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Biobutanol production using pea pod waste as substrate: Impact of drying on saccharification and fermentation

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Abstract

Increasing worldwide energy consumption and limited availability of fossil fuels propelled the researchers to develop advanced fuels (biobutanol) for its commercial development. In the present work, pea pod waste from vegetable sector was investigated for biobutanol production using *C. acetobutylicum* B 527 through series of steps viz. compositional analysis, drying study, saccharification, detoxification, and fermentation. Proximate analysis suggested that pea pod waste is rich in holocellulose content with 32.08% of cellulose and 21.12% of hemicellulose on dry basis and hence has a huge potential to be used as carbon source during biobutanol production. In order to enhance storability and subsequent saccharification, drying kinetics of pea pod waste was carried out in varied temperature range (60-120 °C) and the experimental data was simulated by using moisture diffusion control model. Saccharification of pea pod waste samples resulted into total sugar release of 30-48 g/L. Subsequently 95% phenolics and 30% acetic acid were removed using activated charcoal detoxification. The acetone-butanol-ethanol (ABE) fermentation of detoxified pea pod waste slurries resulted in 4.25-5.94 g/L total solvents with about 50% sugar utilization. Overall, the utilization of pea pod waste will serve as basis for valorization of vegetable waste biomass for ABE production.

Keywords

Biobutanol, Drying, Fermentation, Peas pod waste, Saccharification

Highlights

- Exploitation of pea pod waste; a vegetable waste biomass for biobutanol production
- Mathematical modeling elucidate that drying kinetics follows 1st order dependency
- Effectual total sugar release in range of 30-48 g/L using dilute acid hydrolysis
- Activated charcoal detoxification significantly removed more than 95% of phenolics
- Substantial ABE production by pea-pod hydrolysate was approximately 6 g/L

1 Introduction

Comprehensive environmental concerns on valorization in crude oil prices, energy insecurity and escalation of atmospheric carbon dioxide level necessitates the hunting of an alternative renewable energy sources [1]. Biofuels, has an enormous potential to overcome these energy dependencies. Biobutanol is one of the interesting biofuels that offers many advantages over bioethanol like higher calorific value, low vapor pressure, and good affinity with gasoline in varied ratio [2]. Butanol has also been used in chemical process industries as a reactant to synthesize esters besides its use as a fuel additive. Biobutanol is traditionally produced by acetone-butanol-ethanol (ABE) fermentation using anaerobic *clostridia* [3]. However, second-generation biobutanol production is of great importance to avoid food versus fuel war. Until now, researchers have made an attempt to produce biobutanol from various second-generation feedstocks such as barley straw [4], wheat straw [5], spruce chips [6] and others.

India alone generates around 400 million tons biomass every year that can be utilized in production of value added products [7]. As per indigenous farming, around 90 million metric tons of vegetables are being produced per year in India as a second leading producer in the world [8]. Generally, vegetable processing units generate more than 30% (w/w) waste during harvesting, processing, and marketing. Based on vegetable production statistics, it has been noted that India is leading producer of green pea (*Pisum sativum*) with annual production of around 3.56 million metric tons, which subsequently generates more than 1 million ton of pea pod waste every year [9]. In day-to-day life, a huge amount of vegetable waste being generated is either ploughed back into soil or left as a city waste on a dumping ground, which ultimately causes environmental pollution [10]. The modern driving force in bioconversion of these wastes into biobutanol would be beneficial in context of reducing pollution and indirectly relate to strengthen nation's economy [9].

