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Research paper

Exploiting microbubble-microbe synergy for biomass processing: Application in lignocellulosic biomass pretreatment

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Abstract

The potential of lignocellulosic biomass as a sustainable biofuel source is substantial. The development of an efficient and cost effective pretreatment approach remains challenging. In this study, we have explored a new, relatively cheap pretreatment option that works at ambient temperatures. By using microbubbles generated by fluidic oscillation, free radicals around the gas-liquid interface of the microbubble readily attack and degrade lignocellulosic biomass, rendering it more amenable to digestion. The combination of microbubbles and *Pseudomonas putida*—a robust delignification and cellulolytic microbe, further improved biomass degradation and consequently, increased glucose production from wheat straw in comparison to solo pretreatment of the biomass with microbubbles and *Pseudomonas putida* respectively. The microbubble-microbe approach to make biomass more amenable to sugar production is potentially a valuable alternative or complementary pretreatment technique.

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1. Introduction

Lignocellulosic biomass is gaining increased industrial application due to its abundance and advantages as a raw material for valuable chemical production [1]. Apart from the cost effectiveness and low environmental impact [2], lignocellulosic biomass also represents a crucial option as a renewable energy alternative. Generally, before these benefits can be fully exploited, the biomass must be processed from its raw form to release sugar, the essential nutrient for microbial metabolism.

Owing to the structural complexity of lignocellulosic biomass, however, pretreatment is ideally the first processing option for sugar extraction [3]. Various pretreatment methods have been reported previously, which are classed into thermal, mechanical, physicochemical, and electrical methods [4]. Others Talebnia et al. [5], and Agbor et al. [6], have grouped these methodologies into: physical, physico-chemical, chemical and biological methods.

Regardless of the group, conventional pretreatment methods are energy intensive given the high temperatures and pressures involved especially the physical and thermochemical processes. For instance, Kumar et al. [3] reported that the dilute-acid hydrolysis process requires temperatures and pressures of up to 230 °C and 10 atm respectively. Conversely, biological pretreatment methods offer the advantage of cost effectiveness but this benefit is readily offset by the substantial time consumption of the process. Many chemical methods are simply inefficient, or suitable for some biomass, with low yield and quality [3]. Another concern with traditional methods is their unsuitability for continuous large-scale production. This is important if lignocellulosic biomass is to play a significant role as a consumer goods raw material. Furthermore, heating vessels and variable water pumps account for majority of the capital and operating costs. There are also high costs due to commercial enzyme utilisation.

The key challenge, therefore, is to change from high power and time-consuming systems to energy efficient; time-saving approaches without diminishing biomass yield or quality. One interesting option is the application of microbubbles. Microbubbles offer increased surface area to volume ratio, and have been exploited for their mass and momentum transfer capability in algal culture [7] and microbial harvest [8] and water treatment [9]. Another unique attribute of microbubbles is their low-rise velocity, facilitating efficient dissolution of their content gas in their immediate environment. More importantly, however, microbubbles are known producers of free radicals in aqueous solution [10] [11] [12]. This

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can aid the decomposition of lignin, thereby increasing the digestibility of lignocellulosic biomass. Li et *al.* [11], reported a ~60% decrease in phenol during a 2 h study using microbubbles. In a different study, the authors observed the degradation of polyvinyl alcohol by collapsing microbubbles. Other works have shown microbubbles to be a strong oxidizing/degradation agent [12].

This study sets out to explore the performance of microbubbles and a cellulotic microbe (*Pseudomonas putida* KT 2440) in the pretreatment of lignocellulosic biomass at room temperature. The cellulolytic activity of *Pseudomonas putida* has been previously reported [13] [14] [15]; however, its application as the main cellulolytic agent is yet unexplored. *Pseudomonas putida* produces enzymes that break down cellulose with the robustness to cope with significant toxicity levels resulting from the biomass. This new approach holds the potential of reducing/eliminating the high costs associated with conventional pretreatment techniques, if it proves effective in digesting lignocellulosic biomass to release fermentable sugar.

2. Materials and methods

2.1. Biomass and growth media preparation

This study used wheat straw as a lignocellulosic biomass owing to its high cellulose and hemicellulose content, 29–35% and 26–32% respectively, with low lignin percent (16–21%) [16] and availability.

