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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

**Table 3**
Response of Taguchi design.

| Run No | Loading (% w/v) | Acid Concentration (%v/v) | Time (min) | Cellulose (%) | | |
|---|---|---|---|---|---|---|
| | | | | Experimental | Predicted | Residuals |
| 1 | 10 | 0.25 | 15 | 27.82 ± 0.09 | 27.66 | 0.58 |
| 2 | 10 | 0.50 | 30 | 23.06 ± 0.19 | 24.84 | -7.72 |
| 3 | 10 | 0.75 | 45 | 21.50 ± 0.28 | 22.02 | -2.42 |
| 4 | 10 | 1.00 | 60 | 20.15 ± 0.38 | 19.2 | 4.71 |
| 5 | 12 | 0.25 | 30 | 28.27 ± 0.16 | 29.34 | -3.78 |
| 6 | 12 | 0.50 | 15 | 27.21 ± 0.52 | 24.72 | 9.15 |
| 7 | 12 | 0.75 | 60 | 26.14 ± 0.95 | 23.7 | 9.33 |
| 8 | 12 | 1.00 | 45 | 17.31 ± 1.07 | 19.08 | -10.23 |
| 9 | 15 | 0.25 | 45 | 30.41 ± 0.56 | 31.41 | -3.29 |
| 10 | 15 | 0.50 | 60 | 28.03 ± 0.96 | 28.59 | -2.00 |
| 11 | 15 | 0.75 | 15 | 23.13 ± 0.49 | 22.17 | 4.15 |
| 12 | 15 | 1.00 | 30 | 17.65 ± 0.95 | 19.35 | -9.63 |
| **13** | **17** | **0.25** | **60** | **34.80 ± 0.09** | **33.09** | **4.91** |
| 14 | 17 | 0.50 | 45 | 27.00 ± 0.85 | 28.47 | -5.44 |
| 15 | 17 | 0.75 | 30 | 23.00 ± 1.2 | 23.85 | -3.70 |
| 16 | 17 | 1.00 | 15 | 20.43 ± 0.38 | 19.23 | 5.87 |

hydrolyzed with an enzyme loading of 20 FPU of cellulase and 50 IU of pectinase per gram of peel. Hydrolysis was conducted in a 100 ml screw cap bottle containing peel and enzymes with citrate buffer at pH of 4.8, maintained at 50 °C in a shaking water bath for 48 h At the end of the hydrolysis, the sample was kept in a hot air oven at 110 °C to inactivate enzymes and then the hydrolysate was collected. The reducing sugar content present in the hydrolysate was determined by DNS method.

Fermentation of the hydrolysate was carried out by using baker's yeast, *Saccharomyces cerevisiae*, purchased from the nearest supermarket. Baker's yeast was activated in 20 ml of sterile sucrose solution (50 g/L) by inoculating yeast grains (1 g) and incubating in an orbital shaker with a speed of 100 rpm at 30 °C for 20 h These activated cells were inoculated into the sterile YEPD media containing yeast extract, 10 g/L; peptone, 20 g/L; dextrose, 20 g/L; and agar, 20 g/L and then it was incubated at 30 °C for 7 days. The hydrolysate was sterilized and was inoculated with 5% (v/v) of a 24 h old seed culture of *Saccharomyces cerevisiae*. Fermentation was carried out in a screw cap bottle in an orbital shaker with an agitation of 100 rpm at 37 °C for 72 h Fermented broth was withdrawn at the end of the fermentation and centrifuged for 15 min at 6000 rpm. The supernatant was collected and estimated the ethanol concentration by dichromate method [26].

## 3. Results and discussions

### 3.1. Acid catalyzed steam explosion

Taguchi robust method was utilized to optimize the process parameters within the design space and further to identify the most significant parameter on acid catalyzed steam explosion. The response of each trial is given below in Table 3. The acid catalyzed steam explosion which results in highest cellulose content was selected with the highest and optimum means of each process variable. The optimum conditions for all the variables were observed with the run number 13. The maximum cellulose content after the acid catalyzed steam explosion was found to be 34.80% which is by using a solid loading of 17% and an acid concentration of 0.25% for an exposure of 60 min. Main effect plots are given in Figs. 1–3. The response table for means and analysis of variance of cellulose are given in and Table 4 and Table 5 respectively.

