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Sustainable biobutanol production from pineapple waste by using *Clostridium acetobutylicum* B 527: drying kinetics study

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Highlights

- Characterization of pineapple peel waste
- Drying kinetics of pineapple peel and its mathematical modeling
- Pretreatment and detoxification of wet and dried pineapple peel
- ABE fermentation of detoxified hydrolysates using *Clostridium acetobutylicum*

Abstract

Present investigation explores the use of pineapple peel, a food industry waste, for acetone-butanol-ethanol (ABE) production using *Clostridium acetobutylicum* B 527. Proximate analysis of pineapple peel shows that it contains 35% cellulose, 19% hemicellulose, and 16% lignin on dry basis. Drying experiments on pineapple peel waste were carried out in

the temperature range of 60-120 °C and experimental drying data was modeled using moisture diffusion control model to study its effect on ABE production. The production of ABE was further accomplished via acid hydrolysis, detoxification, and fermentation process. Maximum total sugar release obtained by using acid hydrolysis was 97 g/L with 95-97% and 10-50% removal of phenolics and acetic acid respectively during detoxification process. The maximum ABE titer obtained was 5.23 g/L with 55.6% substrate consumption when samples dried at 120 °C were used as a substrate (after detoxification).

Keywords

Biobutanol, Drying, Fermentation, Pineapple peel, Detoxification

Abbreviations

D_0 - Pre-exponential factor (m^2/s)

D_{eff} - Effective moisture diffusivity (m^2/s)

E - Activation energy (kJ/mol)

k - Drying constant (1/min)

L - Half thickness of samples (m)

M - Moisture content for a given time (g/g dry solids)

M_e - Equilibrium moisture content (g/g dry solids)

M_0 - Initial moisture content (g/g dry solids)

MR - Dimensionless moisture ratio

M_{t1} - Moisture contents at time (t_1)

M_{t2} - Moisture contents at time (t_2)

n - Positive integer (-)

N_m - Mean mass flux of moisture ($kg/m^2/min$)

R - Universal gas constant (kJ/mol/K)

r_M - Rate of drying (g/g dry solid/min)

S - Cross sectional area (m²)

T - Drying air temperature (K)

t - Time (s)

t₁, t₂ - Drying times (min)

W_d - Mass bone dry solid (g)

W_t - Mass for a given time t (g)

ρ_s - Density of solid (kg/m³)

1. Introduction

The rhythm of fossil fuel(s) utilization of current civilization is clearly unsuitable due to high price of crude oil and adverse environmental impact. Biofuel is a high priority alternative energy source because of rapid depletion of fossil fuel and other environmental issues. This instigated an attention to produce biofuels from renewable resources (Ezeji et al., 2013). Currently, a variety of alternative fuels such as butanol, ethanol, and methanol have been proposed to replace fossil fuels. Among them, butanol shows superior chemical properties over ethanol and methanol. It has higher energy density and low octane number that is comparable to gasoline. Further, it has low vapor pressure that eases transportation and shows lesser corrosion of contact material (Hu et al., 2011).

Biobutanol is traditionally produced by acetone-butanol- ethanol (ABE) fermentation with *Clostridium* spp. A biological route of ABE fermentation typically involves four basic steps viz. feedstock selection, pretreatment, detoxification, and fermentation. Among them, selection of feedstock is an important step that contributes around 60-70% of overall process cost. Therefore, scrutinizing low cost feedstock such as lignocellulosic biomass is essential. Recent progress in agricultural sector is fascinating,

and many researchers are exploring the horizons to improve the traditional farming with advanced irrigation and water management (Valipour 2014, 2015; Valipour and Singh, 2016; Yannopoulos et al., 2015). With these modern developments, biomass is considered as renewable, cheap and fourth largest source of energy in the world. India alone generates over 400 million tons biomass every year directly from plants, agricultural waste, food, and vegetable industry waste (Bankar et al., 2013; Valipour and Singh, 2016). Although, few researchers have made attempts to utilize the agricultural biomass for biofuel production, the utilization of fruit and vegetable industry waste is still unattended. India ranks second in the world in fruit production with an annual output of 48 million tons fruits, contributing about 12% of the world's fruit production. Pineapple is one of the leading fruits being produced and utilized largely in India. Incidentally, India ranks seventh in worldwide pineapple production with annual production of 1.3 million tons (FAO, 2015). However, the processing and commercial production of pineapple (juice) generates approximately 20-40% (w/w) waste in the form of peel and core (Nga and Trang, 2015).

