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Published in:
Bioprocess and Biosystems Engineering

DOI:
10.1007/s00449-017-1806-y

Published: 01/07/2017

Document Version
Peer-reviewed accepted author manuscript, also known as Final accepted manuscript or Post-print

Please cite the original version:
Khedkar, M. A., Nimbalkar, P. R., Chavan, P. V., Chendake, Y. J., & Bankar, S. B. (2017). Cauliflower waste utilization for sustainable biobutanol production: revelation of drying kinetics and bioprocess development. *Bioprocess and Biosystems Engineering*, *40*(10), 1493–1506. https://doi.org/10.1007/s00449-017-1806-y

Cauliflower waste utilization for sustainable biobutanol production: revelation of drying kinetics and bioprocess development

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Abstract

Efficient yet economic production of biofuel(s) by using varied second-generation feedstock needs to be explored in current scenario to cope up with global fuel demand. Hence, present study was performed to reveal the use of cauliflower waste for acetone-butanol-ethanol (ABE) production using *Clostridium acetobutylicum* NRRL B 527. The proximate analysis of cauliflower waste demonstrated to comprise 17.32 % cellulose, 9.12 % hemicelluloses, and 5.94 % lignin. Drying of cauliflower waste was carried out in the temperature range of 60-120 °C to investigate its effect on ABE production. The experimental drying data was simulated using moisture diffusion control model. The cauliflower waste sample dried at 80 °C showed maximum total sugar yield of 26.05 g L^{-1}. The removal of phenolics, acetic acid and total furans were found to be 90-97 %, 10-40 %, and 95-97 %, respectively. Incidentally, maximum ABE titer obtained was 5.35 g L^{-1} with 50 % sugar utilization.

Keywords: Biobutanol, Cauliflower waste, Detoxification, Drying, Fermentation

1. Introduction

The present global economy and energy necessities mainly rely on fossil fuels. However, increasing world population and growing number of motor vehicles cause hyper-consumption of non-renewable energy resources that resulted in rapid exhaustion of petroleum fuels. The use of fossil fuels accelerates the amount of toxic gasses in environment. Hence, it focuses on some environmental concerns such as global warming that deleteriously affect human health. The menacing environmental problems can be mitigated by taking ample efforts in development of alternative energy sources, such as use of biofuels [1, 2]. Biobutanol is one of the potential biofuels that has recently received considerable attention in scientific and industrial sectors [3, 4]. Furthermore, butanol being gasoline additive has superior chemical properties over ethanol and methanol and can be blended in different ratio with gasoline unlike bioethanol. Generally, biobutanol is produced along with acetone and ethanol by using *Clostridium* spp. under strict anaerobic condition and commonly referred as acetone-butanol-ethanol (ABE) process. In ABE process, the cost of feedstock plays an influential role and contributes around 60-70 % of final

product cost [5]. Therefore, a cheap substrate source such as lignocellulosic biomass is continuously being encouraged as substitute to produce biofuels. Lignocellulosic biomass is environment and economically feasible due to its abundant availability and non-food nature [1].

India being a farming country generates huge amount of waste biomass (approximately 400 million tons per year) that can be utilized to produce valuable biochemicals [6]. Along with agriculture, the waste generated during vegetable processing is also considered for biofuel production. India ranks second in the world for cauliflower production and accounts 35 % of total global production having a production capacity of 7.89 million tons per year. However, during harvesting, processing, and marketing of cauliflower generates almost 45-60 % (w/w) cauliflower waste [7]. Dumping of cauliflower waste (a non-edible portion of cauliflower) is always a critical problem [8]. Such a huge amount of waste has been dumped as city waste on a dumping ground, which ultimately causes environmental pollution. Additionally, the incineration of waste biomass contributes to rise in atmospheric CO_2 level, which is harmful to living society [9, 10]. Therefore, it would be worthwhile to utilize vegetable waste for biobutanol production which can further help to reduce environmental concerns and also supports to strengthen the nation's economy, by producing new job opportunities [10].

The proximate analysis of cauliflower waste reported by Oberoi et al. [8] shows that, it composed of 16.6 % cellulose, 14.9 % crude protein, 8.4 % hemicellulose, 17 % total sugars, 6.25 % phenolics, 14 % ash and 10 to 20 % minerals on dry basis. Besides, some reports are available, wherein cauliflower waste has been utilized for production of protease [11] and garbage enzymes [12]. Babbar et al. [13] and Gonzales et al. [14] studied the nutritional evaluation, utilization, and influence of different solvents during extraction of phenolics and polyphenols from cauliflower waste. Additionally, the improvement of non-extractable phenolics from cauliflower waste was also studied by Gonzales et al. [15]. Similarly, other studies are also available in the extractions of antioxidants and biochar production from cauliflower waste [16-20].

Vegetable residues including cauliflower waste contain high level of moisture, which may be the hindrance for its further processing [21]. Ribeiro et al. [22] reported moisture content in cauliflower waste to be 90-95 % on wet basis. The high water activity of cauliflower waste can be lowered by subjecting it to drying operation which further reduces the chances of microbial spoilage and deterioration during chemical reaction [23]. Additionally, the drying operation reduces mass and volume of cauliflower waste to ease its storability under ambient temperature, which in turn reduces transportation cost considerably. Moreover, drying of cauliflower waste also prevents dilution effect during pretreatment studies. Although drying of cauliflower waste magnifies its significance, no study is reported till date to the best of author's knowledge, which explores the impact of drying temperature on saccharification and biobutanol production using cauliflower waste as feedstock.

Agricultural residues mainly composed of complex carbohydrate (cellulose and hemicellulose) structure. Hence, a pretreatment step is essential to break the complex structure to further release simple sugars. However, pretreatment processes are prone to form various inhibitors such as furfural, hydroxyl methyl furfural (HMF), ferulic and p-coumaric acids, which can affect the microbial growth during ABE fermentation. Hence, detoxification is an additional step needed to be carried out to remove such inhibitors to improve final product titer [2, 5].

Based on aforementioned discussion, the present investigation was carried out to produce biobutanol using cauliflower waste as a feedstock. The objective of current study was categorized into three parts *viz.* (i) proximate analysis and drying kinetics of cauliflower waste, (ii) pretreatment and detoxification process to obtain fermentable sugars, and (iii) fermentative

production of biobutanol. An emphasis was given on drying study to investigate the effect of dried material on ABE fermentation. Further, the mathematical model was developed to predict moisture content of cauliflower waste with respect to time during drying operations. Subsequently, batch fermentation studies were conducted using *C. acetobutylicum* NRRL B 527 to compare and suggest the feasibilities of drying experiments for solvents production.

2. Materials and Methods

2.1 Materials

All the chemicals and materials used during this study are reported in Table 1.