Proximate analysis of pea pod waste showed to contain 26% cellulose, 20.5% hemicellulose, 3.92% lignin, 20.2% crude protein, and 8.5% ash [11]. The presence of higher holocellulose content in pea pod waste makes it suitable to be used as a potential feedstock for butanol production. Other researchers have already used pea pod waste as a feedstock for gibberellic acid [12] and cellulase production [13]. Babbar et al. [14] extracted phenolic compounds from pea pod waste to study their antioxidant activity. However, majority of vegetable wastes like pea pod waste, baby corn husk and forage, cabbage and cauliflower leaves are perishable due to high moisture content in it (80-90% w/w) [15]. Moreover, moisture rich feedstock is unsuitable for bioproduction, as it is readily prone to microbial spoilage and also adds unnecessary transportation cost. Therefore, drying treatment to the feedstock can be adapted to store these materials throughout year or during the lean period of supply for continuous production process. One of the major bottlenecks in second-generation butanol production is formation of inhibitors during pretreatment [16]. These inhibitors subsequently affect ABE fermentation and hence additional step of detoxification is also necessary [17].

Based on aforesaid discussion, the objective of present work was to produce biobutanol from pea pod waste via series of steps such as drying, pretreatment, detoxification, and ABE fermentation. In current manuscript, drying kinetics of pea pod waste is mainly focused along with mathematical modeling. Furthermore, acid hydrolysis and detoxification were also carried out for maximum fermentable sugars followed by inhibitor removal. Pea pod waste was used as potential carbon source for ABE production by using *C. acetobutylicum* NRRL B-527. To the best of our knowledge, this is the first report on production of biobutanol by using pea pod waste as a feedstock.

2 Materials and Methods

2.1 Materials

Gallic acid, anthrone powder, Folin-Ciocalteu reagent, sodium carbonate, coomassie brilliant blue dye, phosphoric acid, and activated charcoal were obtained from HPLC, India. Yeast extract and meat extract was purchased from Himedia, India. D-glucose, peptone, sodium chloride, L-cysteine hydrochloride, magnesium sulfate, manganese sulfate, iron sulfate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ammonium acetate, biotin, thiamin, p-aminobenzoic acid, sodium hydroxide, sodium potassium tartarate, acetic acid, butyric acid, acetone, butanol, isopropanol were purchased from SRL Ltd, India. Sodium acetate and phenol were obtained from Sigma Aldrich, India. Hydrochloric acid and sulfuric acid were procured from Avra Synthesis Ltd, India. All the chemicals and solvents were of analytical grade (AR). Strain *C. acetobutylicum* NRRL B-527 was a kind gift from ARS (Agriculture Research Services) culture collection, USA.

2.2 Microorganism and inoculum preparation

Initially, the lyophilized cells of *C. acetobutylicum* NRRL B-527 was grown in a revival medium (RM) for 48 h and subsequently inoculated into 6% (w/v) sterile starch solution for spore formation. The starch bottles were incubated for 8-10 days at 37 °C and kept in cool and dry place for further use. The RM used in present study contained (g/L): glucose (5.0), peptone (10), beef extract (10), yeast extract (3.0), sodium chloride (5.0), soluble starch (1.0), L-cysteine hydrochloride (0.5), sodium acetate (3.0) and resazurin (0.001) at pH 6.8. For inoculum preparation, the method proposed by Harde et al. [18] was used. The sterile reinforced clostridial medium (RCM) was used as seed medium in which 2% (v/v) spore suspension was inoculated and incubated for 18-20 h at 37 °C. The composition of RCM includes (g/L): meat extract (10), peptone (5.0), yeast extract (3.0), glucose (30), starch (1.0), sodium chloride (5.0), sodium acetate (3.0) and L-cysteine (0.5) and pH 6.8.

2.3 Compositional analysis of pea pod waste

Fresh green peas (*Pisum sativum*) were procured from local markets of Pune, Maharashtra, India. The pods were separated from kernels and further used for compositional analysis. Prior to analysis, the pods were cut into small pieces and ground (Indica grinder, Power 750W) to get homogenized mass. This mass was dried and characterized for protein, crude fiber, fat, ash, and total volatile solids according to standard methods [19]. Further, cellulose and hemicellulose were estimated using methods reported by Updegraff [20] and Gao et al. [21], respectively. The lignin content was also determined as explained by Kirk and Obst [22].