Pineapple peel waste comprises 35-50% cellulose, 20-35% hemicellulose, and 5-30% lignin (Roda et al., 2014). Hence, the use of pineapple peel waste can be explored in effective bioproduct generation. Kumbhar et al. (2015) reported higher amount of bacterial cellulose production (0.3 g/g) from pineapple peel. Besides, several authors have made an attempts to use pineapple peel as a substrate for production of bromelain, polyphenols (Gautam et al., 2010), biohydrogen, and biogas (Nga and Trang, 2015). Additionally, reports on production of bioethanol from pineapple peel waste using *S. cerevisiae* and *Enterobacter aerogenes* with maximum production of 9.69 g/L and 1.38 g/L ethanol respectively, are also available (Choonut et al., 2014). Pineapple peel usually contains 80-

90% (w/w) moisture on wet basis (Namsree et al., 2012), making it compulsory to reduce moisture level (minimum threshold value ~ 7% w/w) to circumvent microbial spoilage and deterioration by chemical reaction (Kiranoudis et al., 1995). In this connection, drying operation substantially reduces the mass and volume of pineapple peel. With drying operation the storage space, packing size and transportation cost can be reduced. Moreover, it also prevents an additional dilution due to presence of high amount of moisture, during process operations.

Lignocellulosic biomass require crucial step of pretreatment to alter recalcitrant structure. Voluminous reports on pretreatment of lignocellulosic biomass like grain straw (barley, wheat, sorghum, and rice husk), corn stover, and corn fiber (Qureshi et al., 2008) are available in literature. However, such studies pertaining to pineapple peel are scarce. Approaches based on pretreatment, enzymatic saccharification (Roda et al., 2014), physical, and chemical pretreatment methods (Choonut et al., 2014; Rattanapoltee and Kaewkannetra, 2014) are known to enhance the sugar release. During pretreatment processes, the fermentation inhibitors are known to produce that lead to decrease in solvent productions. Thus, detoxification is an essential step to improve solvent yield and productivity.

Sustainable biobutanol production is still lagged behind due to impediments such as high cost of traditional feedstock, low butanol titer due to the limited microbial tolerance, and high product recovery costs because of low production of butanol and the presence of other by-products. A pineapple peel waste in India is yet to be scrutinized in context of their potential for biochemical conversion by *Clostridium* spp. to biobutanol. Hence, present study is focused on the use of inexpensive and abundantly available lignocellulosic biomass to circumvent high-substrate costs. Besides, the development of a simple, yet

economic bioprocess is also desirable to maximize the biobutanol production. Therefore, there is an increasing interest in butanol bioconversion, which leads towards significant efforts in commercial production, or retrofitting same existing bioethanol plants for butanol production (Maiti et al., 2016).

Based on aforementioned discussion, it was thought desirable to undertake systematic investigation to produce biobutanol using pineapple peel waste as a feedstock. The present work is categorized into four parts: (i) characterization of pineapple peel waste, (ii) study of drying kinetics (iii) saccharification and detoxification studies, and (iv) fermentative production of biobutanol from detoxified hydrolysates of pineapple peel waste. This paper essentially gives an emphasis on drying study to evaluate its effect on saccharification and subsequently on ABE fermentation. The mathematical model was developed to formulate correlation between experimental and predicated variables during moisture removal. To the best of our knowledge, no attempts have been made on biobutanol production using pineapple peel waste as a feedstock, till date. The detailed pretreatment and fermentation studies of pineapple peel biomass will be done in following parts of this project and will be presented in next papers.

2. Materials and Methods

2.1 Materials

Dextrose, dipotassium hydrogen phosphate, ammonium acetate, biotin, thiamin, p-aminobenzoic acid, glycerol, sodium potassium tartarate, sulfuric acid, soluble starch, peptone, L-cysteine hydrochloride, sodium chloride, magnesium sulfate, manganese sulfate, isopropanol, iron sulfate, sodium hydroxide, butyric acid, acetic acid, butanol, acetone, ethanol and 3, 5- dinitrosalicylic acid were purchased from SRL Ltd, India.

Sodium acetate and phenol were procured from Sigma Aldrich, India. Folin-Ciocalteu reagent, sodium carbonate, meat extract and yeast extract were obtained from Himedia laboratories, India. Hydrochloric acid and sulfuric acid was obtained from Avra, India. All the chemicals used were of analytical grade.

2.2 Organism and revival medium

Bacterial strain, *C. acetobutylicum* NRRL B-527 was a kind gift from ARS (Agriculture Research Services) culture collection, USA. The lyophilized cells were regenerated by using sterile revival medium (RM) in 100 mL air tight, anaerobic screw cap glass bottles with working volume of 80 mL. It was grown for 48 h at 37 ± 2 °C and subsequently used for the preparation of spore suspension and glycerol stock. Revival medium contained (g/L): dextrose 5; peptone 10; beef extract 10; yeast extract 3; sodium chloride 5; soluble starch 5; sodium acetate 3; L-cysteine HCl 0.5; Resazurin 0.001; water, made up to 1000 mL. Medium pH was adjusted to 6.8 with HCl, if required. The medium was purged with nitrogen to remove dissolved oxygen; sealed and autoclaved at 121 °C for 20 min and cooled at room temperature. L-cysteine HCl was added separately in revival medium using filter sterilization.

