Effect of Pressure on Alkaline Pretreated Sugar Cane Bagasse for Enhanced Bioethanol Production

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Abstract

Global warming has become a major concern as a result of the excessive release of greenhouse gas emissions. An important strategy for achieving carbon neutrality targets is to focus on renewable energy resources. Second generation bioethanol synthesis via sugarcane bagasse (SCB) is another promising approach for the reduction of greenhouse gas emissions. Here in, this study presents the second generation of bioethanol production from sugarcane bagasse with the pretreatment condition adjoined with basic hydrogen peroxide and pressure effect by fermentation using microorganisms *Saccharomyces Cerevisiae* and *Bacillus Subtilis*. The results revealed better production through pretreatment at different operational stages through batch fermentation. Different characterization techniques including Scanning Electron Microscopy (SEM), Fourier Transform Infra-Red (FTIR), High Performance Liquid Chromatography (HPLC), and Thermogravimetric Analysis (TGA) results confirmed the better effects of structural changes of hemicellulose, lignin, and cellulose during treatment, weight loss, thermal stability, and higher concentration of the produced bioethanol in the distillate After pretreatment, the conversion of biomass to bioethanol by using *Saccharomyces Cerevisiae* gives a high production yield (70%), which presents a production of 70g/L from 100g of SCB at the end of 72 h and a yield of bioethanol (0.7g/g) of SCB confirmed through gas chromatography/mass spectrometry qualitative analysis (GC/MS). The pretreatment conditions of alkaline hydrogen peroxide (H_2O_2) were optimized to the values 3h, 50°C, 60 psi, pH 8.6, and 150 rpm. This study sheds light on the effects of pretreatment conditions for bioethanol production from sugarcane bagasse.

Introduction

In recent times, we are witnessing a crisis that can unfold into catastrophy and this crisis is primarily due to vast CO_2 emission because of fuel combustion which totally damages the environment and affects climate globally. Simultaneously, energy security has assumed un-presented importance in global discourse. As the events unfold due to the limited availability of conventional fossil fuels, the major renewable energy source is biomass which is used nowadays potentially and received much attention. Much of these untapped energy resources have huge potential [1]–[3]. The fourth-largest energy source worldwide is biomass after (petroleum, natural gas, and coal) and it offers approximately 10.2% (almost 50.3 EJ per year) of the annual global energy supply [4]. Global warming can be controlled using biofuels if there is a significant enhancement of production from the present production of 9.7×10^6 GJ d^{-1} to 4.6×10^7GJ d^{-1} in the years between 2016 to 2040 [5]. Conventionally sugarcane is the most common and important crop harvested worldwide and its bagasse is considered as waste and byproduct of the sugar industry is either discarded or burned to produce heat energy and it is the most cheaper source of energy for developing countries [6]. It has been estimated that for every 1000 kg of sugarcane about 270-280 kg as residual bagasse and about 140 kg of straw is obtained [7]. There are total 81 sugar mills in Pakistan with an annual capacity of six million tons of sugar produced. The industry crushes 35-45 million tons of sugarcane from these 12 million tons of sugarcane waste is produced annually [8]. The production of all the petroleum products is expensive as well as they are obtained from nonrenewable energy resources,