2.4 Drying of pea pod waste

2.4.1 Experimental procedure

The homogenized mass of pea pod waste was prepared as explained in earlier section and the whole mass was squeezed to take out maximum possible water. Drying experiments were carried out in hot air tray oven (Bio-Technic BIT- 30, India; 400×380×200 mm) under varied temperature range (60-120°C). A fixed amount (101±0.5 g) of sample was weighed, spread over glass plate and dried at different temperatures. After every 10 min, the sample mass and thickness were recorded and the procedure was continued till constant mass was achieved. The moisture content, moisture ratio and drying rates were calculated to elucidate the underlying mechanism during drying of pea pod waste. All the experiments were done at least in triplicate and result reported are average ± standard deviation.

2.4.2 Determination of drying parameters

The experimental moisture content of pea pod waste was 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 a given time t (g), W_d is a mass bone dry solid (g). The experimental drying data was analyzed using following moisture ratio 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 the initial moisture content (g/g dry solid); M_e is the 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 the drying times (min), M_{t1} and M_{t2} are moisture contents of pea pod waste at time t_1 and t_2 , respectively.

2.4.3 Modeling of drying data

An unsteady state shell mass balance was used over a control volume to discern the drying kinetics of pea pod sample (Fig. 1). The basic mass balance can be written as follows:

Fig. 1. Schematic diagram of control volume

Rate of moisture accumulation = (Rate of moisture in – Rate of moisture out + Rate of moisture production)

During drying operation, it can be conveniently assumed that no chemical reaction occurs and hence a 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 moisture mass flux (g moisture/g dry solid/m²/min), S is a surface area perpendicular to the moisture transport, ρ_s is content of dry solid (g/m³). Equation (4) reduces using mathematical definition of first derivative to following differential equation:

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

The drying operation of food materials usually occurs in the falling rate period [23, 24] and moisture transfer during drying is controlled by internal diffusion [24, 25]. Therefore, convective moisture transport can be neglected. Furthermore, if the constant moisture diffusivity without volume change of drying material is assumed, then equation (5) reduces to following equation:

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

The effective diffusivity of moisture (D_{eff}) term considers the change in volume, shape, texture, as well as chemical composition of the drying material. Equation (6) can be solved analytically if the assumptions such as uni-dimensional moisture movement, uniform initial moisture distribution, and negligible external resistances to moisture transfer and isothermal process are made [26]:

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

where, MR is the dimensionless moisture content ratio, M is the average moisture content at time t (g/g of dry solid), M_0 is the initial moisture content (g/g of dry solid), M_e is the equilibrium moisture content (g/g of dry solid), and L is the thickness of the slab (m).

The values of M_e are reasonably small as compared to M and M_0 , therefore M_e can assumed to be equal to zero without a significant error [27]. Further, equation (7) gives satisfactory estimate of the moisture ratio although the first term in the series expansion is considered [28]. Thus, equation (7) simplifies as follows:

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

When experimental values of $\ln(M_0/M)$ are plotted against t, the slope of the curve is a measure of effective diffusivity as:

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

Another simplified approach to predict drying kinetics for the falling rate period of drying is “thin layer equation”:

$$-\frac{dM}{dt} = KM \quad (10)$$

where K is an empirical constant called as drying constant. The assumption of equation (10) is valid, when the material being dried is thin enough, or the air velocity is high enough, so that the drying air conditions are constant throughout the thin material. The following relation is derived by integrating the “thin layer” equation (10) between initial and mean moisture content at time t:

$$\frac{M}{M_0} = \exp(-Kt) \quad (11)$$

Comparing equations (11) and (8), under the above stated assumptions. The drying constant K can be related to moisture diffusivity as follows:

$$K = \frac{\pi^2 D_{eff}}{4L^2} \quad (12)$$

where, the term $8/\pi^2$ is considered equal to unity. Equation (12) relates the empirical drying constant K, to the theoretical property of moisture diffusivity (D_{eff}). It is a valuable tool for K to D_{eff} transformation and thus to predict drying data in situations where the significant computation time needed by the numerical solution is undesirable.