2.3 Spore suspension and inoculum preparation

Spore suspension of *C. acetobutylicum* NRRL B-527 was prepared by using 60% (w/v) starch solution. Actively growing cells of *C. acetobutylicum* NRRL B-527 were inoculated in sterile starch solution and incubated anaerobically for 6-8 days at 37 °C. Spore suspension was stored in a cool and dry place for further studies. A heat shock treatment was carried out to activate the cells in reinforced clostridia medium (RCM) as reported by Bankar et al. (2013). Activated spore suspension 2% (v/v) was grown into 100 mL sterile RCM medium for 18-20 h. After 20 h, 5% (v/v) actively growing cells (with

cell density of $OD_{560} -1.2$) were inoculated into production (P2) medium and in hydrolysates. The P2 medium reported by Bankar et al. (2012) was used as control during this study.

2.4 Characterization of pineapple peel waste

Proximate analysis was carried out for pineapple peel waste as per standardized methods in literature. Moisture and ash content were determined according to Sluiter et al. (2005). Cellulose content in biomass was determined by Anthrone method (Updegroff, 1969) while hemicellulose estimation was carried out as per reports of Gao et al. (2014). The methods proposed by Sluiter et al. (2011) were used to determine fat and lignin content. Analysis of crude fiber, lipid, and total protein content of the pineapple peel were done as described by Rani and Nand, (2004).

2.5 Drying of pineapple peel waste

Drying experiments were conducted to study the effect of temperature, thickness of biomass, and time of drying operation. All the experiments were performed in triplicate and results reported are average of three readings.

2.5.1 Sample preparation and procedure

Pineapple peel was obtained from local markets of Pune, Maharashtra, India. It was ground by using laboratory grinder (Indica, Power 750W). The wet biomass was squeezed out to remove maximum possible liquid from it. A laboratory hot air tray oven (Bio-Technic BIT-30, India; 400×380×200 mm) with variable temperature controller was used for drying experiments. Different pineapple peel samples (10 ± 0.2 g, 67 ± 0.2 g, 160 ± 0.2 g) were weighed and subjected to drying operation. Drying experiments were carried out in the range of 60-120 °C and same material was used for saccharification studies. Pineapple peel mass and thickness was measured for all the temperatures, after every 10 min initially,

and 60 min during later stage of plateau, till it reaches constant. Moisture content, moisture ratio, and drying rate of samples were calculated with the help of data received. All the experiments were carried out at least in triplicate and results reported are average $\pm$ standard deviation.

2.5.2 Drying calculation

The experimental moisture content of pineapple peel is estimated for a given time by following expression:

$$M = \frac{W_t - W_d}{W_d} \quad (1)$$

where M is the moisture content for a given time (g/g dry solid), W_t is a mass for given time t (g), W_d is a mass bone dry solid (g). The experimental drying data is analyzed using following non dimensional equation:

$$MR = \frac{M - M_e}{M_0 - M_e} \quad (2)$$

where MR is the dimensionless moisture ratio, M is the moisture content for a given time (g/g dry solid), M_0 is an initial moisture content (g/g dry solid) and M_e is equilibrium moisture content (g/g dry solid). The experimental drying rate was determined using following expression:

$$\text{Drying rate} = \frac{M_{t2} - M_{t1}}{t_2 - t_1} \quad (3)$$

where t_1 and t_2 are drying times in min, M_{t1} and M_{t2} are moisture contents of pineapple peel at time t_1 and t_2 , respectively.

2.5.3 Modeling of drying data

An unsteady state shell mass balance approach was used to model the drying kinetics, to study structural changes in pineapple sample during drying operation. Mass

balance was made over a thin shell of finite thickness (Δy) perpendicular to the direction of moisture transport. The conservation of mass equation can be expressed by considering a section of sample on control volume.