2.2 Cauliflower waste as feedstock

Fresh cauliflowers were collected from the local vegetable market of Pune, Maharashtra, India. The cauliflowers were cleaned and cut using stainless steel knife. The non-edible portion, comprising upper stem, stalks, and leaf midribs, was further cut into small pieces and ground using laboratory grinder (Indica, Power 750W). The chopped biomass was squeezed to remove maximum possible water content from it. Subsequently, half part was dried at 60 °C in hot air oven for proximate analysis and rest of the portion was stored at 4 °C for drying kinetic studies.

2.3 Proximate analysis of cauliflower waste

Proximate analysis of cauliflower waste was carried out on a dry basis using standard reported methods to estimate the composition for further work. Cauliflower waste dried at 60 °C was used to determine the ash content using a method reported by Sluiter et al. [24]. Furthermore, cellulose and hemicellulose content in biomass was determined by Anthrone method [25], and method reported by Gao et al. [26], respectively. The lignin content was determined by Klason method [27], and fat content was determined using method reported by Sluiter et al. [28]. Analysis of moisture, crude fiber, lipid, and total protein content of the cauliflower waste was accomplished as per methods reported by Baloch et al. [29].

2.4 Drying of cauliflower waste

2.4.1 Experimental setup

Drying studies were conducted to investigate the effect of temperature and time on drying characteristics of cauliflower waste. The experiments were carried out in hot air tray oven (Bio-Technic BIT-30, India; 400×380×200 mm) with the variable temperature controller. Cauliflower waste sample prepared as mention in section 2.2 was weighed (100±0.2 g) and used for drying experiments in the temperature range of 60-120 °C with thickness layer of 10 mm. The mass and layer thickness were measured for all the temperatures studied, after every 10 min. The frequency of measurement was increased to 60 min when sample weight started to approach the constant reading. The calculations of water content, moisture ratio, and drying rate were performed with the help of data received. All the experiments were carried out at least in triplicate and results reported are an average ± standard deviation.

2.4.2 Drying calculation

The moisture content of cauliflower waste obtained for a given time by the following expression:

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

where M is the moisture content of given time (g g^{-1} 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 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 of a given time (g g^{-1} dry solid), M_o is the initial moisture content (g g^{-1} dry solid), M_e is the equilibrium moisture content (g g^{-1} dry solid). The experimental drying rate was determined using the 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 in min, M_{t1} and M_{t2} are moisture contents of cauliflower waste at time t_1 and t_2, respectively.

2.4.3 Simulation of drying data

The moisture is believed to be transported to the surface of a material by several mechanisms like molecular diffusion, capillary motion, liquid diffusion through solid pores, etc during drying operations. However, none of these mechanisms prevails throughout the drying process. Nonetheless, the mechanisms of diffusion have been commonly used to interpret experimental observation using Fick's second law of diffusion:

$$D_{eff} \frac{\partial^2 M}{\partial y^2} = \frac{\partial M}{\partial t} \qquad \text{for } 0 \le y \le L \quad (4)$$

with the following boundary condition:

$$\frac{\partial M}{\partial y} = 0 \qquad \text{in } z = L \quad (5)$$

where M is the moisture content of sample for a given time (g g^{-1} of dry solid), t is the drying time (s), y is the spatial dimension (m), and D_{eff} is the effective moisture diffusivity (m^2 s^{-1}).

The moisture transport takes place due to the concentration gradient within the solid bed, where the concentration is low on a surface, while it is high at the bottom of the solid bed. Effective diffusivity (D_{eff}) considers the change in volume, shape, and texture as well as change in chemical composition of a drying material.

It is a common practice [30] to rewrite Eq. (4) in terms of following dimensionless groups:

$$\tau = \frac{D_{eff} t}{L^2}, \ \xi = y/L, \text{ and } \Psi = \frac{M - M_e}{M_0 - M_e} \quad (6)$$

Rewriting Eq. (4) in terms of dimensionless groups (5) yields:

$$\frac{\partial^2 \Psi}{\partial \xi^2} = \frac{\partial \Psi}{\partial \tau} \qquad \text{for } 0 \le \xi \le 1 \quad (7)$$

$$\frac{\partial \Psi}{\partial \xi} = 0 \qquad \text{in } \xi = 0 \text{ and } \psi = 0 \text{ in } \xi = 1 \quad (8)$$

The analytical solution to Eq. (6) can be obtained by two methods *viz.* (i) method of separation of variables and (ii) Laplace transform if the following assumptions made: (i) moisture movement is uni-dimensional, (ii) initial moisture distribution is uniform, (iii) no chemical reaction takes place during drying operation such as thermal and chemical properties of material, (iv) constant moisture diffusivity with negligible shrinkages, and (v) negligible external resistances to moisture transfer and isothermal process. The step-wise derivation can be found elsewhere [30].

The analytical solution of Eq. (6) is as follows:

$$\Psi = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\left(\frac{\pi^2 D_{eff}\,t}{4L^2}\right)\right] \tag{9a}$$

OR

$$MR=\frac{M}{M_0}=\frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\left(\frac{\pi^2 D_{eff}\,t}{4L^2}\right)\right] \tag{9b}$$

where L is the half layer thickness of samples (m), and n is the positive integer. The value of M_e can be considered small as compared to values of M and M_0. Therefore, M_e can be conveniently assumed equal to zero. Eq. (9) is an algebraic equation which could be solved by successive approximation to determine D_{eff}. The solution of algebraic equation is simple as compared to numerical solution of partial differential equation by finite differences or finite elements. The series of Eq. (9) converges at the first term when MR < 0.7 [30, 31, 32]. Therefore, the first term of expansion series of Eq. (9) was only considered to determine effective diffusivity (D_{eff}) while other terms were neglected.

$$MR=\frac{M}{M_0}=\frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff}\,t}{4L^2}\right) \tag{10}$$

The values of D_{eff} can be obtained for a given temperature by plotting *ln* MR versus drying time. Slope (m) of a straight line gives value of D_{eff} as follows:

$$D_{eff}=\frac{m\times 4L^2}{\pi^2} \tag{11}$$

Effect of temperature on effective diffusivity can be expressed using Arrhenius equation:

$$D_{eff}=D_0\exp\left(-\frac{E}{RT}\right) \tag{12}$$

where D_0 is the pre-exponential factor of Arrhenius equation($m^2\ s^{-1}$), E is the activation energy (J mol^{-1}), R is the universal gas constant (J $mol^{-1}\ K^{-1}$), and T is drying temperature (K). The E and D_0 can be estimated from slope and intercept of the plot of lnD_{eff} versus T^{-1}.