which are in depleting phase [9]. Therefore, the need of the hour is to shift towards renewable green fuels. Bioethanol is one of the best alternatives for conventional petroleum products [10]. Figure 1 shows the renewable energy source bioethanol is the inspiring fuel that is produced and consumed in different parts of the world. According to preliminary work, bioethanol production from biomass (sugarcane bagasse, molasses, etc.) has low emissions of greenhouse gases [11]. This waste is lignocellulosic material mainly containing hemicellulose, lignin, and cellulose [12]. Lignocellusic materials are hard to digest, through different pre-treatment techniques compressed structure can change into conventional biofuel e.g. ethanol, and currently ethanol production capacity of Pakistan is 270,000 tons per annum used for fuel which can readily be increased up to 400,000 tons per annum through the rise in feedstock like sugarcane bagasse waste [13]. Sugarcane production has increased considerably in the last three decades which is about 9.93×10^8 tonnes in 1988 to 1.91×10^9 tonnes in 2018 according to the statistics released by the food and agricultural organization. [14]. A variety of pretreatment methods has been utilized till now to alter the composition of sugarcane bagasse. Biological, physical, physicochemical, and chemical pretreatments were experimented with in previous published work and proved to be successful in enhancing bioenergy production [15]–[17]. Physical pretreatment necessitates a significant amount of energy to break down the complex structure of sugarcane bagasse[18]. By applying pressure during chemical pretreatment will result in fast size reduction of solid particles and alteration of sample composition [19], [20]. However, the most valuable technique for removing lignin from sugarcane bagasse is a physicochemical method and its advantages are simple operation at minimum temperature and pressure [21]. The stated pretreatment conditions produces (1 h, 25°C, 1.84 mL hydrogen peroxide/g bagasse) 416.7kg of glucose from 1 ton of raw bagasse and the ethanol production was 187.87kg from 1 ton of raw bagasse. [22]. Most researchers have tried to optimize fermentation process parameters like temperature, time hydrogen peroxide concentration, and pH [23]. The microorganisms in this process are most effective for biodegradation of long (carbon, hydrogen) catenation into short (carbon, hydrogen) catenation to enhance the bioethanol production [24]. However, *Scheffersomyces Shehatae* was used for fermentation after 48 hours, 9.11 g/l ethanol production (yield 0.38 g/g) was obtained. After 72 hours of fermentation, enzymatic hydrolysate yielded 8.13 g/l ethanol (yield 0.22 g/g) when fermented by *Saccharomyces Cerevisiae* [25]–[27].

In this study physiochemical treatment carried out using highly concentrated hydrogen peroxide with applied pressure by an inert gas which effects more on the compacted structure of substrate as compared to other previous published work and increased bioethanol production achieved.

Methodology

1. Effect of Fermentation time on Bioethanol production

Figure 2 showed the ethanol production from two microorganisms with treated and untreated solid substrates sugarcane bagasse in 72 hours and 20g of raw bagasse was used in each trial. Ethanol production from the treated substrate was obtained 42g/l with *Saccharomyces Cerevisiae* while untreated sugarcane bagasse ethanol production was 22g/l with *Saccharomyces Cerevisiae* [53], [54]. Ethanol

production from treated sugarcane bagasse was obtained 14g/l with *Bacillus Subtilis* and from untreated sugarcane bagasse 8g/l with *Bacillus Subtilis* respectively [55]. However, ethanol production from the treated substrate was obtained higher than untreated sugarcane bagasse because of highly concentrated alkaline chemical 35% hydrogen peroxide treatment, with applied pressure during agitation which affects the conversion rate of fermentation by fast reduction of particle size and breaking the chemical bond of sugar [56]–[58] However, in previous work ethanol production was reported 51.5g/l from 100g raw bagasse with three different methods by reusing of liquor residue with 15% hydrogen peroxide and 5% $Ca(OH)_2$ and enzymes loading [22]. Therefore, it's been observed that the impact of pressure is significant to achieve the increase in ethanol production.

2. Effect of applied pressure on Bioethanol production

Figure (3) represented the relation between applied pressure and the production of bioethanol through batch fermentation. When the pressure was applied during treatment and untreated strongly effected the unconvertable, compacted structure of substrate [28], [59]. Sugarcane bagasse is challenging to ferment without treatment due to the complex matrix consisting of the three main polymers of lignocellulosic biomass (hemicellulose, lignin, and cellulose) and pretreatment process is principally required to break down [60]–[62]. Simultaneously during the fermentation process microorganisms efficiently utilized the carbon content of the substrate. However, when pressure is applied with inert gas, fused in the pore volume of substrate, reduce the strength of the bond and reduce the particle size of substrate [63]. In previous work, pressure has been applied through steam and CO_2, which do not bring any change in pore size of the substrate [64]–[67]. In this work pressure applied with non-reactive gas effecting the conversion rate of substrate indicated by the SEM analysis that amorphous hemicellulose could be easily degraded on applied pressure, reducing the fermentation time and enhancing the bioethanol production, with the pretreated and non-treated substrate. Non-reactive gases are also environment friendly [68].

