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Research Paper

Alkaline treatment for detoxification of acetic acid-rich pyrolytic bio-oil for microalgae fermentation: Effects of alkaline species and the detoxification mechanisms

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ABSTRACT

Bio-oil derived from Pyrolysis of lignocellulosic biomass contains appreciable amounts acetic acid, which can be used as substrate for growing microalgae *Chlamydomonas reinhardtii*. However, the toxic compounds in the bio-oil inhibit the cell growth. This work is to develop alkaline treatment methods to reduce the toxicity and improve fermentability of acetic acid rich bio-oil. When growing in raw bio-oil without any detoxification treatment, the algae can only tolerate up to 0.1 wt% of bio-oil. Treatment with KOH, NaOH and $Ca(OH)_2$ significantly reduced the toxicity and consequently improved the fermentability of bio-oil. The bio-oil tolerant level by microalgae depended on the alkali species used. Among the three alkali species, $Ca(OH)_2$ proved the most effective detoxification reagent. Inhibitory compounds such as furans, phenols, ketones, aldehydes, ethers, esters, alcohols were removed by $Ca(OH)_2$ treatment through precipitation. The detoxification mechanisms by the $Ca(OH)_2$ -based treatment were also explored. The synergistic effect of alkaline pH, high temperature, and presence of Ca^{2+} played an important role for the precipitation of those compounds, and the consequent detoxification. Collectively, the results shows alkali, particularly $Ca(OH)_2$-based, treatment is an effective for reducing the toxicity of the pyrolysis derived bio-oil as fermentative substrate for microalgae growth. The microalgae can tolerant $Ca(OH)_2$-treated bio-oil up to 5.5 wt%, which was 55 times higher than algal tolerance level of untreated bio-oil.

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1. Introduction

Producing fuels and chemicals from lignocellulosic biomass is often achieved through biochemical pathways, which are commonly composed of three steps: pretreatment of biomass to break down its recalcitrant structure; enzymatic hydrolysis of pretreated biomass into reducing sugars; and fermentation of the sugars into various desired products [1]. This process is limited by several technical and economic barriers such as the high costs for pretreatment and enzymes, lack of robust microbes capable of fermenting mixed sugars (hexose and pentose), and under-utilization of lignin compounds in the biomass [2–4].

Thermochemically-based fast pyrolysis is another method converting lignocellulosic biomass into fuels. Fast pyrolysis is the thermal decomposition of biomass in the absence of oxygen, the process can convert biomass into an energy rich liquid (bio-oil), a flammable gas mix (syngas) and a carbon- and nutrient-rich solid (biochar) [5]. Raw bio-oil is an extremely complex mix of chemical compounds. The phenolic oligomers contained in bio-oil can be upgraded into hydrocarbon, which can be further refined into drop-in fuels by using existing petroleum refining technology and infrastructure [6], while other compounds such as levoglucosan and acetic acid, can also be used as fermentative substrates to produce various fuels and chemicals [7–11].

To simplify the bio-oil composition and facilitate further bio-oil refinery, a fractionation system has been developed in Iowa State University to separate raw bio-oil into different stage fractions (SFs) with distinct chemical and physical properties [12]. For example, the stage fraction 1 (SF1) contains the majority of levoglucosan and phenolic oligomers; while stage fraction 5 (SF5) contains the majority of water and acetic acid [12]. The acetic acid contained in the bio-oil is often undesirable due to its high corrosiveness and low heating value [13].

Researchers have attempted to use bio-oil derived acetic acid as road deicers [14]. As a fermentative substrate, the bio-oil derived acetic acid has also been used for microorganism fermentation. For example, Lian et al. (2012) explored yeast fermentation using pyrolytic acetic acid. Our research team also reported the use of acetic acid contained in bio-oil fraction SF5 for the growth of microalgae *Chlamydomonas reinhardtii* [10,11]. The purpose of using *C. reinhardtii* as model organism in these studies is that the algal species is amenable for genetic manipulations [15–17], therefore, it is possible to elucidate the genomic response of the algal cells to this unique substrate. Such an underlying mechanism can then be used as guidance for developing genetic manipulation strategies for other microorganisms to improve the utilization efficiency of the pyrolysis-derived acetic acid.

Use of acetic acid rich bio-oil fraction (SF5) as pyrolytic substrates for microorganism growth, however, still faces a major challenge; i.e., the inhibition of cell growth by the toxic compounds contained in the bio-oil [10,11,18]. Some identified compounds in bio-oil such as phenols and furfural have proven inhibition for microorganism growth, while other compounds are difficult to identify and may also be inhibitory to microorganism growth.

In the near term, the practical approach for efficient utilization of SF5 is to develop various treatment methods for reducing its toxicity. Our previous research has shown that various treatment methods such as activated carbon adsorption [10] and sodium hydroxide (NaOH) can effectively reduce the toxicity of raw SF5 for the alga *C. reinhardtii* fermentation [11]. However, development of an appropriate detoxification method should also consider the process economics. For example, treatment of SF5 by NaOH is very efficient in terms of removing toxic compounds such as furfural, phenols, acetol, and HFM [11]. From an economic production point of view, however, NaOH is a relatively expensive alkali species, and thus, will entail a significant process cost. To develop cost effective treatment methods, it is appealing to explore less expensive alkali species while achieving the similar removing efficiency. In addition, the underlying mechanism of those alkali-based treatments for reducing the toxicity should also be explored. The main objective of this study is to evaluate the various alkaline species for reducing the toxicity while improving fermentability of SF5. The mechanism of detoxification with each alkali, particularly $Ca(OH)_2$, was also investigated.

2. Materials and methods

2.1. Microalgae subculture and the effects of acetic acid concentration on cell growth

Microalgae *C. reinhardtii* ST21 strain was provided by Dr. Martin Spalding from Iowa State University. The strain was used due to its high lipid content and thus, the potential of being used as biofuel feedstock [10,11]. The strain was stored on an agar slant at 4 °C under 12/12 light/dark cycle. To prepare the seed culture, the cells were transferred to 250-mL Erlenmeyer flasks containing 50-mL tris-acetate-phosphate (TAP) medium containing 1 g/L acetic acid. Here, we approximate 1 mL pure glacial acetic acid as 1 g of acetic acid. The medium pH was adjusted to 7 before autoclaving at 121 °C for 15 min. The flasks were placed in an orbital shaker (200 rpm) at 25 °C with continuous illumination at 110–120 μmol s^{-1} m^{-2}.

Initial work was performed to test the effects of initial acetic acid concentration on the microalgae growth. TAP medium containing acetic acid was adjusted to pH 7 via NaOH solution and then filtered via 0.22 μm membrane for sterilization. Cells were grown in 24-well plates. Under aseptic conditions, each well in the plate was added with 1 mL medium containing different levels of acetic acid and 0.1 ml algae seed. The plates were placed on an orbital shaker with a speed of 130 rpm. The temperature was set at 25 °C with continuous illumination at 110–120 μmol $s^{-1}m^{-2}$. The optical density of the culture at 730 nm (OD_{730}) were measured via a BioTek EL × 800 microplate reader (Winooski, VT) on daily basis. The OD_{730} value was then converted into biomass yield (cell dry weight concentration, g/L) through a correlation curve. All the experiment was performed in triplicates.

2.2. Preparation of raw bio-oil and acetic-acid-rich bio-oil stage fraction SF5

The pyrolysis and bio-oil fractionation system for preparing acetic acid-rich stage fraction 5 (SF5) were described