Another approach to study drying kinetic is also widely reported in the literature wherein drying rate is directly expressed as a function of moisture content using "thin layer equation"

$$-r_M=-\frac{dM}{dt}=kM \tag{13}$$

where r_M is the rate of drying and k is a drying constant (s^{-1}). Eq. (9) upon integration between initial and mean moisture content at time t yields:

$$\frac{M}{M_0}=\exp(-kt) \tag{14}$$

Comparing Eq. (6) and (10), the drying constant, k, can be related to moisture diffusivity as follows:

$$k=\frac{\pi^2 D_{eff}}{4L^2} \tag{15}$$

where the term $8/\pi^2$ is considered equal to unity in order to relate drying constant to effective diffusivity. Substituting Eq. (11) into Eq. (9) and using Eq. (8), we get:

$$-r_M = -\frac{dM}{dt} = AD_0 \exp\left(-\frac{E}{RT}\right)M \quad (16)$$

where A is equal to $\pi^2/4L^2$. Eq. (12) combines Fick's second law of diffusion and thin layer equation to express the rate of drying as a function of moisture content and temperature.

2.4.5 Statistical Analysis

The goodness of fit of proposed drying model to the experimental data was assessed using statistical parameters *viz*. coefficient of determination (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 indicate the best fit of the model. Values of statistical parameters are determined as follows:

$$SSE = \frac{1}{N}\sum_{j=1}^{N}\left(M_{exp,j} - M_{pred,j}\right)^2 \quad (17)$$

$$\chi^2 = \frac{\sum_{j=1}^{N}\left(M_{exp,j} - M_{pred,j}\right)^2}{N-z} \quad (18)$$

where $M_{exp,j}$ and $M_{pred,j}$ are experimental and predicted values of j^{th} moisture content, respectively. N is the number of experimental runs and z number of constants.

2.5 Pretreatment of cauliflower waste and detoxification of hydrolysates

The pretreatment of sun dried cauliflower waste was performed in an autoclave at 121 °C for 60 min, with constant solid to liquid ratio of 1:10 (g mL^{-1}) using 2 % (v/v) sulfuric acid, 2 % (v/v) hydrochloric acid, and 2 % (w/v) sodium hydroxide for selection of suitable acids and/or alkali. Furthermore, cauliflower waste samples dried at different temperatures during drying step and non-dried (wet sample) sample of 5.5 g (equivalent to 1 g dried sample) were also pretreated for 15 min using 2% (v/v) sulfuric acid under aforesaid pretreatment parameters. The hydrolysates obtained after pretreatment were analyzed for total sugar release and for presence of inhibitors. Subsequently, detoxification of cauliflower waste hydrolysates was carried out with modification in method reported by Hodge et al. [33]. The pH of hydrolysates was adjusted to 10 with sodium hydroxide solution followed by 5 % activated charcoal treatment. The activated charcoal treatment was continued for 2 h at 200 rpm and at 60 °C. This mixture was then filtered, and the filtrate was used for batch fermentation studies. Total phenolics, total furans, and total acids were analyzed simultaneously before and after detoxification for non-dried and dried cauliflower waste hydrolysates. All the experiments were carried out at least in triplicate and results reported are an average ± standard deviation.

2.6 Production of ABE

2.6.1 Organism and inoculum preparation

C. acetobutylicum NRRL B-527 was generously gifted by Agriculture Research Services (ARS) Culture Collection, USA. Lyophilized cells were regenerated by using sterile revival medium (RM) according to ATCC 1207 protocol, and spore suspension was stored at a dry place for further studies. Reinforced clostridia medium (RCM) reported by Bankar et al. [34] was used as a growth medium, and heat shock treatment was carried out to activate the cells. 2 % (v/v) activated spore suspension was inoculated into 100 mL sterile RCM medium, and grown for 18-20 h. The 5 % (v/v) actively growing cells (with the cell density of OD_{560} 1.32) were inoculated into a production (P2) medium and hydrolysates after 20 h. The P2 medium reported by Bankar et al. [35] was used as control in the present study.

2.6.2 Batch fermentation

Batch fermentation studies were conducted in 100 mL air tight screw cap bottles containing 80 mL working volume. A control experiment with P2 medium was run for 96 h to produce ABE solvents [36]. After detoxification, each hydrolysates were diluted two fold and supplemented with other nutritional components of the P2 medium except glucose. The final total sugar was adjusted to 60 g L^{-1} by using auxiliary glucose for better comparison with other experiments. Medium pH was adjusted to 6.5 and purged with nitrogen to maintain anaerobic conditions. The bottles were sealed using aluminum cap and sterilized at 121 °C for 20 min and cooled. Subsequently, bottles were inoculated with 5% (v/v) actively growing cell suspensions of 20 h old inoculum and incubated at 37 °C for 96 h. Samples were collected and analyzed after fermentation for sugar consumption, ABE, and total acid production.

2.7 Analytical method

Solvents (ABE) and acids (acetic and butyric) were measured using gas chromatography (Agilent Technologies 7890 B) equipped with a flame ionization detector, and AB-INNOWAX capillary column (30m×0.32mm×1μm). The total sugar was analyzed by phenol sulfuric acid method [37]. To find out the total phenolic content (before and after detoxification), a diluted sample (1 mL) was mixed with 1 mL Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 0.8 mL aqueous sodium carbonate (20 %). The solution was allowed to stand for 30 min, and absorbance was measured at 765nm [38]. The standard graph was prepared, and the total phenolic content in a sample was calculated as gallic acid equivalents. Besides, the total furans (Furfural and 5-hydroxymethyl furfural) were estimated using a spectroscopic method based on difference in the absorbance values obtained at 284 and 320nm [39]. Furthermore, the morphological changes in the biomass were monitored using scanning electron microscopy (SEM) (JSM-6380LA, JEOL, Japan). Samples were adhered to carbon tape and sputter-coated with platinum and images were taken at an acceleration voltage of 5 kV. Furthermore, the sun dried and hot air oven dried cauliflower biomass was also characterized by Fourier Transform Infrared Spectroscopy (FTIR) from Bruker in the range of 500-4000 cm^{-1} with a resolution of 2 cm^{-1}. The data obtained was analyzed using Opus software.

3 Result and discussion

3.1 Feedstock and proximate analysis of cauliflower waste

Proximate analysis of cauliflower waste showed that it is a rich source of cellulose (17.32 %), hemicellulose (9.12 %), and lignin (5.94 %). Besides, other compositions such as crude fiber, fat, and total protein content were also analyzed and reported in Table 2. These results are in good agreement with other reports [8].