Accumulation of mass moisture rate = (moisture in rate – moisture out rate + mass moisture production rate)

It can be conveniently assumed that no chemical reaction occurs during drying operation, and hence the production term becomes zero. The mass balance equation can be written as follows:

$$N_m|_y S - N_m|_{y+\Delta y} S = \frac{\partial (\rho_s M \Delta y S)}{\partial t} \quad (4)$$

where N_m is mean mass flux of moisture ($\text{kg/m}^2/\text{min}$), S is the cross sectional area (m^2), ρ_s is density of solid (kg/m^3) and M is moisture content (g/g of dry solid). Using mathematical definition of first derivative, following differential equation can be obtained, which describes mass flux gradient as:

$$-\frac{\partial N_m}{\partial y} = \frac{\partial \rho_s M}{\partial t} \quad (5)$$

Moisture transport during the drying operation was assumed to occur solely by molecular diffusion phenomena. Therefore convective moisture transport can be neglected. If the constant moisture diffusivity without volume change of drying material is assumed, then equation 5 can be reduced as:

$$D_{\text{eff}} \frac{\partial^2 M}{\partial y^2} = \frac{\partial M}{\partial t} \quad (6)$$

Moisture transport takes place due to concentration gradient within solid bed, where concentration is low at surface and high at bottom beneath solid bed. An effective diffusivity (D_{eff}) considers the change in volume, shape, and texture as well as change in

chemical composition of drying material. Equation 6 can be solved analytically if following assumptions are made: (i) moisture movement is unidimensional, (ii) initial moisture distribution is uniform, (iii) no chemical reaction (thermal or chemical) takes place during drying operation, (iv) constant moisture diffusivity, negligible shrinkages and final equilibrium moisture content is close to zero, and (v) negligible external resistances to moisture transfer and isothermal process (Crank, 1975):

$$MR = \frac{M}{M_0} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-(2n+1)^2 \left(\frac{\pi^2 D_{\text{eff}} t}{4L^2} \right) \right] \quad (7)$$

where D_{eff} is effective moisture diffusivity (m^2/s), t is a time (s), L is a half thickness of samples (m) and n is a positive integer (-). Equation 7 can further be simplified to only first term of series, without affecting accuracy of the prediction (Falade and Solademi, 2010; Kara and Doymaz, 2014):

$$MR = \frac{M}{M_0} = \frac{8}{\pi^2} \exp \left(-\frac{\pi^2 D_{\text{eff}} t}{4L^2} \right) \quad (8)$$

The effective moisture diffusivities can be determined for a given temperature when $\ln D_{\text{eff}}$ is plotted against time. A slope (K) of straight line determines the value of effective moisture diffusivity as:

$$K = \frac{\pi^2 D_{\text{eff}}}{4L^2} \quad (9)$$

where K parameter defined by equation 9. The temperature dependence of effective diffusivity can be expressed using Arrhenius relationship:

$$D_{\text{eff}} = D_0 \exp \left(-\frac{E}{RT} \right) \quad (10)$$

where D_0 is the pre-exponential factor (m^2/s), E is an activation energy (kJ/mol), R is universal gas constant (kJ/mol/K), and T is drying air temperature (K). The kinetic

parameters (E and D₀) can be estimated from slope and intercept of a plot of ln D_{eff} verses 1/T.

2.5.4 Statistical Analysis

The goodness of fit of proposed drying model to experimental data was assessed by means of statistical parameters *viz.* coefficient of determination (R²), sum squared error (SSE), and chi- square (χ²). The highest value of R² (~ 1.0) and lowest values of SSE and χ² indicate, best fit of model. The values of statistical parameters are determined as follows:

$$SSE = \frac{1}{N} \sum_{j=1}^N (M_{\text{exp},j} - M_{\text{pred},j})^2 \quad (11)$$

$$\chi^2 = \frac{\sum_{j=1}^N (M_{\text{exp},j} - M_{\text{pred},j})^2}{N-z} \quad (12)$$

where M_{exp, j} and M_{pred, j} are experimental and predicted values of jth moisture content (g/g dry solid), respectively, N is number of experimental runs, and z is number of constants.

2.6 Saccharification of pineapple peel waste by acid hydrolysis

The chemical pretreatment method was employed using 2% (v/v) sulfuric acid, 2% (v/v) hydrochloric acid, and 2% (w/v) sodium hydroxide in both autoclave (solid to liquid ratio 1:10, g/mL, at 121 °C for 60 min) and water bath (solid to liquid ratio 1:10, g/mL, at 100 °C for 60 min) to obtain total sugars from sun dried pineapple peel waste. Further, pretreatment parameters *viz.* concentration of sulfuric acid, solid to liquid ratio, temperature, and time were optimized (unpublished data) to be 1.3% (v/v), 1:5 g/mL, 121 °C, and 15 min, respectively. Pineapple peel samples which were dried earlier at different temperatures (as detailed in sections 2.5.1) were pretreated using optimized condition. Wet

(non-dried) pineapple peel of 4.4 g (equivalent to 1 g of dried sample) was also used as a control, during saccharification experiments and analyzed for total sugar release.

2.7 Detoxification of pineapple peel hydrolysates

Detoxification of pineapple peel hydrolysates were carried out to remove fermentation inhibitors by activated carbon (AC) using the method reported by Hodge et al. (2009). The pH of hydrolysates (pH 2.9) was gradually increased to 10 using sodium hydroxide solution. Subsequently, 5% (w/v) AC powder was added into hydrolysates and kept at constant temperature of 60 °C with continuous shaking at 200 rpm for 2 h. AC was separated by filtration, and filtrate was used for ABE fermentation. Total phenolics content before and after detoxification of dried and non-dried pineapple peel hydrolysates was determined by using Folin-Ciocalteu reagent.

