Efficient lactic acid production through conversion of organosolv pretreated lignocellulosic biomass by Lactic Acid Bacteria

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Lactic acid (LA) is an important bio-product due to its high yield of glucose and widespread applications (John *et al.*, 2007). Specifically, the recent demand for biodegradable and biocompatible poly-lactate polymers (PLA) sharply increased the global interest in LA production (Abdel-Rahman et al., 2013). To date, the successfully realized commercial production of LA has involved the utilization of pure sugars or edible crops. Lignocellulosic biomass is a promising feedstock for LA production considering its great availability, sustainability, and low cost compared to refined sugars (Rahman *et al.*2011). Due to concerns over feedstock costs and the limited worldwide food availability, lignocellulosic resources are being investigated as raw materials for the production of LA (Neureiter *et al.*, 2004; Zhu *et al.*, 2007). During bioconversion, LA accumulation acidifies the fermentation broth, causing inhibition to bacteria metabolism and thus limiting the process efficiency and yield; therefore, much research has been carried out either to alleviate the effect of LA through optimizing the process conditions or through use of genetically engineered strains that are not only more tolerant to acids and low pH but also utilize both pentose and hexose sugars, which are commonly found in lignocellulosic biomass (Taskila and Ojamo 2013). Lactic Acid Bacteria (LAB) consist one of the most industrially important groups of bacteria and hold a crucial role in fermentation, where they are used to convert sugars derived from different raw materials to LA (Makarova *et al.*2006). Bioconversion processes have significant advantages with the most prominent among them being their high selectivity. However, they have long incubation times and typically produce a low concentration aqueous solution of the desirable product. In order to achieve high sugar production, to make the process economically viable and, at the same time, to reduce the environmental impact, the use of high initial solid concentration (high gravity—HG) during saccharification and fermentation can serve as a solution (Kalogiannis *et al.* 2018).

In the present study, mild oxidative organosolv pretreated biomass was used as a substrate for the production of highly-concentrated sugar streams able to support the growth of two LAB strains. Alongside the optimization of pretreatment conditions to maximize the subsequent hydrolysis yields and the screening of saccharification efficiency of the different biomass samples, productivity of lactic acid was evaluated through simultaneous saccharification and fermentation (SSF) using LAB. Organosolv lignocellulosic biomass was treated with aqueous solutions of organic solvents (ethanol, acetone, tetrahydrofuran), at different operation conditions (temperature and reaction time) under an overpressure of O_2 that was crucial in depolymerizing and removing the lignin fraction. Enzymatic hydrolysis was performed using Cellic® CTec2 (Novozymes), a cellulase complex consisting of cellulases, β-glucosidases and hemicellulase, to degrade cellulose and hemicellulose to fermentable sugars. Preliminary screening of biomass saccharification was conducted at 9% initial dry matter (DM) content and 9 mg/g substrate enzyme loading, in 0.1 M citrate-phosphate buffer (pH 5.0), at 50 ºC and 160 rpm. After produced sugars determination, the most promising samples were studied further in order to evaluate the effect of varying enzyme loading and % DM, reaching up to 15% DM. *Lactobacillus delbrueckii sp. bulgaricus* (ATCC 11842) and *Bacillus coagulans* (ATCC 7050) were obtained as freeze-dried stocks from the German collection of microorganisms and cell cultures (ATCC, Germany) and used for the production of LA from organosolv pretreated biomass using SSF approach. *L. delbrueckii* is able to ferment glucose, but not xylose, to efficiently produce LA, while *B. coagulans*, as heterolactic bacteria, ferment xylose contrary to *L. delbrueckii* which is characterized as homolactic bacteria. $CaCO_3$ (at 1/2 of the total sugar concentration in the biomass loading, by weight) and the culture inoculum (10% (*v/v*)) were added to the SSF medium, the initial pH value of which was approximately 6.5. The pH value during the fermentation process was controlled by $CaCO_3$ automatically and could be maintained between 5.0 and 5.5. Glucose release was analyzed using GOD–POD method (Lin *et al.* 1999), while the total amount of reducing sugars (TRS) released was estimated using the dinitrosalicylic acid reagent

(DNS) method (*Miller, 1959*). LA concentration was determined spectrophotometrically at 390 nm by measuring the amount of iron (III) lactate formed after the incubation of iron (III) chloride with LA (Borshchevskaya *et al.* 2016).

Table 1. LA production from *L. delbrueckii* and SSF using organosolv pretreated biomass at 45ºC, 9% initial dry matter, with an enzyme loading of 9mg/ g of biomass, after 168h of fermentation. (*ACO: acetone, EtOH: ethanol, THF: tetrahydrofuran*)

Biomass pretreatment	Cellulose %	Lactic acid (g/lt)	mg lactic acid/ g biomass
H_2O/ACO (50/50%), O_2 8 bar, 160ºC, 120min	66.77	77.11	856
H_2O/ACO (50/50%), O_2 16 bar, 160ºC, 120min	76.63	61.07	678
H_2O/ACO (50/50%), O_2 8 bar, 175ºC, 120min	82.3	50.5	561
H_2O/ACO (50/50%), O_2 16 bar, 175ºC, 30min	79.74	64.56	717
H_2O/THF (50/50%), O_2 16 bar, 160ºC, 60min	68.99	72.96	810
H_2O/EtOH (50/50%), O_2 16 bar, 160ºC, 120min	72.96	52.5	583
H_2O/EtOH (50/50%), O_2 16 bar, 175ºC, 60min	81.28	67.1	745
H_2O/THF (50/50%), O_2 16 bar, 150ºC, 120min	73.09	78.93	877
H_2O/ THF (50/50%), O_2 16 bar, 160ºC, 120min	79.13	85.7	952
H_2O/ THF (50/50%), O_2 16 bar, 175ºC, 60min	85.28	53.23	591

Our results showed that both LAB could efficiently utilize the biomass-derived sugars for the production of LA. Biomass samples which have been treated with tetrahydrofuran as a solvent at 150 ºC and with acetone and ethanol at 160 ºC for 120 min were the most efficient in producing sugars as well as in producing lactic acid with high lactic acid yields, such as 0.95 g lactic acid /g biomass after 168 h of fermentation, at 9% initial DM. Hydrolysis experiments with 15% DM and 9 mg enzyme/ g of substrate showed up to 78% *w/w* cellulose conversion, rendering the high initial solids process very promising for maximizing the yield of lactic acid in free-fall mixers. Apart from the design of high-gravity processes, another challenge is the evaluation of the possibility of the hemicellulose-rich liquid fraction after pretreatment to be used after detoxification as carbon source for the lactic acid bacteria, in order to efficiently utilize all fractions within the frame of *zero-waste* biorefinery concept. The overall results of this work demonstrate that lignocellulosic biomass can be efficiently used for the production for value-added products, such as lactic acid, through environmentally friendly bioconversion processes.

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