3.2 Drying of cauliflower waste

Higher moisture content (80-85 % on wet basis) in cauliflower waste causes microbial spoilage and foul odor after storage. Therefore, drying of cauliflower waste was carried out in a temperature range of 60-120 °C to investigate the drying effect on total sugar release as well as on ABE fermentation process. The experimental moisture content profiles as a function of time are shown in Fig.1. It is evident that the moisture content of cauliflower waste decreases exponentially with time. Drying temperature also shows a significant effect on the moisture content of cauliflower waste. The results showed that drying time decreased substantially when drying

temperature was increased. For example, the drying times required to reach the moisture content of 0.15 g g^{-1} of dry solids were 230, 120, 100, and 60 min at the drying temperature of 60, 80, 100, and 120 °C, respectively. These findings are in good agreement with other reports on agricultural by-products [40-42].

The experimental drying data was converted into moisture ratio and plotted against time to estimate values of drying rate constants for a given temperature (Fig. 2). Fig. 2 shows that Eq. (14) fits well to the experimental data for a given temperature. This confirms that the rate of drying follows the first order with respect to moisture content of the material. The values of effective diffusivities were obtained using Eq. (16) wherein the term $8/\pi^2$ was considered equal to unity. Nevertheless, the term $8/\pi^2$ was considered while estimating values of D_{eff}. Furthermore, it was observed that the experimental values match well with predicted values with percentage deviation of 3.92 %.

The values of effective diffusivity vary between 1.05×10^{-8} m^2 s^{-1} to 2.5×10^{-8} m^2 s^{-1} for temperature range of 60-120 °C. It was observed that effective diffusivity increased with an increase in drying temperature. The increasing effective diffusivity with an increase in drying temperature has reported by Mudgal and Pandey [43] which is good agreement with our results. When drying was carried out at a higher temperature, a greater proportion of heat energy was utilized to increase the activity of water molecules leading to higher moisture diffusivity. Table 3 shows that effective diffusivity was doubled with an increase in temperature from 60 to 120 °C. The temperature dependence of effective diffusivity can be illustrated using Eq. (13). The graph was plotted for effect of drying temperature on effective diffusivity (Fig. not shown). The slope and intercept of a straight line give a quantitative estimation of activation energy and pre-exponential factor, respectively. The values of activation energy and pre-exponential factor were found to be 15.08 kJ mol^{-1} and 2.38×10^{-6} m^2 s^{-1}, respectively. These results are in close agreement with reports from Vega-Gálvez et al. [42] for gooseberry and dry vegetable waste.

The moisture content of cauliflower waste can now be predicted when values of effective diffusivity are used in Eq. (10) for a given temperature. As seen from Fig. 2, the predicted values of moisture content match well with experimental data, confirming the validity of Eq. (10). Moreover, the rate of drying can also be predicted using following expression since Eq. (10) presumes that kinetics of drying follows first order to moisture content:

$$-r_M = -\frac{dM}{dt} = kM \tag{20}$$

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

$$-r_M = 3.53\times\exp\left(-\frac{1815}{T}\right)M \tag{21}$$

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 $\chi 2$ reported in Table 3 indicate that predicted data matches well with experimental data (Fig. 2). The close fitting of data showed the drying kinetics to follow first order dependence on the moisture content.

3.3 Pretreatment of cauliflower waste and detoxification of hydrolysates

Pretreatment play a key role in process efficiency since it disrupts cell wall physical barriers and renders cellulose more accessible. It also helps to alter recalcitrant structure and to

obtain high yield of sugar for bioconversion processes. The pretreatments can be physical, physicochemical or chemical with objective to obtain a high monosaccharide yield along with minimal inhibitor generation [1-2]. A well-known pretreatment method is dilute acid and/or alkali hydrolysis, which selectively affect hemicellulose structure. According to literature reports [35, 44] and our previous experience, sun dried cauliflower waste was subjected to acid (2 % (v/v) sulfuric acid, 2 % (v/v) hydrochloric acid) and/or alkali (2 % (w/v) sodium hydroxide) treatment to evaluate the type of catalyst for higher total sugar release. From pretreatment results, it was observed that sulfuric acid treatment resulted in highest total sugar release of 27.11±0.73 g L^{-1}, followed by hydrochloric acid (23.29±0.3 g L^{-1}), and sodium hydroxide (16.76±0.1 g L^{-1}) (Fig. 3). Hence, 2 % (v/v) sulfuric acid was selected to further treat different oven dried cauliflower waste samples along with non-dried (wet) sample to estimate the effect of drying temperature on total sugar release. Interestingly, samples dried at 60 °C and 80 °C showed highest total sugar release of 25.18±0.6 g L^{-1} and 26.05±0.2 g L^{-1}, respectively. On the other hand, increasing drying temperature to 100 °C and 120 °C resulted in total sugar release of 18.34±0.1 g L^{-1} and 17.67±0.3 g L^{-1}, respectively. As expected, non-dried (control) sample even after acid treatment was able to release lower total sugar of 14.41±0.1 g L^{-1}. Possible reason could be the presence of high moisture content, which either affected acid concentration used during treatment or caused microbial spoilage during storage. Baloch et al. [29] reported that drying of cauliflower is effective in the conservation of chemical composition and deterioration prevention as a result of decreased moisture content. Mudgal and Pande [43] reported the decrease in the carbohydrate content with the increasing drying temperature from 60-90 °C. The carbohydrate content in cauliflower samples ranged from 3.25 to 3.45 g per 100 g as against 5.40 g per 100 g in fresh vegetable indicating a significant loss of nutrients during processing. Furthermore, Avila-Gaxiola et al. [45] studied the effect of drying on agave leaves and reported that when drying temperature was increased beyond 100 °C, resulted in Maillard reaction by turning leaves to be brown in colour.

The pretreatment data of all dried and non-dried (control) cauliflower waste samples are summarized in Table 4. It was observed that samples dried at high temperature (100 °C and 120 °C) accounts for lower total sugar release as compared to samples dried at lower temperature (60 °C and 80 °C). This could be due to the browning of sugars and/or Maillard reaction, which further affected total sugar release. Besides, total sugars obtained from dried samples are more than non-dried sample. This may be due to positive unpredictable structural changes in cauliflower waste samples during drying operation. Therefore, different cauliflower waste samples *viz.* sun dried, dried at 80 °C and acid treated were characterized by using Scanning Electron Microscope (SEM), to confirm the morphological changes during drying and pretreatment process (Fig. 4). As shown in Fig. 4 A., the sun dried sample showed much flatter, smoother and compact surface with quite wrinkly appearance. The partial disorganization with wavy structure for cauliflower waste dried at 80 °C was verified in Fig. 4 B. On the other hand, acid treated cauliflower waste showed corrugated and broken surface, revealing the disruption of highly ordered structure (Fig. 4 C.). Su et al. [46] also observed morphological changes during treatment had a significant effect on total sugar release and reported high reducing sugars of 79.8 % for hydrolyzed bagasse.

