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Effect of lignocellulose degradation products on microbial biomass and lipid production by the oleaginous yeast *Cryptococcus curvatus*

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ABSTRACT

This work investigated effects of lignocellulose degradation products on cell biomass and lipid production by *Cryptococcus curvatus*. Furfural was found to have the strongest inhibitory effect. For the three phenolic compounds tested, vanillin was the most toxic, while PHB and syringaldehyde showed comparable inhibitions in the concentration range of 0–1.0 g/L. Generally little significant differences on the relative cell biomass and lipid contents at the same concentrations of tested compounds were observed between glucose and xylose as a sole carbon source. At 1.0 g/L of furfural, the cell biomass and lipid content decreased by 78.4% and 61.0% for glucose as well as 72.0% and 59.3% for xylose, respectively. *C. curvatus* ceased to grow at concentrations of PHB over 1.0 g/L or vanillin over 1.5 g/L. The strain could survive in the presence of syringaldehyde up to 2.0 g/L for glucose or 1.5 g/L for xylose. The compounds' negative impact was reduced by an increase in inoculum size and a 10% (v/v) seed was detected to be optimal for cell biomass and lipid production. The results demonstrated *C. curvatus* could effectively utilize most of the dominant monosaccharides and cellobiose existing in lignocellulosic biomass hydrolysate in the presence of toxic compounds.

1. Introduction

Lignocellulosic biomass is a promising feedstock that has attracted considerable attention for biofuel production due to its sustainability and abundance [1]. The main constituents of lignocellulosic materials are cellulose, hemicellulose and lignin [2]. Cellulose is a linear polymer of glucose, hemicellulose is a heteropolysaccharide composed of abundant pentoses (xylose, arabinose, etc.) and a few hexoses (glucose, galactose, mannose, rhamnose, etc.), and lignin is an aromatic polymer composed of phenylpropane subunits [3]. However, prior to enzymatically hydrolyzing cellulose and hemicellulose into fermentable sugars, pretreatment processes are required to disrupt the plant cell wall complex. Unfortunately, some by-products are inevitably generated in this process, such as furfural and 5-hydroxymethyl-furfural (HMF) from degradation of pentoses and hexoses, acetic acid from de-acetylation of hemicelluloses, formic acid from breakdown of furfural and HMF, levulinic acid from HMF degradation [3,4], as well as phenolic compounds including syringaldehyde, p-hydroxybenzaldehyde (PHB), vanillin, etc. derived from lignin [5]. These lignocellulose degradation products are usually inhibitory to microorganisms in the down-stream fermentation step [6]. For furfural and HMF, they inhibit alcohol dehydrogenase (ADH), pyruvate dehydrogenase (PDH) and aldehyde dehydrogenase (ALDH), as well as the glycolytic enzymes hexokinase and glyceraldehyde-3-phosphate dehydrogenase [3]; for weak acids, such as acetic, levulinic and formic acid, their inhibitory effects have been mainly attributed to uncoupling and intracellular anion accumulation [7]; for phenolic compounds, although their inhibition mechanisms have not be clarified yet, it is supposed that they probably act on biological membranes, causing loss of integrity, which thereby affects their abilities to function as selective barriers and enzyme matrices [3,8].

During the past decades, the effect of lignocellulose degradation products has been studied extensively on the performance of ethanol-fermenting microorganisms [3,9–11], while limited research has been conducted about their inhibitory effects on oleaginous yeasts, such as *Rhodosporidium toruloides*, *Trichosporon fermentans*, etc. [1,5,12]. Tolerance to inhibitors appears to be strain dependent. For instance, the cell growth of ethanologenic yeast strains was significantly inhibited in the presence of acetic acid at 4.8 g/L [13], whereas the oleaginous yeast *Cryptococcus curvatus* could utilize acetic acid as a sole carbon source at 40 g/L without severe substrate inhibition for lipid production [14]. Additionally, Huang et al. [15] reported that the removal of inhibitors existing in the rice straw hydrolysate by a detoxification method was required to greatly improve both cell biomass and lipid yields by *T. fermentans*. Nevertheless, our previous study proved that the