3. Fourier-transform infra-red spectroscopy

The Fourier transform infrared (FTIR) was used to observe the structural changes of hemicellulose, lignin, and cellulose during chemical and pressure treatments exposed to sugarcane bagasse. Figure (4) and figure (5) represented the FTIR spectrum of the initial solid substrate (sugarcane bagasse) without treatment referred to as standard. The wavelength range is between (3200-3400cm^{-1}) and its intensity peak is alcohol (O-H). The wavelength range between (2800-3000cm-1 indicates sp^3 (C-H) [69]. The region of (1600-1700cm^{-1}) represent (C=C and C=N). Wavelength range between Acyl and phenyl (1100-1350cm^{-1}) (C-O) Trisubstituted alkene (790-840cm^{-1}) [70], [71]. The region has a significant molecular interaction, making the area highly complicated, involving the superposition of numerous lignin and carbohydrate vibration modes. Due to (C-O) stretching of (C-O-C), the region between 1,100 and 1,200cm^{-1} has a substantial proportion of hemicellulose and cellulose, with a maximum value around (1,035cm^{-1}). After acid hydrolysis (red line), the region between (1,100 and 1,000cm^{-1}) exhibits two peaks, showing the elimination of hemicellulose [72]. In the region of (1,247cm^{-1}), hemicellulose elimination is also

visible [73]. The elimination of lignin appears to have an impact on alkaline hydrolysis. The (C=C) stretching of the aromatic ring in lignin causes the band around (1515cm^{-1}). The FTIR spectrum of native sugarcane bagasse, alkaline hydrolyzed cellulignin, and hydrolyzed substrate in the range (2,700-3,900cm^{-1}). Increased under-curve width asymmetry in the range of (3100-3500 cm^{-1}) is significant indication of the production of alcohols [74].

4. Gas chromatography mass spectrometry

Gas chromatography-mass spectrometry (GC/MS) was used in the qualitative analysis of ethanol in the fermentation process. Bioethanol obtained from sugarcane bagasse is an integrated system for the analytical equipment by combined alkaline chemical and applied pressure treatment. GC/MS is used to separate analysts and mass spectrometry is used for its identification. The retention time was (4.190 (min) Ret index 759 and quality index SI is 49) of production which is obtained from treated sugarcane bagasse with microorganism *B. Subtilis*, retention time was (4.380 (min) Ret index 1803 quality index SI 453) of production which is obtained from treated sugarcane bagasse with microorganism *S. Cerevisiae* however both microorganisms used for bioethanol production [52][75].

5. Thermo-gravimetric analysis

The thermal stability and weight loss of the solid substrate sugarcane bagasse were analyzed by TGA at a temperature between 100°C and 600°C as shown in figure (6). The curves that were obtained by the TGA, were divided into different three phases. The different degradation ranges define the removal of cellulose and hemicellulose because sugarcane bagasse mainly consists of lignocellulose, which starts to burn above 250°C. The first phase explained the removal of moisture and in this case, it was noticed at a temperature of 250°C, and during the second phase, a high amount of moisture content was removed at 300°C and 450°C which describes the removal of volatile compounds. The last phase ranges from a temperature of 480°C is shows the significant weight loss was recorded between temperatures 500°C and 600°C is 90% loss of raw sugarcane bagasse, 97% weight loss of treated sugarcane bagasse with microorganism *Saccharomyces Cerevisiae*, 93% weight loss of untreated sugarcane bagasse with microorganism *Saccharomyces Cerevisiae* and 92% weight loss of treated sugarcane bagasse with microorganism *Bacillus Bubtilis*. However the maximum weight loss of 97% showed at this point is the strong evidence of chemical pretreatment and carbon bond degradation at a range between 300°C and 450°C, there was a tremendous amount of volatile matter indicating a combustion reaction [76], [77].

6. High-performance liquid chromatography

Figure 7 shows that a significant quantity of cellulosic sugarcane bagasse is consumed during the process of fermentation. In this work, fermented bioethanol was analyzed which was obtained from non-pretreated sugarcane bagasse with microorganism *Saccharomyces Cerevisiae* and alkaline pretreated sugarcane bagasse with *Saccharomyces Cerevisiae* as well as alkaline pretreated sugarcane bagasse with *Bacillus Subtilis* to find the concentration value of bioethanol with the help of (HPLC) analysis. The produced bioethanol was estimated to be a steep peak at a retention time of (2.920, 2.937, and 2.903

min) respectively, related to marketable ethanol ordinary at holding time of 2.892. The variation of retention time and production yield was due to the presence of different microorganisms, alkaline chemical treatment, and applied pressure changes [50], [78], [79].