The changes in hemicellulose and cellulose structure of cauliflower waste were also investigated using Fourier Transform Infrared (FTIR) spectroscopy for dried and non-dried samples. It also provides information on the structure of lignin and hydrogen-bonding changes within complex biomass. Table 5 shows the FTIR peaks and their assignments which are based on literature values of functional group in the biomass [47-49] for the following treatment: cauliflower waste dried in the range of 60-120 °C, and sun dried (non dried sample - for better comparison,

material was dried at room temperature labeled as sun dried to avoid the moisture band in the spectra) (Fig.5.).

FTIR spectra of sundried cauliflower waste showed a band at 890 cm^{-1}, which represent β-(1-4) glycosidic linkages of cellulose and is attributed to amorphous cellulose. Compared to the cauliflower waste dried at 120 °C, the absorbance of amorphous cellulose was increased after conventional drying. The frequency ranges of 1200-1000 cm^{-1} can be attributed to contributions of hemicellulose and cellulose having maxima at 1040 cm^{-1} due to the C-O stretching mode and 1165 cm^{-1} due to the asymmetrical stretching C-O-C. The band absorption at 1247 cm^{-1} arises due to C-O stretching. This absorption region indicates features of hemicellulose as well as of lignin [44, 49]. The band at 1247 cm^{-1} shows a minor change in hemicellulose through drying operation for sun dried and oven dried samples (60, 80, 100, and 120 °C). The band intensity was less for cauliflower waste dried at 100 and 120 °C as compared to the others giving indication of lowered total sugar. From composition analysis of hydrolysates (Table 4), it can be observed that drying of cauliflower waste at 60 and 80 °C yielded maximum solubilization of hemicellulose after pretreatment. Furthermore, the wavelength region of 1650-1500 cm^{-1} and 1460-1400cm^{-1} reflects aromatic skeletal vibrations and aromatic skeleton of C-H plane deformations in lignin, respectively. The cauliflower waste samples dried at 100 and 120 °C showed lower absorbance values implying aromatic deformations in lignin. From the compositional analysis also (Table 4), it can be observed that drying of cauliflower waste at 100 and 120 °C yielded slightly higher total phenolics. Overall, from compositional analysis and SEM analysis, the reason for higher sugar release seems to be due to changes in the hemicellulose structure as well as deformation in lignin structure at the lower temperature. This hypothesis was also confirmed by FTIR spectra.

Dilute acid pretreatment is known to form inhibitors at high temperature and pressure that negatively affects ABE fermentation. The inhibitors formed during the hydrolysis include total phenolics, acetic acid, furfural, and 5-hydroxy methyl furfural (HMF). The total phenolics were calculated by standard curve equivalent to gallic acid as shown in Fig. 6. In current study, the inhibitors formed in pretreatment process were analyzed which includes total phenolics and acetic acids in the range of 1.4-2.0 g L^{-1} and 1.5-1.7 g L^{-1}, respectively. Besides, the furfural and HMF were also formed in negligible amount (1-1.3 mg L^{-1}) during the process. Activated charcoal detoxification is widely used treatment to remove inhibitors from the hydrolysates by adsorption mechanism. However, the effectiveness of activated charcoal treatment depends on various other factors such as pH, temperature, contact time, and activated charcoal concentration used during process. Mussatto and Roberto, [50] studied the effect of temperature on inhibitor removal during detoxification and reported that as temperature increases; detoxification efficiency enhances till 60 °C and then become constant thereafter. Besides, reports are available wherein researchers made an attempt to optimized detoxification parameters like temperature of 60 °C, time of 2 h, pH 9.0, and activated charcoal concentration within range of 1-5% [50, 51]. Hence, in current study the hydrolysates were detoxified using activated charcoal treatment at 60 °C. The quantification of inhibitors before and after detoxification is listed in Table 4. Based on results, it was observed that activated charcoal detoxification significantly removed total phenolics (more than 90 %) from samples. However, acetic acid concentration was slightly lowered (10-40 %) in all hydrolysates with up to 10 % total sugar loss. Interestingly, more than 98 % of total furans (furfural and HMF) were removed using activated charcoal treatment. Similar results were reported by Qureshi et al. [52] where the pre-adjustment of pH to 10 decreased the inhibitor concentration by 22 % and 20 % for both furfural and HMF, respectively. Additionally, Yamamoto et al. [53] reported the use of activated charcoal to reduce the inhibitor concentration and further improve the ABE fermentation

efficiency. Another study by Nilsson et al. [54] investigated the application of Birch Kraft black liquor hydrolysates for ABE production and found activated charcoal treatment to significantly improve the butanol concentration from 0 to 2.1 g L^{-1}. The study by Hodge et al. [55] reported the use of activated carbon to decrease the inhibitor concentration of 86-96 % of HMF and total phenolics after detoxification and resulted in improved succinate fermentation to be 9.9 g L^{-1}.

3.4 ABE batch fermentation

The hydrolysates obtained from cauliflower waste samples which were dried at different temperatures followed by acid hydrolysis and detoxification were used in batch fermentation of *C. acetobutylicum* NRRL B-527 to produce biobutanol. The additions of nutrients were essential in detoxified slurries for growth and solvent production [56]. Hence, all the hydrolysates were supplemented with other nutritional components similar to P2 medium to get optimum quantity of ABE solvents. Simultaneously, sufficient amount of sugars are also crucial for better comparison and also to avoid substrate deficiency. Therefore, pure glucose was added in the detoxified hydrolysates with sugar adjustment to be around 60 g L^{-1}. Harde et al. [36] did similar studies using *forskolin* root hydrolysates to produce ABE solvents after 96 h batch fermentation. Hence, current study was carried out at batch level for 96 h and samples were analyzed for solvent production and substrate utilization. The total ABE and acids produced with all hydrolysates are summarized in Table 6. Simultaneously, control (P2) experiment using glucose as substrate was also performed wherein the total solvents and total acids were obtained were 12.29 g L^{-1} and 2.53 g L^{-1}, respectively.