The main effect plot of cellulose showed an increasing pattern of cellulose with respect to solid loading as well as the time of exposure whereas it was declined for acid concentration. For any pretreatment process, an optimal substrate loading is necessary to achieve highest cellulose content. 17% was the upper limit of solid

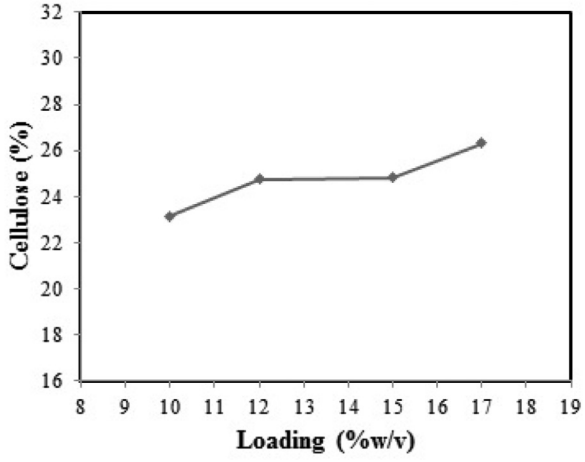


**Fig. 1.** Main effect plots for means of cellulose versus loading.

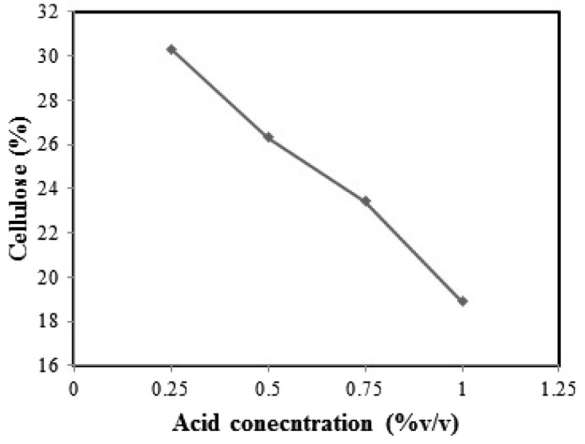


**Fig. 2.** Main effect plots for means of cellulose versus acid concentration.

loading because sweet lime peel was not wet enough to make a uniform mixture of acid and solid slurry beyond that loading. Most of the literature reported the sulphuric acid concentration of 0.5% as the optimum for citrus peel pretreatment by steam explosion. For sweet sorghum, it was reported that 16% solid loading, 0.37% acid as optimum From, our study, it was clear that sulphuric acid concentration of 0.25% (v/v) is enough to get high cellulose con-

