Electronic Journal of Biotechnology 31 (2018) 67–74

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Chemical pretreatment of *Arundo donax* L. for second-generation ethanol production

Juliana Silva Lemões [a,*], Claudia Fernanda Lemons e Silva [b], Sabrina Peres Farias Avila [c], Cândida Raquel Scherrer Montero [d], Sérgio Delmar dos Anjos e Silva [d], Dimitrios Samios [a], Maria do Carmo Ruaro Peralba [a]

[a] *Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Porto Alegre, RS 91509-900, Brazil*
[b] *Centro de Engenharia, Universidade Federal de Pelotas, R. Benjamin Constant, 989, Pelotas, RS 96010-020, Brazil*
[c] *Escola de Química e Alimentos, Universidade Federal do Rio Grande, Av. Itália Km 8, Rio Grande, RS 96203-900, Brazil*
[d] *Embrapa Clima Temperado, Rodovia BR 392, km 78, Pelotas, RS 96010-971, Brazil*

ARTICLE INFO

Article history:
Received 18 July 2017
Accepted 23 October 2017
Available online 13 November 2017

Keywords:
Acid pretreatment
Alkaline pretreatment
Arundo biomass
Biofuel
Enzymatic hydrolysis
Giant reed
Glucose
Lignocellulose
Response surface methodology
Xylose
Raw material

ABSTRACT

Background: Pretreatment of lignocellulosic biomass is essential for using it as a raw material for chemical and biofuel production. This study evaluates the effects of variables in the chemical pretreatment of the *Arundo* biomass on the glucose and xylose concentrations in the final enzymatic hydrolysate. Three pretreatments were tested: acid pretreatment, acid pretreatment followed by alkaline pretreatment, and alkaline pretreatment.
Results: The amounts of glucose and xylose released by the enzymatic hydrolysis of the *Arundo* biomass obtained from acid pretreatment ranged from 6.2 to 19.1 g/L and 1.8 to 3.1 g/L, respectively. The addition of alkaline pretreatment led to a higher yield from the enzymatic hydrolysis, with the average glucose concentration 3.5 times that obtained after biomass hydrolysis with an acid pretreatment exclusively. The use of an alkaline pretreatment alone resulted in glucose and xylose concentrations similar to those obtained in the two-step pretreatment: acid pretreatment followed by alkaline pretreatment. There was no significant difference in 5-hydroxymethylfurfural, furfural, or acetic acid concentrations among the pretreatments.
Conclusion: Alkaline pretreatment was essential for obtaining high concentrations of glucose and xylose. The application of an alkaline pretreatment alone resulted in high glucose and xylose concentrations. This result is very significant as it allows a cost reduction by eliminating one step.

1. Introduction

Lignocellulosic biomasses are a promising raw material for bioethanol production as they are the most abundant carbon source on the planet [1,2]. Second-generation bioethanol production uses lignocellulosic biomasses such as sugar feedstock, which are converted into ethanol through a fermentation process. The main advantages of this technology lie in the fact that the raw material is low cost, renewable, and sustainable [3].

However, lignocellulosic biomass has a complex composition that mainly includes cellulose, hemicellulose (carbohydrate polymers), and lignin [4,5,6]. Because of its composition, pretreatment of the biomass is an essential step of the second-generation biofuel process [5,7]. The goal of the pretreatment is to improve the digestibility of the lignocellulosic biomass. Pretreatment processes remove hemicellulose and lignin, increasing the porosity of the biomass and reducing cellulose crystallinity, thus making the cellulose more accessible for conversion into fuels [8].

Pretreatments can be divided into physical, physicochemical, chemical, and biological methods or a combination of any of these [3,5]. The choice of a pretreatment method depends on the biomass characteristics. It should improve the yields of sugars and avoid degradation products that are inhibitory to the subsequent steps of the process [9].

Chemical pretreatments are useful for improving the digestibility of a lignocellulosic biomass. Acid pretreatment is the most commonly employed method. Diluted or concentrated acids can hydrolyze the hemicelluloses of most lignocellulosic raw materials, promoting enzymatic action and increasing the yield from the biomass hydrolysis [2,10,11]. Different acids such as hydrochloric, nitric, and phosphoric acids can be utilized, but sulfuric acid is usually used [5,12].

* Corresponding author.
E-mail addresses: julianalemoes@gmail.com (J.S. Lemões), sergio.anjos@embrapa.br (S.D.A. Silva).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2017.10.011

Alkaline pretreatment allows the removal of lignin, acetyl groups, and uronic acids by cleavage of the linkage between lignin and hemicellulose. It causes the cellulose to swell, thus decreasing its crystallinity and degree of polymerization and making it more accessible to cellulases [3,4,13].