For non-dried detoxified slurries, the total ABE production was obtained to be 4.31 g L^{-1} with a yield of 0.12 g g^{-1}. While, the highest total solvent of 5.31 g L^{-1} and yield of 0.17 g g^{-1} were achieved when the samples were dried at 80 ºC. Therefore, it can be inferred that the total solvent and yield of dried samples were higher when compared with non-dried sample that signifies 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 ºC resulted in total ABE production and yield of 3.62 g L^{-1} and 0.14 g g^{-1}, respectively. Additionally, sample dried at 60 ºC showed total ABE production and yield of 4.21 g L^{-1} and 0.16 g g^{-1}, respectively. The reason behind increment in solvent production at lower drying temperature is still unclear. Further, the consumption of total sugars during fermentation was found to be in the range of 40-50 % (w/v) and can be seen in Table 6. Therefore, drying of cauliflower waste is efficient at low temperatures (60-80 ºC), which showed positive effect on sugar release as well as on ABE fermentation. Oberoi et al. [8] also investigated the effect of different drying methods on glucoamylase production by using dried cauliflower waste as a feedstock, and reported highest glucoamylase production for biomass dried at 50 °C (44 U mL^{-1}). They also observed lower enzyme production in samples dried at higher temperatures (100 °C). In addition, the results of current study are also compared with other literature reports when different feedstock materials are used to produce biochemicals as shown in Table 6. Hence, it can be concluded that the current study is highly comparable with other published reports for ABE production, when different strains of *Clostridia* including*, C. acetobutylicum* ATCC 824*, C. acetobutylicum* XY16 and *C. acetobutylicum* DSM 792 have been used [36, 57, 58].

Table 6 also showed that almost 4 g L^{-1} of total acids (acetic and butyric acids) was observed after the fermentation, which ultimately affects the ABE production. Hence, selectivity of ABE was calculated as the total ABE produced with respect to the total acids produced. The selectivity for

non-dried sample and sample dried at 80 °C was 0.895 and 1.20, respectively. Cho et al. [59] reported that the weak acids (lower concentrations) are more toxic to *C. acetobutylicum,* influencing the total ABE production. Ezeji et al. [2] reported that 0.3 g L^{-1} p-coumaric and ferulic acids were inhibitory to *C. beijerinckii* BA101 and decreased ABE production significantly. Cauliflower waste has also been utilized along with cane molasses for the production of ethanol [7]. Another report by Sharma et al. [10] reviewed the utilization of cauliflower waste as a substrate for production of industrially suitable enzymes such as cellulases, amylases, pectinases, and others.

The present study reveals that the drying of cauliflower waste eventually aids the higher sugar release through pretreatment and improved solvent production. Therefore, ABE fermentation process provides evident of cauliflower waste exploitation for large-scale production with additional modification in bioprocess. Valorizations of cauliflower waste sample for large-scale production require typical butanol production modifications such as fed-batch, or continuous mode with *in-situ* solvent recovery module. Therefore, the efforts to improve ABE yield and productivity will be targeted in near future by reducing inhibitory components present in a production medium. Further, higher amount of acids that are produced during ABE fermentation will also be addressed either by modified cultivation methods (co-culturing) or by genetic engineering aspects. Besides, efficient yet economic bioprocess is also desirable for biobutanol large-scale production. Hence, there is an extensive need to improve the ABE yields and productivities by an economical way, to foresee its future commercialization.

4. Conclusions

The effect of cauliflower waste drying on total sugar release and subsequent ABE production by using *C. acetobutylicum* NRRL B-527 was investigated in current study. The drying kinetics followed first order kinetics with a following drying rate expression:

$$-r_M = 3.53 \times \exp\left(-\frac{1815}{T}\right) M$$

Pretreatment with 2 % (v/v) sulfuric acid resulted in total sugar release (g L^{-1}) of 25.18, 26.05, 18.34 and 17.6 from cauliflower waste, which were dried earlier at 60, 80 100 at 120 °C, respectively. Subsequently, detoxification treatment efficiently removed phenolics, and total furans to be 90-97 % whereas the removal of acetic acid was in the range of 10-40 % with acceptable sugar loss. The detoxified hydrolysate of non-dried and dried cauliflower waste (at 80 °C) resulted in total solvent production to be 4.3 and 5.3 g L^{-1} with ABE yield of 0.12 and 0.17 g g^{-1}, respectively. The highest yield of 0.17 g g^{-1} with 34 % improvement in ABE yield was obtained after drying compared with non-dried sample. The selectivity of ABE against acids for P2 and cauliflower waste dried sample (80 °C) was also found to be 4.8 and 1.2, respectively. Moreover, the effect of drying on morphological changes, and chemical composition in samples were also verified by SEM and FTIR analysis. The FTIR bands interrelated with (minor changes) band intensity of cellulose, hemicellulose and lignin during drying operation we also studied. In conclusion, although the total solvent and yield from this study are inadequate, the attempt to improve final product titre is underway to check its feasibility at large-scale production.

Acknowledgment

The authors gratefully acknowledge Department of Science and Technology (DST) of Ministry of Science and Technology, Government of India, for providing financial support under the scheme of DST INSPIRE faculty award, (IFA 13-ENG-68/July 28, 2014) during the course of this investigation. Authors are also thankful to Radhika Malkar and Manoj Kamble from Institute

of Chemical Technology, Mumbai for their help in SEM analysis. Authors are also thankful to Akshay Khade from Bharati Vidyapeeth Deemed University College of Engineering, Pune, India, for his help in FTIR analysis.

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Figure legends

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 different temperatures

Fig. 3. The effect of acid and alkali on cauliflower waste to obtained maximum total sugar release

Fig. 4. SEM images of cauliflower waste after drying and preatreatment: A: sun dried (control) sample; B: sample dried at 80 °C; C: acid treated sample

Fig. 5. The FTIR spectra of cauliflower waste for dried (60 - 120 °C) and non-dried samples

Fig. 6. The standard curve of total phenolic estimation using Folin–Ciocalteu reagent

Table 1 Material used for experimentation and its details
NA- Indicates % purity data is not reported for this material

Materials	Make
Sodium acetate (98% purity), Phenol (99%)	Sigma-Aldrich, India
Hydrochloric acid (36%), Activated charcoal (NA)	Avra Pvt Ltd., India
Thiamine (98.5%), Sodium potassium tartarate (99%) Sulphuric acid (98%)	SRL, India Thomas Baker, India
Sodium carbonate (99%)	Merck Pvt. Ltd., India
Folin Ciocalteu (99.9%), Starch soluble (98%), Brilliant blue dye (99%), Gallic acid (98%), Mangnase sulphate (98%)	HPLC, Lab reagent, India
Peptone (NA), Yeast extract (NA), Meat extract (NA) Sodium chloride (99.9%), Magnesium sulphate (99%), Isopropanol (99%), Iron sulphate (98%), Sodium hydroxide (99%), Biotin (98%), L-cysteine hydrochloride (99%), n-Butanol (99.5%), Acetone (99.9%), Ethanol (99.9%), Butyric acid (99.8%), Acetic acid (99.8%), Glucose (99.5%), Potassium dihydrogen phosphate (99%), Dipotassium hydrogen phosphate (99%), Ammonium acetate (98%), P-aminobenzoic acid (98%), Glycerol (99%)	Himedia Pvt. Ltd. India Sisco Research Laboratories Pvt. Ltd.(SRL) – India

Table 2 Proximate analysis of cauliflower waste

Parameters (%)	Current study	Baloch et al. 2015 [29]	Dhillon et al. 2007 [7]
*Moisture content	81.1±0.21	90.62	85.5
#Moisture content	89.7±0.18	-	-
Ash	4.32±0.32	5.76	14
Total fat	0.49±0.19	2.24	-
Total crude fiber	34.58±0.48	18.59	-
Total protein	13.8±0.11	19.06	14.9
Cellulose	17.32±0.25	16.0	16.6
Hemicellulose	9.12±0.22	8.0	8.4
Total lignin	5.94±0.31	3.68	6.25

* moisture content of ground and squeezed biomass on wet basis
# moisture content of ground and non- squeezed (after crushing) biomass on wet basis

Table 3 Statistical parameters applied to assess the drying model of cauliflower waste. Standard deviation is less than 5%.