Wheat straw was prepared for pretreatment by mechanically reducing biomass particle size to ≤1 mm. The biomass was then washed using distilled water and oven-dried at 80°*C* for 24 h. Wheat straw solution (1% w/v) was prepared with distilled water, and the pH was set at 3, using concentrated HCl (Sigma-Aldrich, UK). The growth medium with a composition of: 1% wheat straw, 0.5% yeast extract, 0.02% magnesium sulphate and 0.02% ammonium phosphate dibasic was prepared according to Abdul-Kadhim and Jarallah, [17]. The medium was sterilised by autoclaving at 121°*C* for 15 min and 1 bar pressure before cultivating with *Pseudomonas putida* KT2440.

2.2. Experimental procedure

The experimentation was divided into four (4) groups to study the effects of each pretreatment. The first group was a control group consisting with biomass in liquid at pH 3. The second group was pretreatment with microbubbles. In this case, wheat straw was treated for 3.30 h at pH 3, by sparging with fluidic oscillator generated microbubbles [8]. The time of pretreatment, 3.30 h, was selected after preliminary studies revealed no additional glucose was produced after 4 h. The third group was the combined pretreatment in which wheat straw was pre-treated with microbubbles for 3.30 h and then, the pre-treatment continued with the application of *Pseudomonas putida* for an additional four (4) hours. Conversely, the fourth group entailed pretreatment with only *Pseudomonas putida*.

After pH adjustment of the wheat straw solution to 3, this solution was introduced into the pretreatment column, which was connected to the fluidic oscillator [18]. Fig. 1 shows the experimental rig, which consists of a fluidic oscillator, a micro-porous diffuser and a pretreatment column. On the other hand, the biological pretreatment was achieved using *Pseudomonas putida* KT2440 in a 500 ml Erlenmeyer flask at 30°*C*. All experiments were conducted at room temperature (~ 25 °*C*), and untreated biomass was used as a control group to compare with other treated biomass. Samples were assayed daily by centrifuging for 15 min at 13000 × g and then filtered with a syringe filter unit (Whatman® Anotop® 25 Plus syringe filter, pore size 0.2 μm, Sigma- Aldrich) to measure glucose concentration.

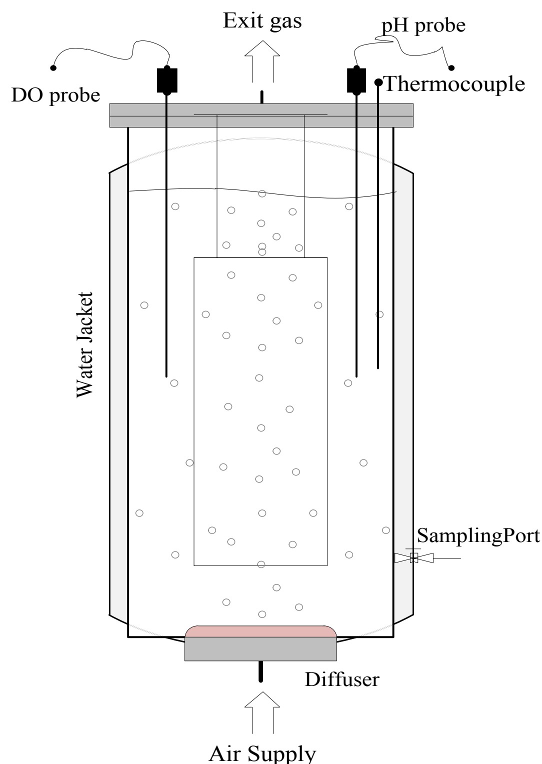

Fig. 1. Schematic representation of the experimental set-up. Compressed air (1 bar) is fed into the oscillator, and there are two outputs from the fluidic oscillator. While, one feeds the microbubbles diffuser, the other is bleed-off.

2.3. Bubble size measurement

The measurement of the microbubbles size distribution was conducted using a Spraytech (Malvern Instrument, UK).

2.4. Glucose concentration

Glucose concentration was measured using a standard glucose assay kit (Sigma-Aldrich, UK) during and after treatment for all experiments.

2.5. Growth pattern of Pseudomonas putida on wheat straw medium

Bacterial growth on wheat straw was monitored optically using a spectrophotometer (DTSTM-1700, 1900 NIR) at 600 nm. The optical density was measured on a daily basis, and the experimental duration was determined based on the measured/observed growth pattern.

2.6. Morphological changes of biomass

Scanning electron microscopy (Model S-360, Phillips) was used to study the morphological changes of the biomass after pretreatment with microbubbles, *Pseudomonas putida* and the combination of both these pretreatment methods. This biomass was coated with gold and set to operate at 15 KV.

2.7. Changes in the functional groups of biomass

FTIR-ATR (Perkin Elmer, UK) was used to examine changes in the functional groups after biomass pretreatment for all experimental

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