7. Scanning-electron-microscopy

The structural and morphological changes on the SCB, before and after the chemical and pressure pretreatment was investigated and presented in figure (8). The untreated, raw substrate biomass showed a representative surface of the rigid, orderly, smooth structure. The treated SCB, on the other hand, presented a characteristic vast surface changes, loosened surface structure, due to chemical treatment and applied pressure. The most visible outcome of pretreatment is the loosening of the fibrous structure. The combined Pretreatment of pressure and high concentration of hydrogen peroxide removes the hemicellulose from SCB, weakening the cell wall and pronounced the loose compact structure. The clear presence of tiny pores was observed by the combined pretreatment of SCB. However, before pretreatment structure of native SCB was thick-walled fiber cells compacted with pith. The parallel stripes make up the Fibers which are covered on outside constituted by parallel as well as compacted, as applied pressure during chemical treatment which effects the highly compacted matrix of the substrate to reduce the long chain of carbon and packed fibers with an open structure of carbon loose strong unconvertable structure of hemicellulose, the increased pore size of substrate for faster chemical reaction on SCB. SEM results are strong evidence of pretreatment of SCB with hydrogen peroxide and pressure improved cellulose recovery and effective lignin and hemicellulose removal for enhanced production of fermentable sugars [80]–[83].

Conclusion

The sugarcane bagasse was pretreated to improve its digestibility without the need for continuous fermentation and continuous pretreatment of substrate. This study examined the effect of pretreatment using orbital shaker agitation at 130, 140, 150 rpm were changed with time 1, 2, 3h.and 20ml of hydrogen peroxide with 35% concentration at pH 8.6. Although the concentration of peroxide with an applied pressure of non-reactive helium gas with variable pressure 20, 40, 60psi. The highest production of bioethanol 42g/l with the treated substrate by microorganism *Saccharomyces Cerevisiae* was obtained at 60psi pressure. The lowest production of bioethanol 14g/l was obtained with the treated substrate by microorganism *Bacillus Subtilis.* Both batch fermentation time was 72h. When compared to lime and CO_2 pretreatment, which reduces energy costs and maximizes raw material use, this method improves process economics. In comparison to lime, CO_2 pretreated bagasse, and other pretreatment approaches, the fermentability of the hydrolysate from peroxide and pressure prepared sugarcane bagasse was efficient. Increased ethanol production was achieved by combining the alkaline and pressure effects with fed-batch hydrolysis.

Declarations

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Authors 'contributions:

Azhar Uddin**:** Experimentation and write up, Rabia Liaquat: Conceptualization and supervision**,**

Ali Abdullah: Characterization, Asif Hussain Khoja: Analysis, Muhammad Muddasar: Write up, Sami Ullah: Testing