Substituting the drying constant to equation (10), the "thin layer" equation is transformed to:

$$-\frac{dM}{dt} = \frac{\pi^2 D_{eff}}{4L^2} M \quad (13)$$

D_{eff} can be related to the temperature using Arrhenius equation:

$$D_{eff} = D_0 \exp\left(-\frac{E}{RT}\right) \quad (14)$$

where R is the universal gas constant (8.314 KJ/ mol /K), E is the activation energy (KJ/mol), D_0 the Arrhenius factor (m^2/s) and T is the absolute temperature (K). Both kinetic parameters (E and D_0) can be estimated from the slope and intercept of the plot $\ln D_{eff}$ versus $1/T$.

2.4.4 Statistical Analysis

The statistical analysis of the drying data was carried out by means of statistical parameters *viz.* regression coefficient (R^2), sum squared error (SSE), and chi- square (χ^2). The highest value of R^2 (~ 1.0) and lowest values of SSE and χ^2 indicates the best fit of the model. The values of statistical parameters are determined as follows:

$$SSE = \frac{1}{N} \sum_{j=1}^N (M_{exp,j} - M_{pred,j})^2 \quad (15)$$

$$\chi^2 = \frac{\sum_{j=1}^N (M_{exp,j} - M_{pred,j})^2}{N - n} \quad (16)$$

where $M_{exp,j}$ and $M_{pred,j}$ are experimental and predicted value of j^{th} moisture content (g/g dry solid), respectively. N is the number of experimental run and n is the number of constant in the drying model.

2.5 Saccharification and detoxification processes

Saccharification of all dried pea pod samples was carried out using dilute acid catalyst. For treatment, 5 mL of 1.3% (v/v) sulfuric acid was added into known amount (1 g) of sample in 50 mL conical flask. Further, the sample was hydrolyzed in an autoclave at 121 °C for 15 min and filtered successively. Similar procedure was repeated for all samples, which were dried at different temperature range (60-120 °C). At the same time, a wet sample (non-dried) of 3.8 g (corresponding to 1 g dried sample) was also hydrolyzed under abovementioned conditions. Finally, the total sugars in all respective hydrolysates were quantified by phenol sulfuric acid method [29]. Additionally, individual sugars at beginning and after fermentation were determined by using a high performance liquid chromatography (HPLC) system (Dionex India Ltd.) equipped with a refractive index detector. An ion exclusion column (Bio-Rad Aminex, HPX 87-H) was used at a temperature of 30 °C with 0.008 M H_2SO_4 as a mobile phase, at a flow rate of 0.6 mL/min and a sample volume of 20 μ L. In addition, three different pea pod waste samples were characterized by Fourier transform infrared spectroscopy (FTIR) from Bruker in the range of 500-4000 cm^{-1} with a resolution of 2 cm^{-1} and the data obtained was analyzed using Opus software.

During these pretreatment processes, many fermentation inhibitory compounds such as furfural, 5 hydroxymethylfurfural (HMF), weak acids, and lignin (phenolics) are formed [30,

31]. These inhibitors were removed using activated charcoal method [32]. pH of all pretreated hydrolysates was adjusted to 10 using sodium hydroxide solution. Further, the hydrolysates were detoxified by using 5% (w/v) activated charcoal at 60 °C with continuous stirring at 200 rpm for 2h. After detoxification, the activated charcoal was recovered by filtration and the filtrate was later analyzed for presence of inhibitors and for total sugars to estimate the sugar loss. The total phenolics of hydrolysates before and after detoxification were determined using Folin-Ciocalteu reagent [33]. Gallic acid (20-100 µg/mL) was used as standard and absorbance of samples were measured in UV spectrophotometer (UV-3000+, Labindia). All the experiments were carried out at least in triplicate and results reported are average $\pm$ standard deviation.