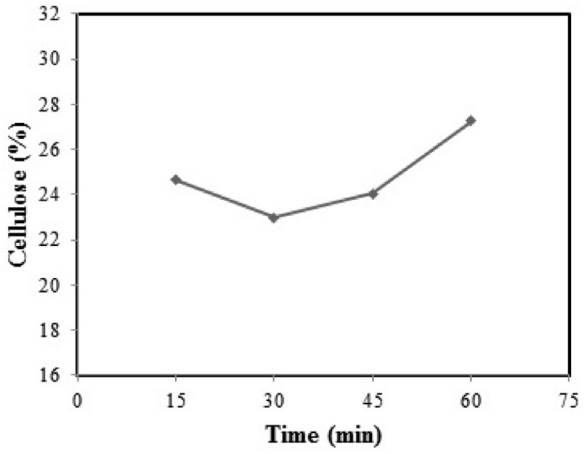


**Fig. 3.** Main effect plots for means of cellulose versus time.

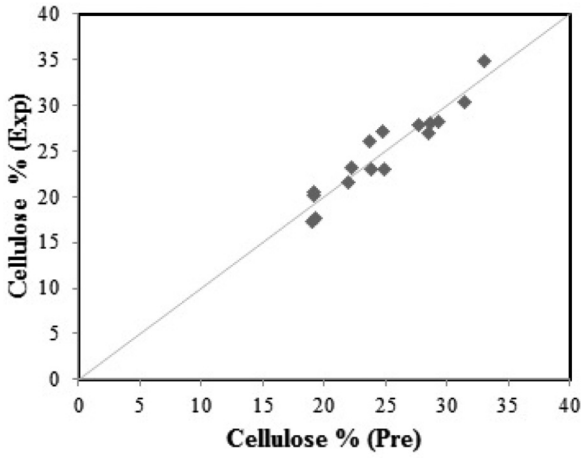


**Fig. 4.** Parity plot of experimental and predicted results.

**Table 4**
Response table for means.

| Level | Means of cellulose (%) for | | |
|---|---|---|---|
| | Loading | Acid concentration | Time |
| 1 | 23.13 | 30.32 | 24.65 |
| 2 | 24.73 | 26.32 | 22.99 |
| 3 | 24.81 | 23.44 | 24.06 |
| 4 | 26.31 | 18.88 | 27.28 |
| **Delta** | 3.17 | 11.44 | 4.29 |
| **Rank** | **3** | **1** | **2** |

**Table 6**
Composition of sweet lime peel before and after steam pretreatment.

| Parameter (%) | Before pretreatment | After pretreatment |
|---|---|---|
| Cellulose | 25.4 | 34.8 |
| Hemicellulose | 9.4 | 4.52 |
| Lignin | 23.6 | 8.34 |
| Pectin | 17.1 | 6.73 |

centration. The increase in acid concentration reduced the cellulose content which could be due to more degradation and resulting inhibitor formation. Since the higher temperature was not provided for the steam explosion, longer time was required for better results. The severity of the pretreatment process increased with the increase in time of exposure [27].

The analysis of variance (ANOVA) study was carried out to understand the effect of each parameter on the yield of cellulose recovered after acid catalyzed steam explosion. The Fishers test (F test) gives the measure of the statistical significance of a process variable [28]. A confidence level of 95% was selected in this study. Higher the F value, greater is the impact of the parameter in the process. F-test with a very low p-value ($<0.05$) gives a very high significance of the empirical relationship developed [29]. ANOVA results showed that the most influencing parameter among three variables was acid concentration, followed by exposure time and loading. Regression analysis was also carried out and the regression equation is as follows.

$$Cellulose = 26.58 + 0.39\,Loading - 14.88\,Acid\ concentration + 0.06\,Time \quad (1)$$

The goodness of fit of the regression model was determined by the coefficient of determination ($R^2$). The highest values of $R^2$ were generally chosen to determine the best fit [30–32]. For a model to be good enough, the $R^2$ value should be closer to 1.0. The coefficient of determination ($R^2$) for the response of cellulose was found to be 0.98 which indicates that 98% of experimental data confirms the accuracy and suitability with the data predicted by the developed empirical relationships [33,34]. The difference between the experimental and predicted cellulose content was less than 5% for the optimum results. The parity plot of experimental and predicted results is shown in Fig. 4. The composition of sweet lime peel before and after the pretreatment is given in Table 6. At the optimum process conditions, the maximum cellulose recovery and delignification are observed which is apt for bioethanol production.

### 3.2. Enzymatic hydrolysis and fermentation

Since the focus of this work was on the optimization of acid catalyzed steam pretreatment process, saccharification and fermentation studies were not investigated in a detailed manner. To prove that sweet lime peel can produce bioethanol, enzymatic hydrolysis and further fermentation were carried out using the optimized pretreatment process parameters. In our earlier study, the enzymes cellulase and pectinase were isolated from *Aspergillus Niger*. This is to make use of a single microorganism for the production of both the extracellular enzymes and which will be useful for the consolidated bioprocess in future. At end of the enzymatic hydrolysis for 48 h, the enzymes were inactivated and then hydrolysate was collected by centrifugation. The amount of reducing sugar present in the hydrolysate was determined by DNS method. It was observed that 7.09 mg of reducing sugar/ml of hydrolysate was produced

**Table 5**
Analysis of variance for cellulose.

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Loading (%w/v) | 3 | 20.181 | 20.181 | 6.727 | 8.78 | 0.013 |
| Acid concentration (%v/v) | 3 | 278.676 | 278.676 | 92.892 | 121.30 | 0.000 |
| Time (min) | 3 | 39.897 | 39.897 | 13.299 | 17.37 | 0.002 |
| Error | 6 | 4.595 | 4.595 | 0.766 | | |
| Total | 15 | 343.349 | | | | |

from the optimized pretreatment process. *Saccharomyces cerevisiae* is a proven microorganism for the production of bioethanol. Fermentation using *Saccharomyces cerevisiae* usually experiments at 30 °C. The hydrolysate was fermented for 72 h and the ethanol was produced from reducing sugar. The quantity of ethanol present in the fermented broth was determined by dichromate method. The yield of ethanol after 72 h of fermentation was calculated as 18% in this work. Zhou et al., 2008 reported an ethanol yield of 4% from orange peels. This indicates that sweet lime peel can produce bioethanol more effectively than orange peels.