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Figures

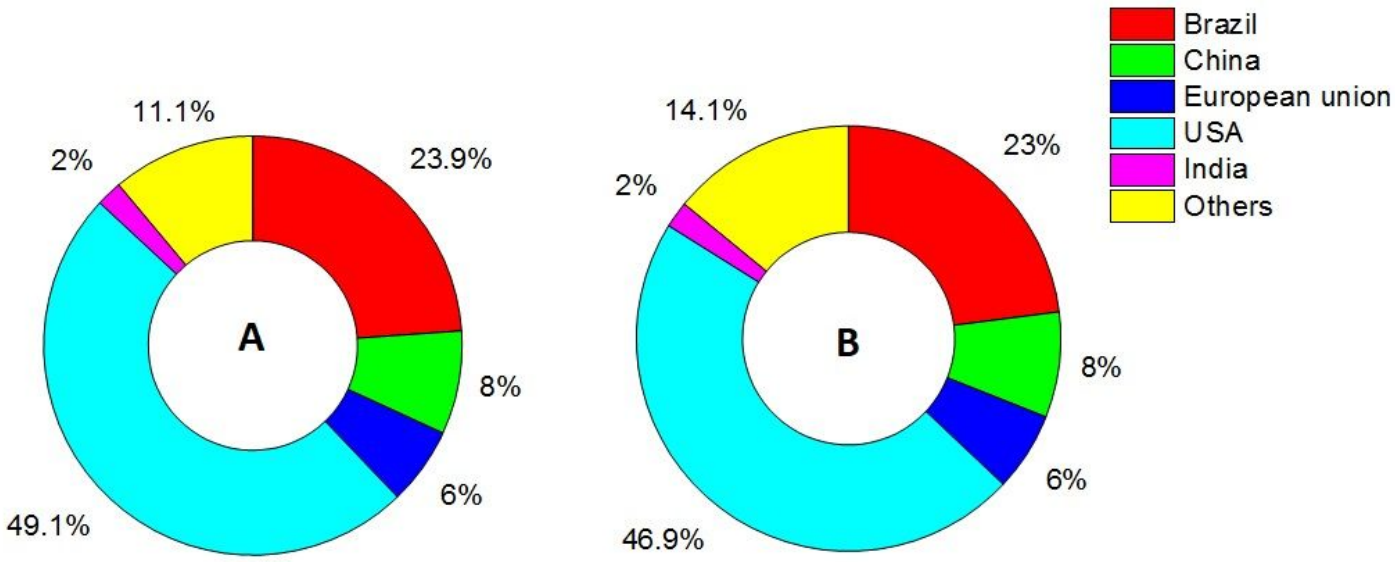

Figure 1

(A) World different countries Bioethanol production in 2020 and (B) World different countries bioethanol consumption in 2020