2.6 Batch fermentation of detoxified pea pod waste

Batch fermentations of all detoxified slurries were performed in 100 mL air tight screw cap bottles with 80 mL working volume, by using *C. acetobutylicum* B-527. The standard P2 medium reported by Bankar et al. [34] includes components as (g/L): glucose (60), magnesium sulfate (0.2), sodium chloride (0.01), manganese sulfate (0.01), iron sulfate (0.01), dipotassium hydrogen phosphate (0.5), potassium dihydrogen phosphate (0.5), ammonium acetate (2.2), biotin (0.01), thiamin (0.1) and p-amino benzoic acid (0.1) and pH 6.5. The control (model P2) experiment was designed according to individual sugar composition (pentose+hexose) of detoxified slurry, for better comparison. The model P2 and detoxified slurries were supplemented with other nutritional components of standard P2 medium and auxiliary glucose, if required. Bottles containing production medium were then purged with nitrogen for about 5-10 min, sealed, and autoclaved at 121 °C for 20 min. The fermentation was started by inoculating 5% (v/v) 20 h old inoculum (OD_{560} 1.23) and maintained at 37 °C for 96 h [18]. Fermentation kinetics of both control and detoxified slurry were studied by withdrawing samples after every 24h and data was plotted to compare fermentation profiles. Solvents (ABE) and acids (acetic and butyric) produced were analyzed using gas chromatography (Agilent Technologies 7890B) [35]. AB-INNOWAX capillary column (30m $\times$ 0.32mm $\times$ 1µm) and flame ionization detector were used and sample of 0.5 µL was injected. All the experiments were performed at least in triplicate and results reported are average $\pm$ standard deviation.

3 Results and discussion

3.1 Compositional analysis of pea pod waste

Proximate analysis of pea pod waste suggested that it is a rich source of holocellulose content (32.08% cellulose and 21.12% hemicellulose). It was also observed that pea pod waste contains significant amount of protein (10.58%) and ash (5.2%). The other compositional details are listed in Table 1. Similar findings have previously been reported on composition of pea pod waste [9, 10, 13]. The presence of high moisture content of about 73.5% (w/w) reveals that pea pod waste is highly prone to the microbial spoilage and affects dilution during pretreatment experiments. Hence, pea pod waste was initially dried and used further in fermentative production of biobutanol.

Table 1

Composition of pea pod waste (%) on dry basis

Parameters	Value (%)	Parameters	Value (%)
Moisture content on wet basis	73.5 $\pm$ 0.28	Protein	10.58 $\pm$ 0.02
Ash	5.20 $\pm$ 0.14	Cellulose	32.08 $\pm$ 1.00

Total fat	0.41±0.01	Hemicellulose	21.12±0.50
Crude fiber	5.42±0.01	Lignin	21.58±0.44

3.2 Drying of pea pod waste

Drying study of pea pod waste was carried out in the temperature range of 60-120 °C to elucidate the effect of drying temperatures on total sugars and subsequently on fermentation. The effect of drying temperature on moisture content is shown in Fig. 2 for a given temperature. The moisture content was decreased exponentially with an increase in temperature and drying time. Therefore, time required to achieve a moisture content lower than 0.10 g moisture/g dry solid at 60 °C was 170 min, nearly the doubled the time needed to reach same moisture content at 80 °C (90 min), and closely four times the time required at a temperature of 120 °C (40 min). Similar findings were reported by other researchers for different feedstocks such as potato [36], apple and pumpkin [37]. Further, model presented in equation (8) signifies presence of only falling rate period during drying operation of pea pod waste.

The plot of $\ln(M_0/M)$ against time for given temperatures yield a straight line, with slop that gives value of drying rate constant which could be converted into effective diffusivity using equation (12). Fig. 3 shows the plot of $\ln(M_0/M)$ Vs time. Linear fitting confirms the validity of equation (11), indicating drying rate follows ‘first order’ with respect to moisture content.

Fig. 2. Effect of temperature on moisture ratio during drying

Solid lines in Fig. 3 indicate the predicted values of moisture content with respect to time, obtained using equation (11). Predicted values match well with experimental values. Table 2 shows the values of effective diffusivity for a given temperature. The values of effective diffusivity confirmed that increase in drying temperature subsequently increases drying rate.