## 4. Conclusion

Lignocellulosic biomass is a suitable and alternative raw material for the production of bioethanol. Among the lignocellulosic biomass, citrus peel waste is an attractive source for bioethanol production due to the richness in carbohydrates. In this study, the acid catalyzed steam explosion was explored for the pretreatment of the sweet lime peel. The optimization of pretreatment process by Taguchi robust method identified the most significant parameter on acid catalyzed steam explosion as acid concentration. The highest cellulose content of 34.80% was observed with a solid loading of 17% and acid concentration of 0.25% for an exposure of 60 min. The difference between the experimental and predicted cellulose content was less than 5% for the optimum results. Thus acid catalyzed steam explosion requires very less acid concentration for high solid loading which will be much useful in the large-scale production of bioethanol. By obtaining an ethanol yield of 18%, it is proved that sweet lime peel is a promising source for the production of bioethanol.

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## References

[1] A. Demirbas, Bioethanol from cellulosic materials: a renewable motor fuel from biomass, Energy Sources 27 (2005) 327–337.
[2] I. John, M.S.G. Siddarth, P. Kalaichelvi, A. Arunagiri, Characterization of citrus peels for bioethanol production, in: Mater. Energy Environ. Eng., Springer, 2017, pp. 3–12.
[3] R. Crawshaw, Co-product feeds: animal feeds from the food and drinks industries R Crawshaw Nottingham University Press, Nottingham, 2001 pp 285, price{\pounds} 30.00 (paperback) ISBN 1-897676-35-2, J. Sci. Food Agric. 83 (2003) 362.
[4] F.R. Marín, C. Soler-Rivas, O. Benavente-García, J. Castillo, J.A. Pérez-Alvarez, By-products from different citrus processes as a source of customized functional fibres, Food Chem 100 (2007) 736–741.
[5] W. Zhou, W. Widmer, K. Grohmann, Developments in ethanol production from citrus peel waste, Proc. Fla. State Hort. Soc. 121 (2008) 307–310.
[6] M. Pourbafrani, G. Forgács, I.S. Horváth, C. Niklasson, M.J. Taherzadeh, Production of biofuels, limonene and pectin from citrus wastes, Bioresour. Technol. 101 (2010) 4246–4250.
[7] W. Widmer, W. Zhou, K. Grohmann, Pretreatment effects on orange processing waste for making ethanol by simultaneous saccharification and fermentation, Bioresour. Technol. 101 (2010) 5242–5249.
[8] I.S. Choi, Y.G. Lee, S.K. Khanal, B.J. Park, H.-J. Bae, A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production, Appl. Energy. 140 (2015) 65–74.
[9] G. Santi, S. Crognale, A. D'Annibale, M. Petruccioli, M. Ruzzi, R. Valentini, M. Moresi, Orange peel pretreatment in a novel lab-scale direct steam-injection apparatus for ethanol production, Biomass Bioenergy 61 (2014) 146–156.
[10] I.S. Choi, J.-H. Kim, S.G. Wi, K.H. Kim, H.-J. Bae, Bioethanol production from mandarin (Citrus unshiu) peel waste using popping pretreatment, Appl. Energy. 102 (2013) 204–210.
[11] M.R. Wilkins, W.W. Widmer, K. Grohmann, Simultaneous saccharification and fermentation of citrus peel waste by Saccharomyces cerevisiae to produce ethanol, Process Biochem. 42 (2007) 1614–1619.
[12] M. Lohrasbi, M. Pourbafrani, C. Niklasson, M.J. Taherzadeh, Process design and economic analysis of a citrus waste biorefinery with biofuels and limonene as products, Bioresour. Technol. 101 (2010) 7382–7388.
[13] K. Grohmann, R.G. Cameron, B.S. Buslig, Fractionation and pretreatment of orange peel by dilute acid hydrolysis, Bioresour. Technol. 54 (1995) 129–141.
[14] K. Grohmann, E.A. Baldwin, Hydrolysis of orange peel with pectinase and cellulase enzymes, Biotechnol. Lett. 14 (1992) 1169–1174.