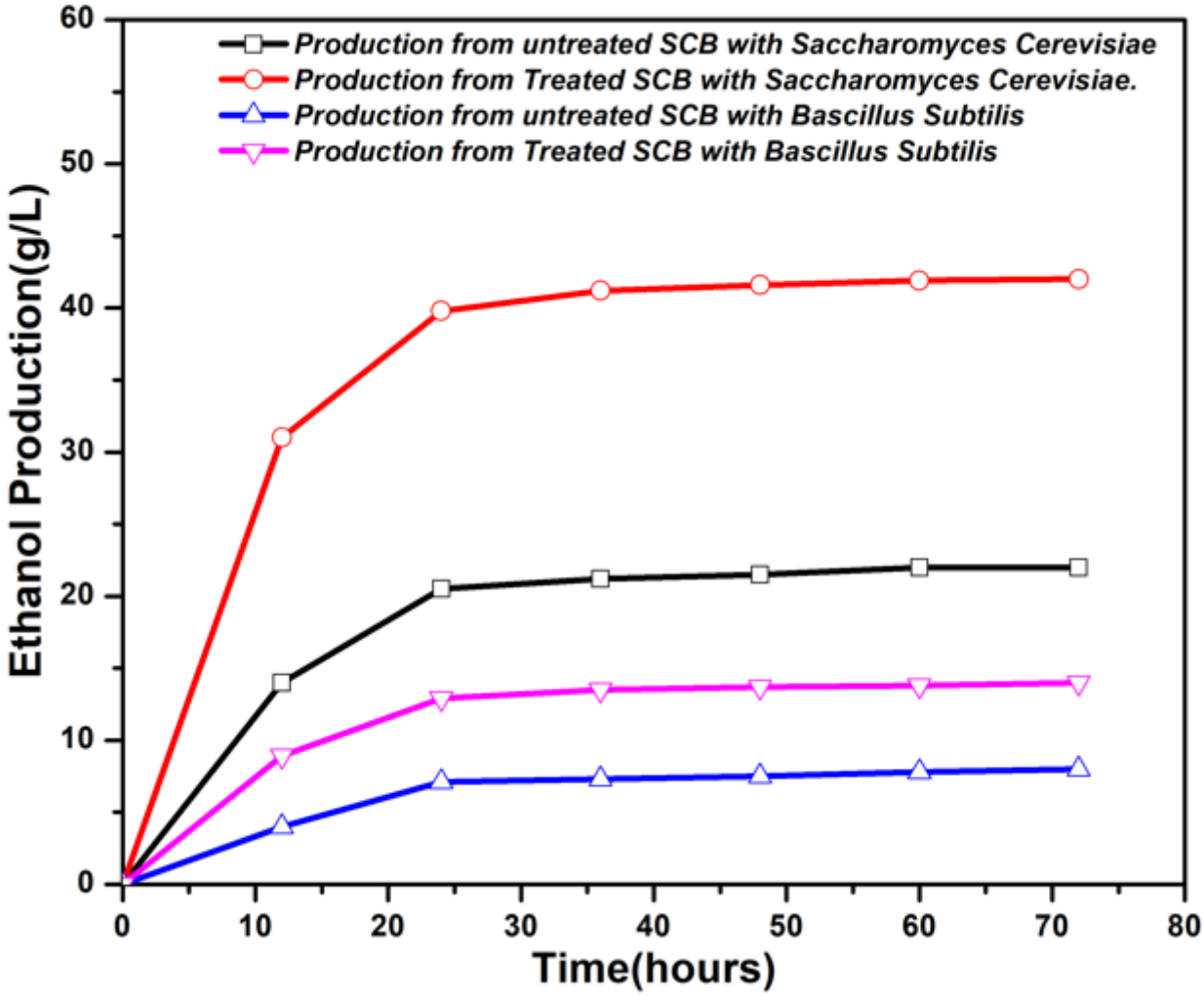

Figure 2

Effect of fermentation time on ethanol production (g/l).

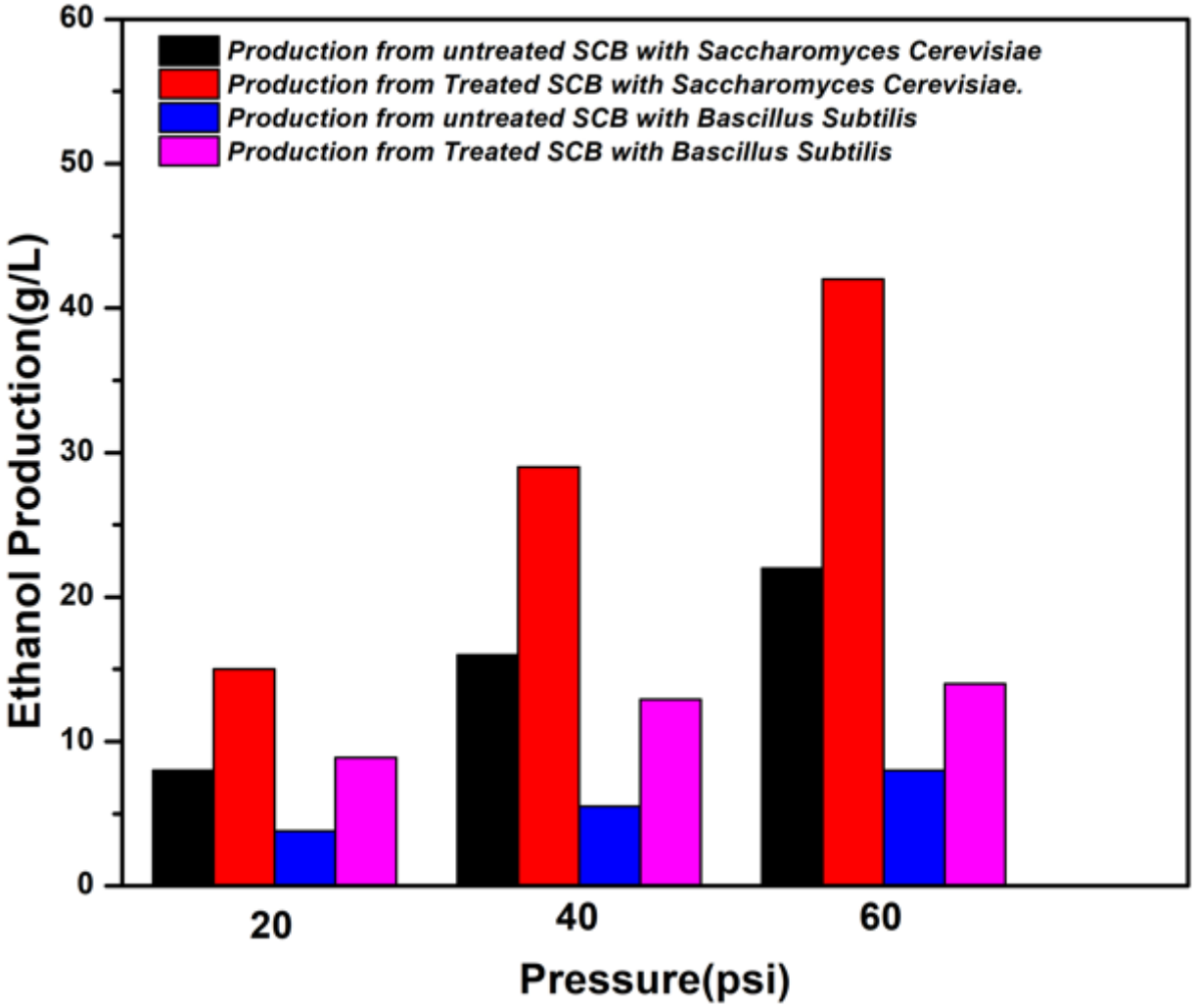

Figure 3

Effect of pressure (psi) on ethanol production (g/l).