Table 2

Statistical parameters applied to assess the drying model and effective diffusivity

Temperature, (°C)	Statistical parameter			K (1/s)	Effective diffusivity $\times 10^8$, (m ² /s)
	R ²	SSE	χ^2		
60	0.991	0.0041	0.0043	0.0003	1.28
80	0.992	0.0027	0.0025	0.0005	2.16
100	0.986	0.0024	0.0026	0.0008	3.58
120	0.995	0.0021	0.0025	0.0013	5.54

Fig. 3. Moisture ratio of four different air temperatures

Fig. 4 shows the plot of $\ln D_{\text{eff}}$ against reciprocal of absolute temperature. A linear relationship exists between effective diffusivity and temperature with statistical parameter value (R^2) equal to 0.99. From the slope of this line, values of activation energy and Arrhenius factor were determined to be 26.02 kJ/mol and 1.14×10^{-2} m²/s respectively. Similar report has been demonstrated by Doymaz et al. [38] for carrot drying.

Fig. 4. Correlation between effective diffusivity and absolute temperature

Rate of drying can be expressed as a function of drying temperature and moisture content using equations (13) and (14):

$$-\frac{dM}{dt} = AD_0 \exp\left(-\frac{E}{RT}\right)M \quad (17)$$

where A is constant and equal to $\pi^2/4L^2$. Substituting the values of A, activation energy, and Arrhenius factor, following rate expression can be written:

$$-r_M = -\frac{dM}{dt} = 282.11 \times \exp\left(-\frac{3200}{T}\right)M \quad (18)$$

Fig.5 shows the plot of drying rate against time. It can be seen that equation (18) fits well with the experimental data within operating conditions. Further, it is also clear that the rate of drying decreases exponentially as the moisture content reduces, close to zero.

Fig. 5. The rate of drying versus time at various temperatures

Overall, drying kinetics of pea pod waste was studied at different temperatures and experimental moisture content data were simulated by moisture diffusion model (Fig. 3). The activation energy was calculated (Fig. 4) and was used in unique simulated equation to estimate drying rate for pea pod waste biomass provided that moisture content and temperature are known (Fig. 5).

The goodness of fit of proposed model was assessed by estimating statistical parameters (R^2 , SSE, and χ^2). Values of R^2 , SSE, and χ^2 that are reported in Table 2 indicate that predicted data matched well with the experimental data. The close fitting of data showed that drying kinetic follow first order dependence on moisture content.

3.3 Saccharification and detoxification processes

Lignocellulosic biomass is generally composed of complex structure of cellulose, hemicellulose, and lignin. Saccharification process uses acid or alkali to break recalcitrant structure of biomass to get fermentable sugars. Various researchers reviewed different saccharification processes such as physical (milling and grinding), chemical (dilute acid or alkali), biological, and others [39, 40]. Each method has its own pros and cons. Hence, selection of suitable method for maximum sugar release with less inhibitor generation is of great importance.

Based on previous experience and other literature reports [32, 41, 42], dilute sulfuric acid treatment was selected and pea pod waste samples were hydrolyzed with 1.3% (v/v) acid concentration at 121 °C for 15 min. The total sugar release after saccharification (for all five hydrolysates) is shown in Fig. 6. As expected, wet (non-dried) sample resulted in total sugar release of 30.33 g/L which is less as compared to other dried samples. Further, it was observed that drying at lower temperature resulted in less total sugar release (40.37 g/L) when compared with sample (48.07 g/L) dried at higher temperature (100°C). This signifies that there is no thermal degradation of sugars during drying operation at higher temperature. Furthermore, higher temperature may also have positive effect on structural distribution that might result in hemicellulose accessibility to acidic hydrolysis. Moreover, reports from literature shows that drying at lower temperature takes longer time, which ultimately hardens the material, and hinders sugar release [43, 44].