Because of the characteristics of these chemical pretreatments, a combination of acid and alkaline pretreatments can increase the enzymatic hydrolysis yield compared to an individual acid pretreatment or alkaline pretreatment. Guo et al. [14] evaluated the two-stage acid–alkaline hydrothermal pretreatment conditions of *Miscanthus* biomass and compared them with single-stage acid and alkaline pretreatments. The results showed higher glucose and xylose concentrations after the two-stage pretreatment compared to the single-stage pretreatments. Similarly, Wang et al. [15] investigated corn stover pretreatments and obtained a higher glucose yield for an acid–alkaline two-stage pretreatment than for either acid or alkaline pretreatment. However, Guilherme et al. [16] applied different pretreatments to sugar cane bagasse and found higher glucose and xylose concentrations after alkaline pretreatment alone compared with a combined acid and alkaline pretreatment.

Arundo donax L., also known as giant reed, is a perennial grass belonging to the Poaceae family. This plant presents advantages as a raw material for ethanol production such as high biomass production, rapid growth, low agronomic input, low production costs, and the ability to grow in different kinds of environments [17,18].

The goal of this study was to evaluate the effects of chemical pretreatments on glucose and xylose production from *A. donax* L. biomass aiming for ethanol production.

2. Materials and methods

2.1. Raw material

Arundo biomass was harvested in the São Gonçalo watercourse in Pelotas, Brazil (latitude 31°46′33″ south and longitude 52°21′34″ west). The biomass was milled and air-dried. Then it was milled again to reduce the particle size. The particle size distribution was determined by sieving through 1.00, 0.50, 0.25, 0.105, and 0.053 mm sieves.

2.2. Compositional analysis

The biomass was analyzed for extractives [19], cellulose, hemicellulose, lignin, and ash [20], following the National Renewable Energy Laboratory (NREL) methods.

2.3. Biomass pretreatment

Arundo biomass was first subjected to acid pretreatment, and the liquid and solid fractions were separated. The pretreated biomass, named acid cellulignin (ACCL), was used for the second alkaline pretreatment step. The *Arundo* integral biomass was also subjected to alkaline pretreatment only. The biomasses resulting from the three pretreatments were then hydrolyzed enzymatically (Fig. 1), and the released sugars were quantified using high-performance liquid chromatography (HPLC).

2.3.1. Acid pretreatment

Arundo biomass (100 g DM) was treated with sulfuric acid and autoclaved at 120°C. Variations in the sulfuric acid concentration (x_1), exposure time (x_2), and solid-to-liquid ratio (S:L ratio) (x_3) followed a central composite rotational design.

After pretreatment, the biomass (solid fraction) was separated from the liquid fraction. The ACCL was repeatedly washed with water to remove the excess acid until the pH was 4.5–5.0. It was then dried in an oven at 65°C.

2.3.2. Alkaline pretreatment after acid pretreatment

The second step of the pretreatment was the alkaline treatment. A solution of sodium hydroxide (0.5 M) at a S:L ratio of 1:20 g/mL was added to 50 g DM of ACCL, obtained from acid pretreatment under the conditions in Section 2.3.1, and allowed to react for 30 min at 127°C. Afterward, the liquid and biomass fractions were separated, and

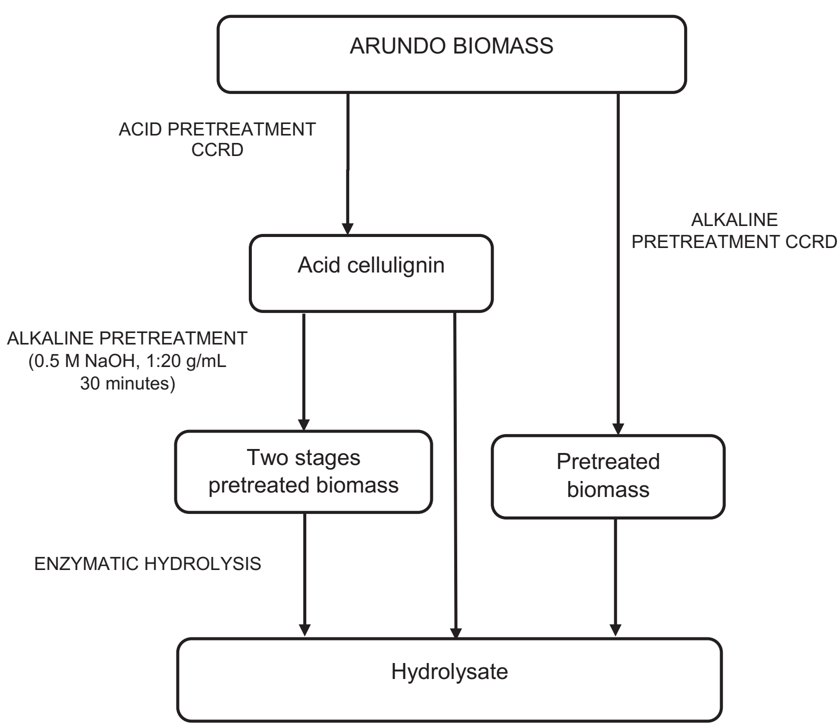

Fig. 1. Flow chart for pretreatment of *Arundo* biomass.