2.8 Fermentative production of biobutanol

Fermentation experiments were carried out in 100 mL air-tight screw cap bottles with 80 mL working volume by using *C. acetobutylicum* B 527. Detoxified hydrolysates obtained from dried and non-dried samples were supplemented with other nutritional components, similar to P2 medium. Each hydrolysate, after AC detoxification was supplemented with glucose to reach total sugar concentration to 60 g/L for better comparison of diversified experiments. A medium pH was adjusted to 6.5 using hydrochloric acid if required. Anaerobic conditions were maintained by purging nitrogen in bottles containing medium, which were sealed with butyl rubber stopper fastened with an aluminum crimp and autoclaved at 121 °C for 20 min. All sterile hydrolysate bottles were inoculated with 5% (v/v) 20 h old inoculum and incubated at 37 °C for 96 h (Harde et al., 2016). A control experiment with P2 medium was carried out under same

experimental conditions. Samples were collected and used to analyze the solvents and sugar consumption.

2.9 Analytical method

Phenol sulfuric acid method was used to calculate total sugar (TS) in the hydrolysates (DuBois et al., 1956). Solvents (acetone, butanol and ethanol) and acids (acetic acid and butyric acid) were quantified by using gas chromatography as described by Bankar et al. (2013). Gas chromatography (Agilent Technologies 7890B) equipped with a flame ionization detector and AB-INNOWAX capillary column (30m×0.32mm×1µm) was used. Injector and detector temperature were maintained at 200 °C and 250 °C, respectively. The total phenolics were determined with Folin-Ciocalteu reagent using gallic acid as standard (20-100 µg/mL) and absorbance of sample was measured spectrophotometrically (UV-3000⁺, Labindia analytical) at 760 nm (Maurya and Singh, 2010).

3 Result and discussion

3.1 Feedstock and characterization of pineapple peel

Pineapple fruit generally consists of three parts: outer cover (pineapple peel), crown (top parts), and pulp (inner part). During pineapple fruit processing, a huge amount of pineapple waste (peel, core, stem and leaves) is generated which accounts almost 50% (w/w) of total pineapple mass. Among them, peel accounts for higher portion of about 30-42% (w/w) (Roda et al., 2014). However, due to inconsistency in composition of pineapple biomass, it is essential to carry out proximate analysis of pineapple peel. Proximate analysis experiments showed that pineapple peel contained 35% cellulose, 19.7% hemicellulose, and 16% lignin. Therefore, pineapple peel has a huge potential to be

considered as promising feedstock for biobutanol production. Further the total ash, total fat, total crude fiber, and total proteins were found to be 4.7%, 0.46%, 23.71%, and 0.33%, respectively. Importantly, pineapple peel also showed to have large moisture content in the range of 75-80% on wet basis, which is in close agreement with the reports presented by Rani and Nand (2004).

3.2 Drying of pineapple peel

Higher moisture content of pineapple peel (77.2% w/w on wet basis) makes it highly susceptible to microbial spoilage and also affects the dilution during saccharification experiments. Therefore, it is desirable to dry the pineapple peel samples to reduce moisture content in order to enhance storability and reduce transportation cost. Fig. 1 shows the declination of moisture content of pineapple peel with respect to time for a given temperature. All curves show an exponential tendency of moisture content (g/g of dry solid) decreasing rapidly with an increase in drying temperature. It was also observed that the time require to reduce total moisture content to fixed values increases, as the temperature decreases. The drying times required to reach minimal moisture content values of samples were 200, 120, 80 and 50 min at 60, 80, 100 and 120 °C, respectively. These results are in good agreement with other reports of drying fruits and vegetables such as potato (Akpinar, 2006), apple and pumpkin (Karabulut et al., 2007) wherein the falling rate period was observed to explain drying operation.

The experimental drying data is converted into moisture ratio using equation 2 and plotted against time to estimate values of effective diffusivity for a given temperature (Fig. 2). The values of effective diffusivities are obtained using equation 9. An effective diffusivity value varies in the range of $1.35 \times 10^{-8} \text{ m}^2/\text{s}$ to $5.27 \times 10^{-8} \text{ m}^2/\text{s}$ for varied temperatures (60-120 °C). It was observed that effective diffusivity was increased with an

increase in drying temperature. When drying was carried out at higher temperature, a greater proportion of heat energy is utilized to increase the movement of water molecules, leading to higher moisture diffusivity (Xiao et al., 2010). Table 1 shows that effective diffusivity increased five-fold with an increase in temperature from 60-120 °C. The temperature dependence of effective diffusivity can be illustrated using equation 10. A straight-line graph of drying temperature vs effective diffusivity (not shown in this paper), gave quantitative estimation of activation energy and pre-exponential factor, with slope and intercept, respectively. The values of activation energy and pre-exponential factor were found to be 24.60 kJ/mol and $6.12 \times 10^{-3} \text{ m}^2/\text{s}$, respectively. These results are in good agreement with reports from Antonio et al. (2012) for olive cake and dry vegetable waste.