Temperature, (ºC)	Statistical parameter			Effective diffusivity × 10^8, (m^2s^{-1})
	R^2	SSE	$\chi 2$	
60	0.994	0.040	0.041	1.01
80	0.993	0.034	0.036	1.38
100	0.995	0.041	0.044	1.89
120	0.995	0.031	0.036	2.29

Table 4 Composition of cauliflower waste before and after detoxification

Cauliflower waste sample	Hydrolysates composition after pretreatment		
	Total sugar (g L^{-1})	Acetic acids (g L^{-1})	Total phenolics (g L^{-1})
Non-dried	14.4±0.2	1.51±0.11	1.45±0.21
dried at 60 ºC	25.18±0.6	1.34±0.12	1.81±0.23
dried at 80 ºC	26.05±0.2	1.65±0.12	1.93±0.34
dried at 100 ºC	18.34±0.1	1.77±0.11	1.96±0.15
dried at 120 ºC	17.6±0.3	1.75±0.11	2.00±0.24
Composition after activated charcoal detoxification			
Non dried	13.1±0.1	1.02±0.11	0.04±0.21
dried at 60 ºC	23.25±0.3	1.05±0.99	0.01±0.32
dried at 80 ºC	25.32±0.1	1.09±0.11	0.03±0.21
dried at 100 ºC	17.5±0.2	1.21±0.12	0.04±0.36
dried at 120 ºC	16.9±0.3	1.35±0.1	0.01±0.25

Table 5 Bands in FTIR spectra of the sun dried and dried sample at different temperature
(60-120 °C - Indicate cauliflower waste dried in the range of 60-120 °C, sun dried - used as control sample)

Band region in wave number (cm^{-1})	Functional group assignments	% Transmittance of dried and sundried Cauliflower waste				
		60°C	80°C	100°C	120°C	Sundried
~835	C-H out of plane vibration in lignin	0.63	0.63	0.59	0.57	0.6
~897	C-H deformation in cellulose	0.63	0.63	0.59	0.57	0.6
1040-1060	C-O stretch in cellulose and hemicellulose	0.62	0.62	0.58	0.55	0.58
1160-1170	C-O-C vibration in cellulose and hemicellulose	0.63	0.63	0.59	0.57	0.6
1240-1260	guaiacyl ring breathing, C-O stretch in lignin	0.63	0.63	0.59	0.57	0.6
1320-1330	syringyl ring breathing in lignin	0.63	0.63	0.59	0.57	0.59
1370-1380	C-H deformation in cellulose and hemicellulose	0.63	0.63	0.59	0.57	0.59
1420-1430	aromatic skeleton vibration (methyl) in lignin combined with C-H plane deformation in carbohydrates	0.63	0.62	0.59	0.56	0.59
1450-1460	aromatic C-H deformation in lignin; asymmetric in CH3, and -CH2	0.63	0.63	0.59	0.57	0.59
1510-1520	aromatic C=C stretch from aromatic lignin	0.62	0.62	0.59	0.57	0.58
1600-1610	aromatic skeletal vibration plus C=O stretch	0.62	0.62	0.58	0.56	0.59
1630−1640	absorbed O-H, conjugate C=O, ketone	0.62	0.62	0.58	0.56	0.59
~1705	C=O stretch unconjugated ketone, esters in xylan	0.62	0.62	0.59	0.57	0.59
2900−2910	C-H stretching, from methyl, methylene groups	0.63	0.63	0.56	0.53	0.57
3300−3400	O-H vibration from aromatic and aliphatic groups	0.62	0.62	0.55	0.51	0.56

Table 6 Effect of cauliflower waste drying on ABE yield

Feedstocks	Initial sugar ($g\ L^{-1}$)	Sugar consumed ($g\ L^{-1}$)	Butanol ($g\ L^{-1}$)	Acetone ($g\ L^{-1}$)	Ethanol ($g\ L^{-1}$)	Total acids ($g\ L^{-1}$)	Yields of ABE ($g\ g^{-1}$)	References
Non dried	57.0±0.3	35.05±0.2	3.13±0.11	0.6±0.1	0.5±0.1	4.89	0.12	Current study
dried at 60 °C	58.5±0.2	26.26±0.3	2.80±0.10	1.1±0.1	0.2±0.1	4.05	0.16	Current study
dried at 80 °C	59.7±0.2	30.25±0.2	2.99±0.10	2.0±0.1	0.3±0.1	4.41	0.17	Current study
dried at 100°C	57.4±0.3	25.84±0.1	3.06±0.11	0.6±0.1	0.2±0.1	5.02	0.15	Current study
dried at 120°C	58.4±0.2	24.76±0.2	2.46±0.10	1.0±0.1	0.2±0.1	4.9	0.14	Current study
P2 control	60.5±0.1	50.5±0.1	6.54±0.11	2.5±0.1	1.2±0.1	2.53	0.24	Current study
oil palm fruit bunch	25.4	18.76	1.94	0.58	0.09	7.78	0.13	[61]
Sago pith residue	23.51	21.84	2.23	1.73	0.26	11.23	0.20	[62]
C. forskohlii root hydrolysates	50.0	26.51	3.45	1.2	0.69	5.14	0.21	[36]

