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Statistical optimization of acid catalyzed steam pretreatment of citrus peel waste for bioethanol production

Indulekha John, Prasanthi Yaragarla, Perumalsamy Muthaiah, Kalaichelvi Ponnusamy, Arunagiri Appusamy*

Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli - 620015, Tamil Nadu, India

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ABSTRACT

Citrus waste is an attractive lignocellulosic biomass for the production of bioethanol due to the richness in carbohydrates and low lignin content. In this study, sweet lime peel was chosen as the lignocellulosic biomass. To increase the cellulose for enzymatic hydrolysis, the statistical optimization of process parameters namely, solid loading, time of exposure and sulphuric acid concentration for pretreatment of sweet lime peel were accomplished by Taguchi orthogonal array design. The sweet lime peel was exposed to acid catalyzed steam pretreatment for solid loading [10%, 12%, 15% and 17% (w/v)], time of exposure [15 min, 30 min, 45 min and 60 min] and sulphuric acid concentration [0.25%, 0.5%, 0.75% and 1% (v/v)]. The cellulose content was found to be an optimum at 35% for 17% (w/v) solid loading and 0.25% (v/v) acid concentration and steam exposure for 60 min. With these optimized process parameters, enzymatic hydrolysis of pretreated sweet lime peel was investigated at 50 °C for 48 h using in vitro isolated enzymes, viz., cellulase and pectinase from *Aspergillus Niger* with an activity of 1.7 FPU/ml and15 IU/ml respectively. 7.09 mg of reducing sugar/ml of hydrolysate was released in enzymatic hydrolysis which was estimated by DNS method. For the production of bioethanol, fermentation of hydrolysate was carried out at 30 °C for 72 h using baker's yeast. The yield of ethanol was 18%. From this study, it is proved that citrus waste is a promising source for the production of bioethanol.

1. Introduction

The production of biofuel from the agro-waste material is one of the best remedies to minimize both crude oil consumption and environmental pollution [1,2]. Bioethanol is the most common and worldwide used biofuel in the transportation sector. Ethanol can be utilized as a fuel either in a pure form or in blend with gasoline. It is a high-octane fuel and it lessens the release of smog and carbon monoxide [1]. The conversion of lignocelluloses to bioethanol and other value-added products is promising because of its abundance as an unutilized biomass, and cost-effectiveness. Moreover, it does not affect the land use and food production. The production of bioethanol from any lignocellulosic biomass involves three major steps, viz., pretreatment, hydrolysis, and fermentation. Pretreatment process disrupts the recalcitrant cell wall and makes the carbohydrates accessible for hydrolysis. In hydrolysis, cellulose and hemicellulose are broken down into simple sugars which can be utilized by the microorganism in the fermentation step to convert it into ethanol. Lignocellulose is composed of carbohydrates such as cellulose, hemicellulose and aromatic polymer lignin. The composition of these carbohydrates varies in various lignocellulosic biomasses. The feedstock should be more in carbohydrates and less in lignin for bioethanol production [3].

Citrus waste is an attractive lignocellulosic biomass for the production of bioethanol due to the richness in carbohydrates and low lignin content [2]. Orange peel waste (OPW), the solid rejected after the juice extraction process, is an important lignocellulosic feedstock for the production of bioethanol. It consists of peel, juice sacs, rag (cores and segment membranes), and seeds, that amounts to 50–70% of the fresh fruit weight [3]. Statistically, the annual worldwide citrus fruit production is more than 88 Tg [4], around 55% of which being orange fruit. Thus, the annual supply of OPW should be about 21 Tg, and 33% of which being easily accessible for further usage in the orange processing plants. The use of OPW as raw material for ethanol production has been so far assessed to a great extent, both at pilot plant [5] and lab [6–9] scales.

* Corresponding author.
E-mail address: aagiri@nitt.edu (A. Appusamy).

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Pretreatment is necessary to enhance the accessibility of cellulosic biomass to cellulose degrading enzymes [10]. A variety of physicochemical pretreatments of OPW were investigated to either enhance the selective removal of non-fermentable sugars and various inhibitors [7,11] or to increase the vulnerability of cellulose to hydrolysis. As per most of these studies, the steam explosion and dilute-acid hydrolysis using sulphuric acid are seem to be the suitable pretreatment methods to increase the amenability of OPW to hydrolysis and to reduce the inhibitory compounds in the hydrolysate. The primary bottlenecks of bioethanol production from OPW are as follows: the high contribution of the pectin and feed solid contents to the viscosity of the medium to be fermented and distilled [5], the process heat duty [12], the present cost of cellulolytic and pectolytic enzymes, the unavailability of genetically altered microorganisms to ferment both pentoses and hexoses [9]. Citrus peels have 0.8–1.6% D-limonene, which act as an inhibitor for yeast fermentation [11]. At this point, when utilizing citrus waste for bioethanol production, acidic steam explosion pretreatment is required to bring down the limonene concentration to 0.05% on the grounds that D-limonene restrains microbial growth [11,13].