[15] M. Boluda-Aguilar, L. García-Vidal, F. del Pilar González-Castañeda, A. López-Gómez, Mandarin peel wastes pretreatment with steam explosion for bioethanol production, Bioresour. Technol. 101 (2010) 3506–3513.
[16] D. Klein-Marcuschamer, P. Oleskowicz-Popiel, B.A. Simmons, H.W. Blanch, The challenge of enzyme cost in the production of lignocellulosic biofuels, Biotechnol. Bioeng. 109 (2012) 1083–1087.
[17] D. Martinez, R.M. Berka, B. Henrissat, M. Saloheimo, M. Arvas, S.E. Baker, J. Chapman, O. Chertkov, P.M. Coutinho, D. Cullen, others, Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocrea jecorina), Nat. Biotechnol. 26 (2008) 553–560.
[18] R.P. de Vries, J. Visser, Aspergillus enzymes involved in degradation of plant cell wall polysaccharides, Microbiol. Mol. Biol. Rev. 65 (2001) 497–522.
[19] D.H. Stamatis, TQM Engineering Handbook, CRC Press, 1997.
[20] G. Taguchi, Introduction to quality engineering: designing quality into products and processes, 1986.
[21] M. Mubarak, A. Shaija, Ultrasonication : an effective pre-treatment method for extracting lipid from Salvinia molesta for biodiesel production, Resour. Technol. 2 (2016) 126–132, doi:10.1016/j.reffit.2016.07.005.
[22] J. Antony Mike Kaye, A. Frangou, A strategic methodology to the use of advanced statistical quality improvement techniques, TQM Mag 10 (1998) 169–176.
[23] D.M. Bryne, S. Taguchi, Taguchi approach to parameter design, Qual. Prog. (1987) 19–26.
[24] D. de Oliveira, T.L.M. Alves, A kinetic study of lipase-catalyzed alcoholysis of palm kernel oil, Appl. Biochem. Biotechnol. 84 (2000) 59–68.
[25] P.J. van Van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597.
[26] H.-B. Seo, H.-J. Kim, O.-K. Lee, J.-H. Ha, H.-Y. Lee, K.-H. Jung, Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process, J. Ind. Microbiol. Biotechnol. 36 (2009) 285–292.
[27] Q. Hu, X. Su, L. Tan, X. Liu, A. Wu, D. Su, K. Tian, X. Xiong, Effects of a steam explosion pretreatment on sugar production by enzymatic hydrolysis and structural properties of reed straw, Biosci. Biotechnol. Biochem. 77 (2013) 2181–2187.
[28] M. Ganesapillai, M. Mathew, A. Singh, P. Simha, Influence of microwave and ultrasound pretreatment on solvent extraction of bio-components from walnut (Julgans regia L.) shells, Period. Polytech. Chem. Eng. 60 (2016) 40.
[29] M.G. Pillai, P. Simha, A. Gugalia, Recovering urea from human urine by biosorption onto microwave activated carbonized coconut shells: equilibrium, kinetics, optimization and field studies, J. Environ. Chem. Eng. 2 (2014) 46–55.
[30] M.G. Pillai, Thin layer drying kinetics , characteristics and modeling of plaster of paris, Chem. Eng. Res. Des. 91 (2013) 1018–1027, doi:10.1016/j.cherd.2013.01.005.
[31] M. Ganesapillai, P. Simha, K. Desai, Y. Sharma, T. Ahmed, Simultaneous resource recovery and ammonia volatilization minimization in animal husbandry and agriculture, Resour. Technol. 2 (2016) 1–10, doi:10.1016/j.reffit.2015.12.001.
[32] P. Simha, A. Zabaniotou, M. Ganesapillai, Continuous urea–nitrogen recycling from human urine: A step towards creating a human excreta based bio–economy, J. Clean. Prod. (2017).
[33] P. Simha, M. Mathew, M. Ganesapillai, Empirical modeling of drying kinetics and microwave assisted extraction of bioactive compounds from Adathoda vasica and Cymbopogon citratus, Alexandria Eng. J. 55 (2016) 141–150.
[34] P. Simha, A. Ramanathan, B. Thawani, P. Jain, S. Hussain, M. Ganesapillai, Coal fly ash for the recovery of nitrogenous compounds from wastewater: Parametric considerations and system design, Arab. J. Chem. (2016).