The moisture ratio of pineapple peel can now be predicted when value of effective diffusivity is used in equation 8 for a given temperature. Fig. 2 shows that predicted values of moisture ratio with respect to time match well with experimental data, confirming validity of equation 8. Moreover, the rate of drying can also be predicted using following expression, since equation 8 presumes that kinetics of drying follows first order with respect to moisture content:

$$-r_M = -\frac{dM}{dt} = kM \quad (13)$$

where k is a drying constant (1/min) and is equal to $\pi^2 D_{\text{eff}}/4L^2$. The rate of drying can be expressed using values of activation energy and pre-exponential factor as a function of drying temperature and moisture content.

$$-r_M = 151.15 \times \exp\left(-\frac{2960}{T}\right) M \quad (14)$$

Fig. 3 shows the fitting of equation 14 to experimental data, estimated using equation 3. It can be seen that equation fits well to the experimental data within the operating conditions. Further, it is also clear that the drying rate decreases exponentially as the moisture content reduces close to zero (when moisture is removed completely). The goodness of fit of proposed model was also assessed by estimating statistical parameters (R^2 , SSE, and χ^2). The values of R^2 , SSE, and χ^2 are reported in Table 1, indicate that predicted data matched well with experimental data. The close fitting of data showed the drying kinetics follows first order dependence on a moisture content.

3.3 Saccharification of pineapple peel waste by acid hydrolysis

Saccharification is an important step to release total sugar from complex structure of pineapple peel. An acid hydrolysis conditions can be deliberated as equilibrium between high hemicellulose and cellulose alterations for efficient bioconversion. Therefore, various physical, chemical, and biological pretreatment methods have been reported in the literature. One such well-known chemical pretreatment method is dilute acid and/or alkali hydrolysis, which specifically affects hemicellulose structure. Based on preliminary reports by different researchers (Amiri and Karimi, 2015; Bankar et al., 2013a; Karimi et al., 2013; Karimi et al., 2015; Rattanapoltee and Kaewkannetra, 2014), dried biomass was treated with both acids and alkalis 2% (v/v) to perceive the total sugar release from pineapple peel waste. In current investigation, 2% (v/v) sulfuric acid pretreatment of dried biomass at 121 °C for 60 min resulted in highest total sugar release of 44.25±4.05 g/L. Besides, 2% (v/v) sulfuric acid pretreatment in water bath (at 100 °C for 60 min) also showed total sugar release to be 40.09±0.79 g/L. Likewise, 2% (v/v) hydrochloric acid resulted in total sugar yield of 41.63±2.979 g/L and 36.87±0.79 g/L in autoclave and water bath, respectively.

Furthermore, the treatment with 2% (w/v) sodium hydroxide resulted in 27.5 ± 0.55 g/L of total sugar in both autoclave and water bath experiments. From aforesaid results, it was concluded that treatment at higher temperatures (autoclave 121°C) resulted in higher total sugar release than treatment at relatively lower temperatures (water bath). In addition, 2% (v/v) sulfuric acid treatment was selected and further optimized (unpublished data) to get maximum total sugars. The optimized parameters include treatment with 1.3% (v/v) sulfuric acid, solid to liquid (g/mL) ratio of 1:5, and treatment time of 15 min. By using optimized conditions, the pretreatment experiments were undertaken to understand the effect of drying on total sugar release from pineapple peel waste (dried and non-dried).

Total sugar release by previously dried samples (60 - 120°C) of pineapple peel using optimized pretreatment parameters is summarized in Table 2. Interestingly, highest total sugars of 95.82 g/L and 97.25 g/L were obtained from the samples dried at 100°C and 120°C , respectively. This could be due to alteration in hemicellulose components of pineapple peel waste. The further characterization is needed to confirm this hypothesis and studies are in progress. Furthermore, time required for drying at 100 and 120°C was much lower than at 60 and 80°C . This ultimately leads to less energy requirement for the overall process. Concurrently, the total sugar release from non-dried sample was observed to be 58.53 g/L, which was lower than other dried samples. This may be due to the dilution effect during pretreatments and favorable unpredictable structural changes in pineapple peel samples during drying operation. Moreover, the amount of acid required would be higher for non-dried samples to release higher sugars per unit. Additionally, the non-dried samples were stored for 8-10 days, before its processing, and hence there is a possibility of utilization of sugars during microbial spoilage. Based on overall results, the drying operation has shown significant effect on total sugar release as compared to non-dried