The enzymatic saccharification of comminuted OPW was firstly considered by Grohmann and Baldwin [14], who demonstrated the necessity of pectinolytic and cellulolytic enzymes in order to achieve high hydrolysis yields. In the enzymatic hydrolysis, pectinolytic, xylanolytic, and cellulolytic enzymes are generally used to break the plant cell walls and to catalyze the breakdown of complex carbohydrates into their simple monosaccharide units (i.e., saccharification) [10,15]. To a great extent, bioethanol production from CPW has been conducted using commercially available enzymes; thus, the cost of cellulosic based bioethanol is very high. The expense can be drastically decreased if in-house-produced enzymes are used for this process [16]. *Aspergillus* and *Trichoderma* are the most utilized microorganisms that release abundant xylanolytic, cellulolytic and pectinolytic enzymes. *Trichoderma* species have been mostly investigated for their cellulolytic enzymes [17] whereas *Aspergillus* species often have pectinolytic and xylanolytic enzymes [18]. Both the fungal species are considered as producers of cell wall-degrading extracellular enzymes for industrial applications [8].

The design of experiments (DOE) is the most useful statistical tool employed in many areas for design comparison, variable identification, design optimization, process control and product performance prediction. Taguchi experimental design is a quick and extensive method for optimization of conferring important outcome in a synchronous study of various factors, making its imprint in quality products supplemented with better process execution, and rendering high output and improved stability [19–21]. Better quality at the economical rate is the main purpose for generation of Taguchi orthogonal array design of experiments (DOE) and it also accesses to maximize the robustness of processes and products [22]. The essential rule included is the encompassment of extensive experimental data as orthogonal (unbiased) array in deciding the impact of different factors which control the reaction occurring, resulting in less experimental error with enhanced efficiency of the experimental result. Taguchi design established the significance of statistically aligned analyses in speculating the settings of product (and/or processes) on different variables [23,24].

The current study solely emphasizes the Taguchi optimization method for various pretreatment process variables such as sweet lime peel loading, sulphuric acid concentration and exposure time and further to evaluate the possibility of bioethanol production from sweet lime peel using in-house-produced enzymes for hydrolysis.

Table 1
Factors and levels of acid catalyzed steam explosion.

Factors	Level 1	Level 2	Level 3	Level 4
Solid loading % (w/v)	10	12	15	17
Time of exposure (min)	15	30	45	60
Sulphuric acid % (v/v)	0.25	0.50	0.75	1.00

Table 2
Taguchi orthogonal array design.

Run No	Loading (% w/v)	Acid concentration (%v/v)	Time (min)
1	1	1	1
2	1	2	2
3	1	3	3
4	1	4	4
5	2	1	2
6	2	2	1
7	2	3	4
8	2	4	3
9	3	1	3
10	3	2	4
11	3	3	1
12	3	4	2
13	4	1	4
14	4	2	3
15	4	3	2
16	4	4	1

2. Materials and methods

2.1. Pretreatment

Sweet lime peel was collected from local juice shop in National Institute of Technology Tiruchirappalli, Tamil Nadu, India. The biomass was sun dried and screened to get particle size of 1 mm. All the chemicals were of analytical grade purchased from Merck. The acid catalyzed steam explosion was selected for the pretreatment of biomass. All the steam explosion experiments were carried out in an autoclave at a temperature of 121 °C and pressure of 15 psi. The statistical optimization of process parameters for pretreatment of sweet lime peel was accomplished by Taguchi orthogonal array design using MINITAB software. The process parameters to be optimized were solid loading, time of exposure and sulphuric acid concentration (Table 1).

Based on this Taguchi method, an orthogonal array of 16 experiments (L_{16}) was designed to optimize the process parameters for the acid catalyzed steam explosion (Table 2). The “response” from the Taguchi design is the amount of “cellulose” present in the peel after the pretreatment. Cellulose content was determined by subtracting acid detergent lignin (ADL) from acid detergent fiber (ADF) as given by Van Soest fiber analysis [25]. All the experiments for ADF and ADL were done in duplicates and the average was taken to calculate cellulose content. Response values were then evaluated to interpret the main effects of these factors on pretreatment. To investigate the factors which were statistically significant, analysis of variance (ANOVA) was carried out. By applying the optimal process parameters in the regression equation, the optimum cellulose content was predicted.

2.2. Enzymatic hydrolysis and fermentation

With the optimized process parameters of the acid catalyzed steam explosion, enzymatic hydrolysis of pretreated sweet lime peel was investigated using isolated enzymes. In our previous study, two in vitro enzymes namely cellulase and pectinase were isolated from *Aspergillus Niger*, purchased from MTCC, Chandigarh. The activity of cellulase and pectinase was found to be 1.7 FPU/ml and 15 IU/ml respectively. 3 g of pretreated biomass was