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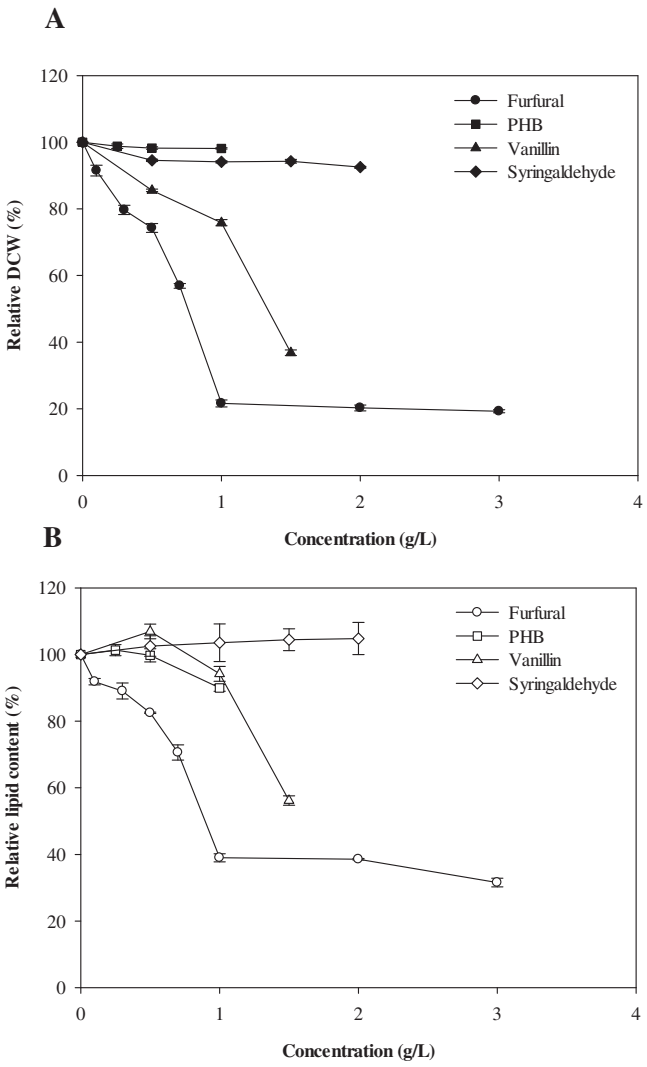

Fig. 1. Effect of lignocellulose degradation products on cell biomass and lipid content by *C. curvatus* when glucose was the sole carbon source (A) DCW; (B) lipid content. At the concentrations of compounds more than the tested values, *C. curvatus* ceased to grow.

