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Ultrasonic-assisted simultaneous saccharification and fermentation of pretreated oil palm fronds for sustainable bioethanol production

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HIGHLIGHTS

- Palm fronds were pretreated using ultrasonic-assisted organosolv/H_2O_2 method.
- Simultaneous saccharification and fermentation (SSF) of palm fronds by ultrasound.
- Optimum conditions: 5 h, 40 °C, pH 5.0, yeast conc. 15 g/l, solid loading 10% w/v.
- Maximal ethanol conc. (18.15 g/l) and yield (57.02%) at optimum SSF conditions.

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ABSTRACT

Ultrasonic-assisted simultaneous saccharification and fermentation (SSF) of modified organosolv pretreated oil palm fronds (OPFs) for sustainable production of bioethanol was investigated for the first time in this study. Ultrasound application in industrial processes is able to reduce the process temperature and time, which eventually improves the energy utilization for sustainable production. According to this study, the ultrasonic-assisted SSF of pretreated OPFs was found to improve the bioethanol yield significantly at shorter times compared to the SSF processes without ultrasound. Optimization of the ultrasonic-assisted SSF process resulted in maximal bioethanol concentration (18.2 g/l) and yield (57.0%) at optimum SSF time (5 h), temperature (40 °C), pH (5.0), yeast concentration (15 g/l) and solid loading (10% (w/v)). SSF coupled with ultrasound was found as a sustainable way of producing high bioethanol titre from organosolv/H_2O_2 pretreated OPFs.

1. Introduction

Since the year 2000, there has been consistent increase in the rates of bioethanol production and consumption with annual growth of about 6–12% worldwide [1,2]. For instance in 2009 and 2010, the global total quantity of bioethanol that were produced for fuel purposes were about 89 billion liters and 93 billion liters respectively with consumption capacities of 63 billion liters and 79 billion liters respectively [2]. The greatest volume of bioethanol that is produced and consumed in the world presently is obtained mainly from wide range of feedstocks with about 80% sourced from corn and sugarcane together [2]. All over the world, the major lignocellulosic feedstocks for bioethanol production are abundant (about 1.7 billion tonnes) especially in Asian countries with the main components being palm wastes, wheat straw and rice husks [2]. Oil palm fronds (OPFs) form the largest percentage of oil palm wastes that are generated by the oil palm industry annually [2]. In order to achieve bioethanol production sustainability, these wastes referred to as second generation feedstocks could replace the edible energy crops, which are currently used to produce bioethanol.

In a bioethanol biorefinery, apart from the pretreatment unit, the saccharification and fermentation units are also crucial units that affect the production costs of the refinery. Combining the saccharification and fermentation processes in one vessel is found to be better alternative to separate hydrolysis and fermentation (SHF) in terms of cost perhaps due to reduced process time, lower energy requirement and high ethanol yield [3,4]. Other advantages of simultaneous saccharification and fermentation (SSF) are the high bioethanol yields at high solid loading compared to SHF. However, one major problem associated with simultaneous saccharification and fermentation (SSF) of biomass is the difference in optimum temperatures for hydrolysis (50–55 °C) and fermentation (30–35 °C) [4]. Coupling of SSF process with ultrasound can accelerate the production rate of bioethanol at shorter time. In this study, SSF of OPFs was combined with ultrasound irradiation to assess the efficiency of the process on bioethanol yield.

Sonication is a physicochemical method of treating materials whereby energy in the form of sound waves is applied. A sound

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of frequency greater than 20 kHz is referred to as ultrasound [5] and it is usually associated with cells disruption thus mostly used in industrial applications. Ultrasound waves produce cavitation and acoustic streaming when they are applied to liquid slurry and these cavitations are able to generate bubbles which result in increase in temperature and pressure in the cavitation area. There is evolution of powerful hydromechanical shear forces through the slurry which disrupt the cells of the materials present in the cavitation area. Sonication of lignocellulosic biomass for bioethanol production depends on factors such as ultrasonic intensity, sonication time, temperature, pH of medium, ultrasonic frequency, concentration of medium etc. For instance, microorganims like enzymes are found to be susceptible to damage by ultrasound at high intensities during lignocellulosic biomass fermentation [6]. Jomdecha and Prateepasen [7] have evaluated the effects of ultrasound irradiation on the fermentation of the yeast, *Saccharomyces cerevisiae*. An ultrasound which is applied at a frequency of 2.2 MHz and electrical power input of 14 W to a fermentation medium has been reported to destroy about 25% of the *S. cerevisiae* cells after 1 h [8]. However, low intensity ultrasound (0.2–0.8 W cm^{-2}) and frequency of 20–40 kHz have been found to facilitate the rapid growth of *S. cerevisiae* and also reduce the fermentation time by 50–64% [7]. Bioethanol production coupled with intermittent ultrasound was found to enhance the bioethanol yield by 20% [9] compared to non-sonicated fermentation. Ur Rehman et al. [10] have concluded that for efficient biomass pretreatment, power ultrasound is preferable as it sufficiently energizes the biomass thus effective for producing high yields at high biomass loading. Nonetheless, low-intensity sonication has the potential to improve the conversion of sugars to ethanol during fermentation. During pretreatment of biomass for bioethanol production, the cell wall of the biomass gets disintegrated which eventually exposes the hemicellulose and cellulose for enhanced sugar production during saccharification. Ultrasonication of pretreated lignocellulosic biomass is found to increase bioethanol yield and improve the process' efficiency at short time and high substrate concentrations hence making it a profitable technology for bioethanol production [11,12]. Ofori-Boateng and Lee [13] have made a first report on high cellulose recovery from oil palm fronds after pretreatment using ultrasound assistance at low temperature and high solid loading as similarly reported by Nazir et al. [14] for alkaline pretreated oil palm empty fruit bunches (EFBs) and Yunus et al. [15] for acid pretreated EFBs. Sono-assisted organosolv/H_2O_2 pretreatment and subsequent SSF into bioethanol has been extensively assessed by Ofori-Boateng and Lee [16] to be exergetically efficient and thermodynamically sustainable compared to separate hydrolysis and fermentation of organosolv pretreated OPFs [17]. Again, comparing the economic and thermodynamic sustainability of OPFs bioethanol pretreatment methods, organosolvation was found to be more efficient compared to steam explosion of OPFs [17,18].