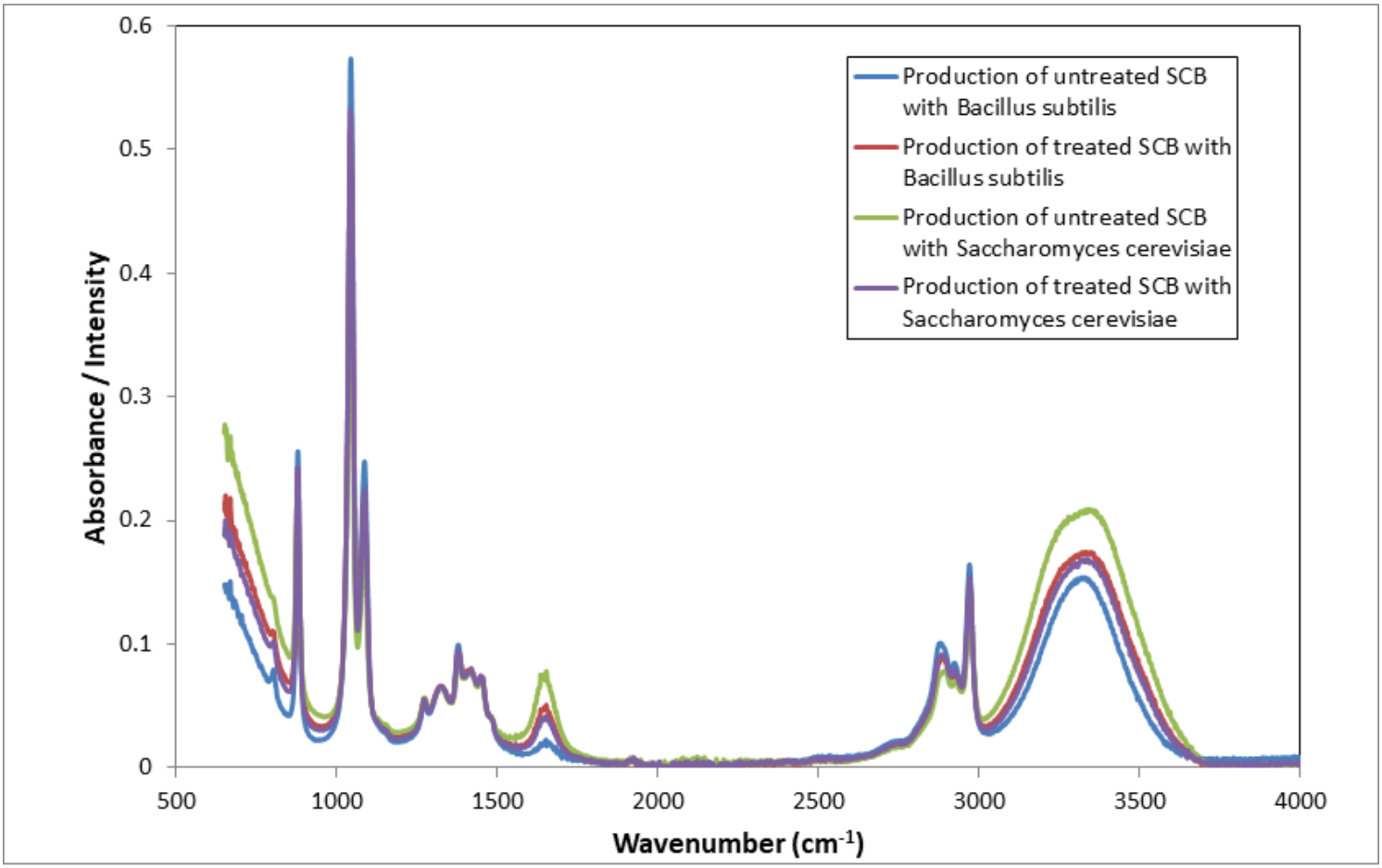

Figure 4

Fourier transform Infra-Red of ethanol production treated and untreated of Sugar Can Bagasse