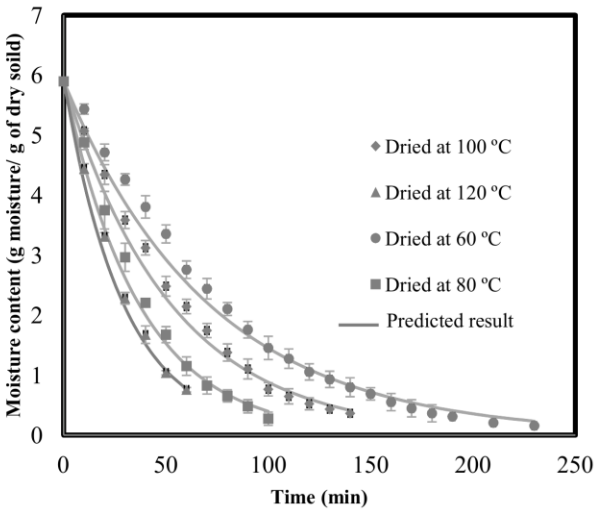

Fig. 1. Effect of temperature on moisture content versus time during drying

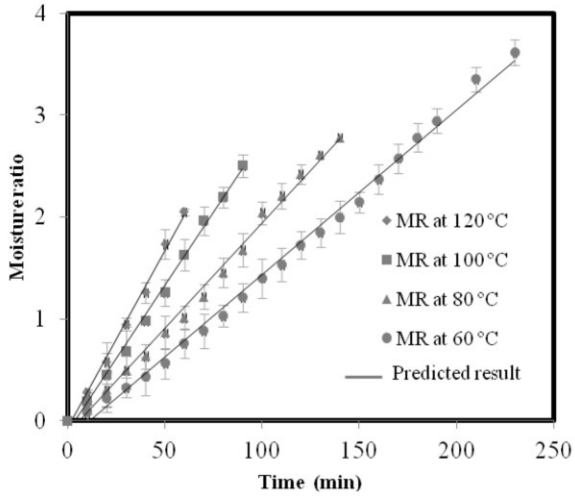

Fig. 2. Kinetic of drying showed by plotting moisture ratio versus time at different temperatures

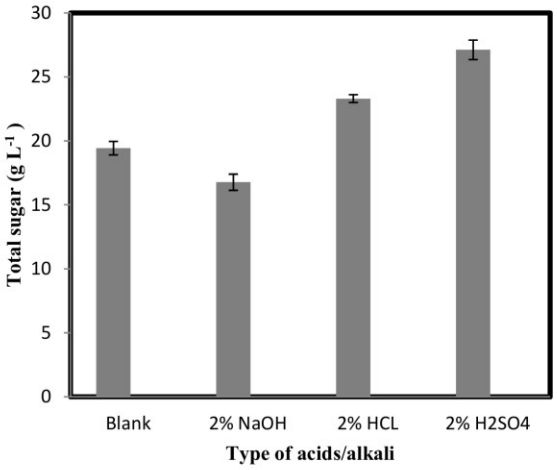

Fig. 3. The effect of acid and alkali on cauliflower waste to obtained maximum total sugar release

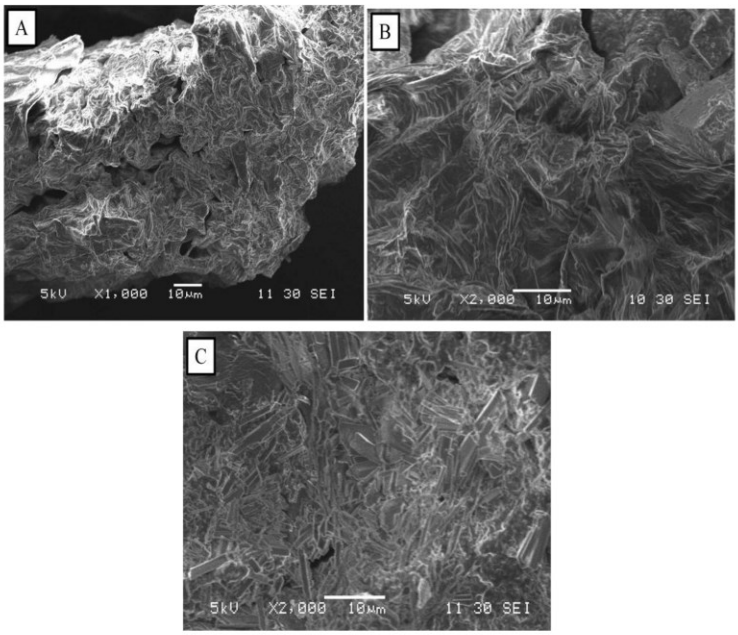

Fig. 4. SEM images of cauliflower waste after drying and preatreatment: A: sun dried (control) sample; B: sample dried at 80 °C; C: acid treated sample

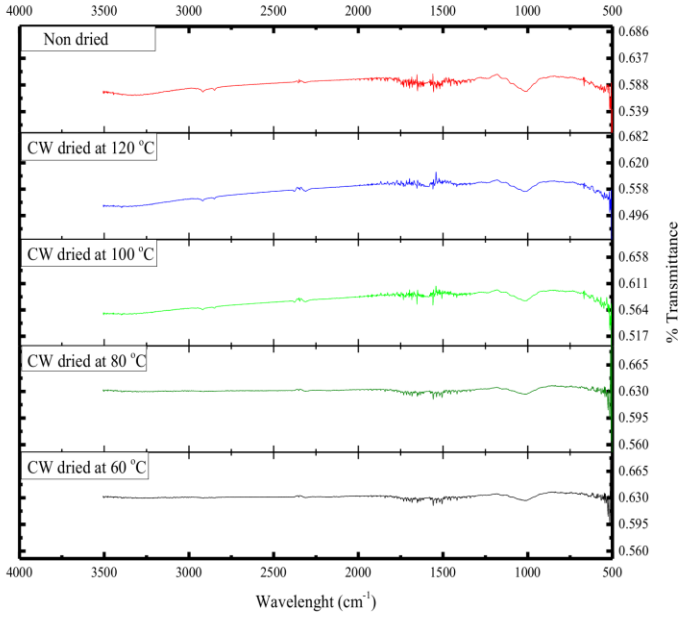

Fig. 5. The FTIR spectra of cauliflower waste (CW) dried in the range of 60 - 120 °C, and non dried

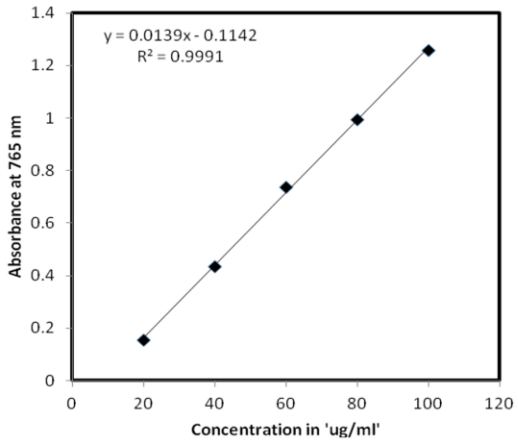

Fig. 6. The standard graph of total phenolic estimation using Folin–Ciocalteu reagent