Fig. 6. Total sugars after saccharification and detoxification

To clarify the compositional changes during different operating procedures, three pea pod samples namely ‘sundried’, ‘dried at 100 °C’ and ‘acid treated’ were investigated using FTIR spectroscopy. Fig. 7 shows variations in peak intensities for C-H, C-O, O-H, N-H, C-N bonds occurs at different frequency level. Especially, 1000-1200 cm^{-1} range corresponds to holocellulose having maxima at 1040 cm^{-1} and 1165 cm^{-1} due to C-O stretching and asymmetrical C-O-C stretching, respectively. Further, band absorption at 1247 cm^{-1} result due to C-O stretching and point out feature of hemicellulose along with lignin. On comparing Fig. 7A and 7B, the band intensity for aforesaid frequency level is nearly similar indicating slight changes in hemicellulose conformation during drying process. However, Fig. 7C shows drastic drop in band intensity which confirms hemicellulose solubilization occurred due to acidic treatment. Similar finding was reported by Pal et al. [45] for steam exploded bagasse. Moreover, the quantitative assessment of resulted liquid fraction after saccharification also showed presence

of pentose sugars thus signifying partial removal of hemicellulose with deformation in lignin orientation.

Fig. 7. FTIR spectra of pea pod waste: A- sundried; B- dried at 100 °C; C- dried (100 °C) and acid treated

Although, saccharification is of prime important step in biobutanol production, it also generates various sugar degradation products during the process. The processes operated at high temperature and pressure results in generation of furfural, hydroxymethyl furfural, acetic acid, ferulic acid, levulinic acid and many other phenolic compounds. This in turns negatively affects ABE fermentation process leading to low solvent yield and productivities. To overcome this concern, all pretreated hydrolysates were subjected to detoxification step. Activated charcoal is highly selective in removal of furans and phenolics with minimal sugar losses [46] and hence was used to detoxify the hydrolysates. Our previous study also showed effectiveness of activated charcoal detoxification to remove fermentation inhibitors [47]. The inhibitory data before and after detoxification process are listed in Table 3. Activated charcoal detoxification mainly removed more than 95% phenolics and 10-30% acetic acid from all hydrolysate samples with very less sugar losses (around 10%) (Fig. 6). The results are in close agreement with other reports on activated charcoal detoxification for other feedstocks [46, 48].

Table 3

Inhibitor removal by activated charcoal

Pea pod waste	Inhibitors	Non detoxified hydrolysates (g/L)	Detoxified hydrolysates (g/L)
Wet (non-dried) 60 °C	TP	1.74±0.1	0.03±0.001
	AA	5.22±0.01	3.62±0.01
	TP	2.19±0.3	0.05±0.003
	AA	2.99±0.01	2.64±0.01

80 °C	TP	2.47±0.2	0.05±0.004
	AA	2.72±0.01	2.20±0.01
100 °C	TP	2.70±0.2	0.06±0.001
	AA	2.47±0.01	1.84±0.01
120 °C	TP	2.82±0.1	0.07±0.003
	AA	2.57±0.01	1.99±0.01

Results are mean ± SD of three determinations; TP: Total phenolics; AA: Acetic acid

3.4 Batch fermentation of detoxified pea pod waste slurries

After drying, saccharification, and detoxification, pea pod waste samples were subjected to batch fermentation using *C. acetobutylicum* B-527. The prerequisite of any fermentation process is to have an appropriate balance of nutritional components for microbial growth and subsequent product formation [49]. Therefore, all pea pod waste slurries were supplemented with minerals and vitamins as per standard P2 medium and auxiliary glucose was added to maintain the final total sugar concentration to be 60 g/L for efficient comparison. Furthermore, all pea pod waste slurries were consisting of pentose (xylose and arabinose) along with hexose (glucose and fructose) as main carbohydrates. Individual sugar profiling before and after fermentation is as shown in Fig. 8. As mentioned earlier, the fermentation of both control (designed as per composition of hydrolysates) and diluted detoxified slurries were performed until 96 h and then analyzed for solvent production. The detoxified slurries showed substantial solvent production with consumption of around 50% sugars in order as: glucose > fructose > xylose > arabinose (Fig. 8).

