tip. Generally, the ultrasonic probe was dipped at 2 cm into the solution, and the cattail cake was ultrasonically pretreated for 15 min at 60–120 W. To prevent overheating, the samples were kept in an ice bath during the pretreatment process.

2.4. Microalgae cultivation in media containing cattail juice

A microalgal strain of *Chlorella* spp. was cultured with de-ionized water, Bristol medium, or proteose medium. Bristol medium consists of the following ingredients: $NaNO_3$ (2.94 mM), $CaCl_2{\cdot}2H_2O$ (0.17 mM), $MgSO_4{\cdot}7H_2O$ (0.3 mM), K_2HPO_4 (0.43 mM), KH_2PO_4 (1.29 mM), and NaCl (0.43 mM). Proteose medium was made by adding 1 g proteose peptone into 1 L of Bristol medium. When scaling-up the culture to a larger volume, a volume of 10% of algal seed was used (Zhang et al., 2014).

The cattail juice was added to DI water or Bristol medium to prepare 1, 2, 5 and 10% (v/v) juice media. Microalgae cultivation was conducted in 150 mL Wheaton glass bottles containing 100 mL algal culture (*i.e.* 90 mL medium and 10 mL algal seed) at room temperature and the light intensity of 600 μmol m^{-2} s^{-1}. The bottles were manually agitated at least once a day. Optical density (OD) of the microalgal culture was measured every other day by using a Thermo Scientific GENESYS 20 Spectrophotometer (Waltham, MA). The correlation (*i.e.* Eq. (1)) between the optical density of *Chlorella* spp. at 680 nm and the cell number was determined in our previous publication (Hasan et al., 2014):

$$\text{Cell number (cell/mL)} = 8 \times 10^6\, OD_{680} + 425897 \quad (1)$$

2.5. Enzymatic hydrolysis and fermentation of cattail cake

Screw-capped 250 mL Erlenmeyer flasks were used as reaction vessels and were agitated at 150 rpm in a constant temperature incubator shaker. The cattail cake was loaded into the reactor to give an initial glucan concentration of around 1 g-glucan/100 mL liquid, and hydrolyzed with a cocktail of enzymes, which included cellulase (Novozyme, NS50013) at a loading of 15 FPU g/glucan, β-