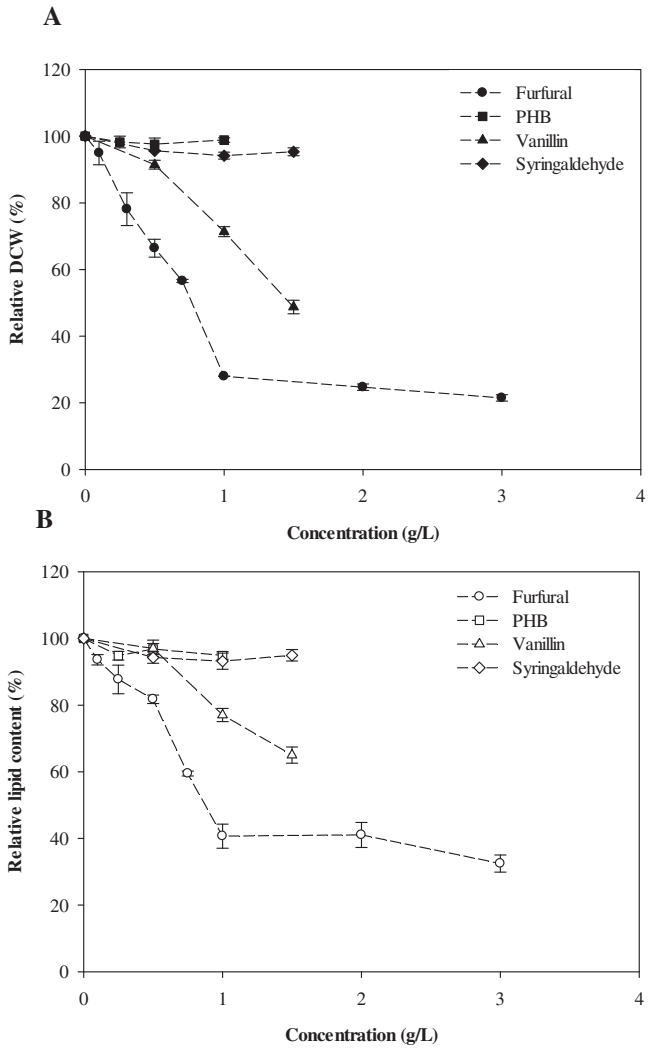

Fig. 2. Effect of lignocellulose degradation products on cell biomass and lipid content by *C. curvatus* when xylose was the sole carbon source (A) DCW; (B) lipid content. At the concentrations of compounds more than the tested values, *C. curvatus* ceased to grow.

detoxification of wheat straw hydrolysate was not necessary for optimal yeast growth and lipid production by *C. curvatus* [16].

A variety of substrates can be utilized by *C. curvatus*, such as industrial glycerol [17], beet molasses [18], whey permeate [19], food waste [20], and wheat straw hydrolysate [16]. *C. curvatus* also has the capacity of accumulating lipid up to 60% (w/w) of the dry cell weight with a similar fatty acid composition to that of conventional vegetable oils. With these characteristics, *C. curvatus* has been considered as a favorable feedstock for a sustainable biodiesel industry [21]. In our previous study, although *C. curvatus* was able to use the hydrolysate from dilute sulfuric acid pretreatment of wheat straw without prior detoxification, effects of the individual degradation products existing in the lignocellulosic hydrolysate on its cell biomass and lipid production were not investigated thoroughly. Therefore, the aim of this study is to explore the performance of *C. curvatus* in the presence of lignocellulose degradation products in terms of cell biomass and lipid production when glucose or xylose is used as a sole carbon source. The significance of the work is two-fold: (1) it fills the information gap on the tolerance of *C. curvatus* to the degradation products originated from lignocellulosic materials; (2) it provides a major criterion of the lignocellulosic biomass pretreatment processes, consequently optimizing the pretreatment conditions for microbial oil production. Acetic acid and HMF have been reported to show little inhibitory effects on *C. curvatus*'s growth [14,16], therefore, other representative compounds, furfural and lignin derivatives including syringaldehyde, p-hydroxybenzaldehyde (PHB) and vanillin were selected as the main focus in this work. Firstly we studied the effects of representative lignocellulose degradation products on the cell growth and lipid accumulation by *C. curvatus*. Then the impact of inoculum sizes on their toxic effect was examined. Finally, we discussed the effects of these degradation products on different sugar utilizations as well as the physical and chemical properties of microbial lipid-derived biodiesel.

2. Materials and methods

2.1. Media and yeast strain preparation

C. curvatus (ATCC 20509) was kept at −80 °C and recovered in YPX medium containing 10 g/L yeast extract, 20 g/L peptone and 20 g/L xylose at 30 °C in an orbital shaker at 150 rpm for 24 h as a pre-culturing step. Seed inoculums were then added to the 50 mL basic culture medium prepared as follows: 20 g/L glucose or xylose, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 2.0 g/L KH_2PO_4, 0.003 g/L $MnSO_4 \cdot H_2O$, 0.0001 g/L $CuSO_4 \cdot 5H_2O$, and 1.5 g/L yeast extract, and the pH in the media was adjusted to 5.5. The basic culture media were sterilized by passing through a 0.22 μm membrane (Millipore, MA) and then ready for further fermentation use.