Fig. 8. Individual sugar profiling before and after fermentation stage (for detoxified slurries). D: Dried; IS: Initial total sugar; RS: Residual total sugar

The total solvent and acids produced during fermentation are summarized in Table 4. As predicted, model P2 was able to produce higher total solvent of 8.80 g/L with 0.74 g/L of total

acids. The solvent yield and productivity of 0.24 g/g and 0.09 g/L.h, respectively were achieved. Slurries obtained from samples, which were dried at higher temperatures (100 °C and 120 °C) showed fairly equal solvent production (5.94 g/L) and yield (0.20 g/g). Similar findings were reported by Avila-Gaxiola et al. [44] during ethanol fermentation using agave tequilana leaves as a feedstock.

Table 4

Performance of ABE fermentation using model P2 and pea pod waste slurries

Sample	Initial sugars (g/L)	Sugar consumed (g/L)	Butanol produced (g/L)	Total ABE (g/L)	Total acids (g/L)	Solvent yield (g/g)
Model P2	60±0.2	36±0.02	4.87	8.80	0.74	0.24
Wet	59.92±2.00	31.64±0.08	2.31	4.80	4.04	0.15
60 °C	57.40±1.70	29.67±0.11	2.76	4.25	4.30	0.14
80 °C	59.67±2.20	29.93±0.05	3.18	5.24	4.92	0.17
100 °C	60.14±1.50	29.61±0.11	3.82	5.94	3.21	0.20
120 °C	60.36±1.00	30.30±0.26	3.59	5.64	4.59	0.18

Results are mean ± SD of three determinations

Table 5 enlist different feedstocks used from agricultural sector for biobutanol production and compares to that with current study.

Table 5

List of selected feedstocks used from agricultural sector for ABE production by using *Clostridia*.

Feedstock	Microorganism	Solvent titer (g/L)	References
Corn stover	<i>C. acetobutylicum</i>	6.26	[50]
Cauliflower waste	<i>C. acetobutylicum</i>	5.35	[51]
Corn fiber	<i>C. beijerinckii</i>	1.70	[52]
Apple pomace	<i>C. beijerinckii</i>	8.32	[53]
Sugar beet pulp	<i>C. beijerinckii</i>	9.00	[54]
Pea pod waste	<i>C. acetobutylicum</i>	5.94	current study

Although, detoxification step was implemented before ABE fermentation, the total solvent production was seemingly low. Hence, detailed fermentation profiles of control and sample experiments were compared which varied significantly after 48h fermentation (Fig. 9). Experiments with detoxified slurries showed higher total acids which ultimately hampered *Clostridial* growth thereby decreased total ABE in fermentation broth. This may be due to the accumulation of higher amounts of acids during fermentation.

Fig. 9. Evaluation of ABE fermentation profiles: ● control; ○ 100 °C dried detoxified slurry

From current investigation, it can be inferred that the pea pod waste has a high potential to be used in biobutanol production with further modifications. Although, ABE solvent production is bit lower when compared with other feedstocks, we are in a continuous process to

improve it further. Simultaneously, efforts will be made to reduce large amount of acids produced during ABE fermentation, either by incorporating genetic engineering aspects or by bioprocess modification. The fed batch and continuous ABE process development studies are also underway to further improve the yield and titers.

4 Conclusions

The utilization of pea pod waste for biobutanol production by using *C. acetobutylicum* B-527 was studied. The drying of pea pod waste resulted into high total sugar release and in turn aided efficient ABE production. The drying kinetics followed first order with respect to moisture content as follows:

$$-r_M = 282.11 \times \exp\left(-\frac{3200}{T}\right)M$$

Dilute acid pretreatment resulted in total sugar release of 48.07 g/L from pea pod waste sample (dried at 100 °C). Activated charcoal detoxification successfully removed phenolics and acetic acid to minimal level. Further, the fermentative production of ABE from detoxified pea pod waste slurry resulted in total solvent of 5.94 g/L with about 50% sugar utilization.

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