This study aims at pretreating OPFs using ultrasound irradiation for enhanced bioethanol production by the technology of simultaneous saccharification and fermentation (SSF). During the ultrasonic-assisted SSF of OPFs, process conditions such as sonication time, temperature, yeast concentration and solid loading were optimized for maximal recovery of OPFs' bioethanol titer.

2. Method

2.1. Preparation of lignocellulosic materials

OPFs were obtained from the oil palm plantation at the Engineering campus of Universiti Sains Malaysia. The petioles were shredded into smaller pieces (about 10–20 mm long) and washed thoroughly with tap water after removing the leaflets. The washed fronds were dried in an oven (Memmert Beschicking-Loading Modell 100-800) at 105 °C for 16 h to a moisture content of about 10%. The dried fronds were ground (Analytical mill IKA(R) A11, Retsch, Germany) and then made to passed through a 1 mm AS 200 sieve shaker (Retsch, Germany). The dried fronds were stored in zipped poly bags in a cool place until further use.

2.2. Chemicals and microorganisms

Analytical grade ethanol (absolute) was purchased from Fisher Scientific, UK. Sodium hydroxide, ACS grade hydrogen peroxide (H_2O_2) and sulfuric acid (98.0%) were also purchased from Sigma–Aldrich (St. Louis, MO, USA). Commercial cellulase from *Trichoderma reesei* (Celluclast 1.5 L) and ß-glucosidase from *Aspergillus niger* (Novozyme 188) were obtained from Novozymes, Denmark. The standard sugars (glucose and xylose) were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.3. Ultrasonic-assisted organosolv/H_2O_2 pretreatment (UOP) of OPFs

Ultrasonic-assisted organosolv/H_2O_2 pretreatment (UOP) was carried out in an ultrasonic bath (Elma Hans Schmidbauer GmbH & Co. KG, Germany) at a frequency of 37 kHz and ultrasonic power of 200 W. The procedure for the UOP of OPFs in this study followed the same method for optimized conditions as described in our previous studies [13]. Briefly, the extractive-free OPFs (2 g) was mixed with 40 ml of 1.4% aqueous NaOH and 80% aqueous ethanol (1:4 v/v) in a 500 ml Erlenmeyer flask. The flask was then placed in the ultrasonic cleaning bath and sonicated at 75 °C for 30 min. The residue was washed several times with distilled water and subsequently delignified at room temperature for 16 h using 3% aqueous H_2O_2. The delignified OPFs were washed several times with deionized water until the pH was 7. The dried residue (cellulose) from this stage was used as substrate for simultaneous saccharification and fermentation (SSF).

2.4. Production of fermentable sugars from OPFs' cellulose

2.4.1. Enzymatic hydrolysis of pretreated OPFs

In order to study the rate of formation and degradation of sugars in OPFs' cellulose, enzymatic saccharification of the cellulose was carried out using Celluclast 1.5 L (15 filter paper unit (FPU)/g cellulose) and Novozyme 188 (15 International units (IU)/g cellulose). Briefly, in a sterilized flask with total working volume of 100 ml, 5% (w/v) pretreated OPFs was mixed with the enzyme solutions and the medium was maintained at pH 4.5 using 0.05 M sodium citrate buffer (after autoclaving for 15 min at 121 °C). 0.05 g/l tetracycline was added to the fermentation medium to prevent microbial growth. The flask and its content were incubated in an incubator shaker (150 rpm) at 50 °C for 72 h with agitation at 150 rpm. Sample aliquots (2 ml) were taken at various time intervals (6, 12, 24, 48, 72 h), filtered and analyzed for sugar concentrations after dilution.

2.4.2. High performance liquid chromatography (HPLC) Analysis

Analysis of sugars were carried out with an Agilent series 1200 infinity high performance liquid chromatography system which was equipped with a 385-ELSD (evaporative light scattering detector) and operated at 80 °C with nitrogen as the carrier gas. The column used was a 300 mm × 7.7 mm Hi-Plex Ca column. The HPLC-ELSD's spray chamber temperature was set at 40 °C. Deionized water was used as the mobile phase at a flow rate of 0.6 ml/min and 20 µl injection volume was used. Samples and standards were filtered using 0.45 µm and 0.20 µm regenerated