pineapple peel samples (Table 2). Albanese et al. (2014) and Al-Harashsheh et al. (2009) reported an importance of drying during tomato pomace processing to prevent microbial spoilage because of high moisture content. Moreover, some researchers also used dilute sulfuric acid (0.5-4% v/v) treatment for pineapple peel processing to release maximum sugars with minimum fermentation inhibitors (Rattanapoltee and Kaewkannetra, 2014; Huang et al., 2011). Besides, it is worth investigating the effective pretreatment processes with higher bioconversion capacity, minimum energy requirements, minimum fermentation inhibitor formation, and simple methodology. This can be achieved by modification in pretreatment methods or blend of different pretreatment methods.

3.4 Detoxification of pineapple peel hydrolysates

Saccharification methodologies and neutralization are mostly accompanied with inhibitor formation that negatively influences ABE production. The inhibitors released during acid hydrolysis include furfural, 5-hydroxymethyl-furfural (HMF), acetic acid, and phenolic compounds. Moreover, effectual detoxification can be prescribed as medicine to eradicate fermentation inhibitors present in hydrolysate. The removal of inhibitors has been previously investigated by different methods such as overliming, vacuum evaporation, ion exchange resin, and adsorption using AC (Harde et al., 2014). Each method has its own characteristics and a specific mechanism of action for inhibitor removal. The AC detoxification method is commonly used due to high adsorption capacity with minimum total sugar loss. Hence, in present study, the detoxifications of five hydrolyzates by AC were carried out. Detoxification treatments lead to removal of 95-97% phenolics (total phenolics concentration 0.065 to 0.11 g/L for all hydrolysates). Similarly, AC detoxification also removed acetic acid upto 50% when used in different concentrations with only 10 to 13% total sugar loss (Table 2). The mechanism of AC

detoxification includes adsorptive removal of phenolics and dilute acids (Dina et al., 2012; Tiruneh et al., 2016). Adsorption phenomenon may be driven by physicochemical interaction. Physical adsorption is either due to weak forces or electrostatic interactions whereas adsorption resulting from chemical interaction between adsorbent and adsorbate. Due to heterogeneity of inhibitory compounds in hydrolysates, the adsorption mode is expected to be a mixture of physical and chemical adsorption (Tiruneh et al., 2016). Yamamoto et al. (2014) reported the use of activated charcoal to reduce the inhibitor concentration and further improve ABE fermentation efficiency. Qureshi et al. (2008) also reported the pre-adjustment to pH 10 decreases inhibitory concentration in dilute acid hydrolysate by using 20% AC detoxification. Similarly, Hodge et al. (2009) reported the use of activated carbon to decrease the inhibitor concentration of 86-96% of HMF and total phenolics after treatment. The application of AC as a detoxifying agent can be an economic process if cheap sources of AC or economic regeneration methods are available.

3.5 Fermentative production of biobutanol

Lignocellulosic biomass has been widely explored for ABE fermentation by *Clostridia* species is evident for biphasic fermentation viz. acidogenic phase and solventogenic phase. In current study, the hydrolysates obtained after drying at different temperatures followed by acid hydrolysis and detoxification methods were used for ABE fermentation by using *C. acetobutylicum* B 527. Additionally, Khamaiseh et al. (2014) pointed out the addition of nutrients other than carbon was essential for the fermentation process to get optimum ABE solvents. Therefore, all hydrolysates were supplemented with nutrient similar to standard P2 medium. In order to maintain total sugar concentration (for effective comparison) to be 60 g/L, auxiliary glucose was added to the detoxified hydrolysates. Likewise, Harde et al. (2016) studied the fermentation of ABE using root

hydrolysates and achieved maximum total solvents after 96 h batch fermentation. Based on this study, all the fermentation experiments were carried out at batch level for 96 h and samples were analyzed for its solvent production and substrate utilization. ABE fermentation titers and yields are shown in Table 3. Standard P2 medium containing glucose as carbon substrate was used as a control and it produced total solvents and total acids to be 10.92 g/L and 1.55 g/L, respectively.

For non-dried detoxified hydrolysates, the total solvent production was obtained to be 4.11 g/L with yield of 0.12 g/g. While, the highest total solvent of 5.23 g/L and yield of 0.15 g/g were achieved when the samples were dried at 120 °C. It can be inferred that the total solvent and yield of dried samples were higher when compared with non-dried sample signifying effectiveness of drying operation. Further the variations in drying temperatures have shown significant effect on total solvent production and yield. For example, the sample dried at 120 °C resulted in total solvent of 5.23 g/L whereas sample dried at 60 °C showed total solvent of 4.16 g/L. The reason behind increment in solvent production at higher drying temperature is still unclear. Overall sugar consumption during fermentation was in the range of 50-55% and individual results of sugar consumption for varied dried samples are as shown in Table 3. Hence, drying of pineapple peel waste is efficient at high temperature that does not show any negative effect on total sugar release as well as on ABE fermentation. This study apparently seems contradictory to Oberoi et al. (2007) who reported that the cauliflower wastes dried at 50 °C retained the color and resulted in significant glucoamylases production. However, current study is in line with the reports by Avila-Gaxiola et al. (2016) who presented positive effect of drying temperature on agave tequilana leaves and showed improved ethanol fermentation.

As can be seen from Table 3, all the fermentation experiments showed high amount of total acids (acetic and butyric acids) production that directly affects ABE production. Ezeji et al. (2007) reported that presence of 3 g/L furfural and HMF are stimulatory to ABE production, whereas 0.3 g/L p-coumaric and ferulic acids were inhibitory to *C. beijerinckii* BA101 and decreased ABE production significantly. Ezeji et al. (2013) showed another way to enhance butanol production by integrated fed-batch fermentation system coupled with gas stripping. Although, the final solvent results out of this study may not seem to be promising, we are in a process to continuously improve it. Furthermore, the efforts to improve ABE solvent titer and yield will be targeted to reduce inhibitory components present in the medium. Besides, the large quantity of acids produced during ABE fermentation will also be addressed by genetic engineering aspects as well as by bioprocess modification. The fed batch and continuous ABE process development are additional target areas to check its commercial feasibility.

4 Conclusions

Drying of pineapple peel waste showed significant influence on total solvent production, solvent yield, and sugar consumption with *C. acetobutylicum* B 527. The dried and non-dried samples were treated at different temperatures and highest total sugar yield was found to be 97 g/L for samples previously dried at 120 °C. The detoxified hydrolyzates were subjected to fermentation that resulted in maximum total solvents and yield to be 5.23 g/L and 0.15 g/g respectively, with maximum sugar utilization. This process may allow the use of drying at industrial scale and therefore helps to reduce the storability and transportation cost.

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Figure captions

Fig. 1. Effect of temperature on moisture content versus time during drying

Fig. 2. Kinetic of drying showed by plotting moisture ratio versus time at various temperatures

Fig. 3. The rate of drying versus time at various temperatures

Table captions

Table 1: Statistical parameters applied to assess the drying model and effective diffusivity

Table 2: Composition of hydrolyzates before and after AC detoxifications

Table 3: Effect of pineapple peel drying on total solvents and yield of ABE

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Table 1: Statistical parameters applied to assess the drying model and effective diffusivity

Temperature, (°C)	Statistical parameter			Effective diffusivity $\times 10^8$, (m ² /s)
	R ²	SSE	χ^2	
60	0.992	0.012	0.013	1.35
80	0.993	0.010	0.011	2.43
100	0.996	0.014	0.016	3.72
120	0.992	0.016	0.019	5.27

Table 2: Composition of hydrolysates before and after AC detoxifications

Pineapple peel sample	Hydrolysates after dilute acid treatment			Hydrolysates after AC detoxification		
	TS (g/L)	AA (g/L)	TP (g/L)	TS (g/L)	AA (g/L)	TP (g/L)
Non-dried sample	58.53	6.58	2.55	52.23	3.99	0.069
Dried at 60 °C	75.23	5.97	3.48	66.85	3.21	0.065
Dried at 80 °C	86.06	4.45	3.40	74.12	3.25	0.096
Dried at 100 °C	95.82	3.81	3.71	85.28	2.82	0.085
Dried at 120 °C	97.25	3.40	3.84	84.25	3.30	0.111

AC: Activated carbon, TS: Total sugar, AA: Acetic acid, TP: Total phenolics

Table 3: Effect of pineapple peel drying on total solvents and yield of ABE

50% hydrolysates plus water after AC	IS (g/L)	SC (g/L)	Total (g/L)	ABE (g/L)	TA (g/L)	Yields of ABE (g/g)	Productivity of ABE (g/L/h)
P2 control	60.67	50.54	10.92	1.55	0.21	0.11	
Non-dried sample	59.22	29.62	4.11	3.52	0.12	0.04	
Dried at 60 °C	63.00	32.40	4.16	3.62	0.13	0.04	
Dried at 80 °C	62.00	32.15	4.67	3.75	0.14	0.04	
Dried at 100 °C	60.78	34.34	5.00	3.31	0.14	0.05	
Dried at 120 °C	60.66	33.07	5.23	3.73	0.15	0.05	

IS: Initial sugar, SC: Sugar consumed, TA: Total acids