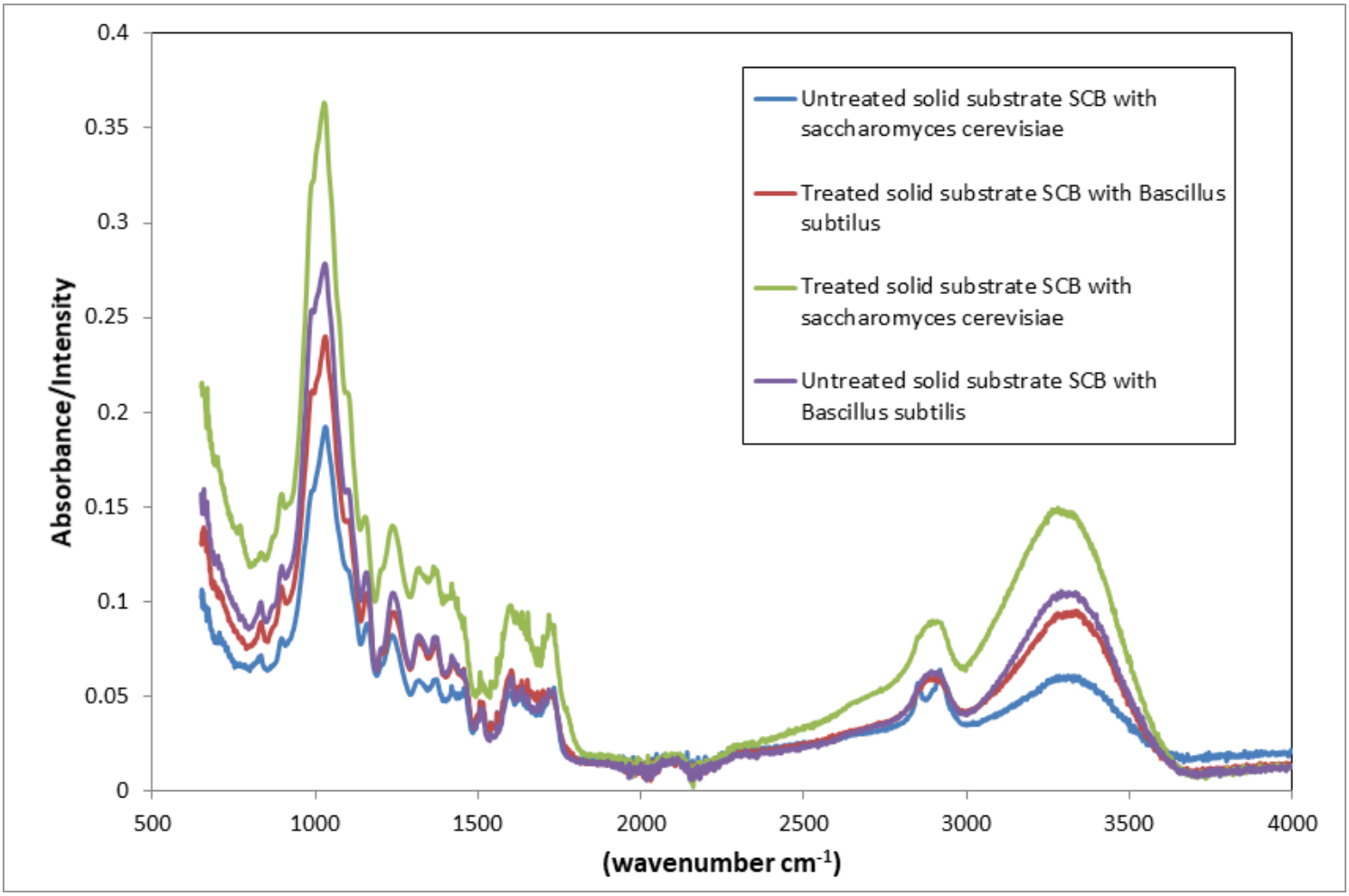

Figure 5

Fourier transform Infra-Red spectra of Solid substrate before and after treatment of Sugar Cane Bagasse.

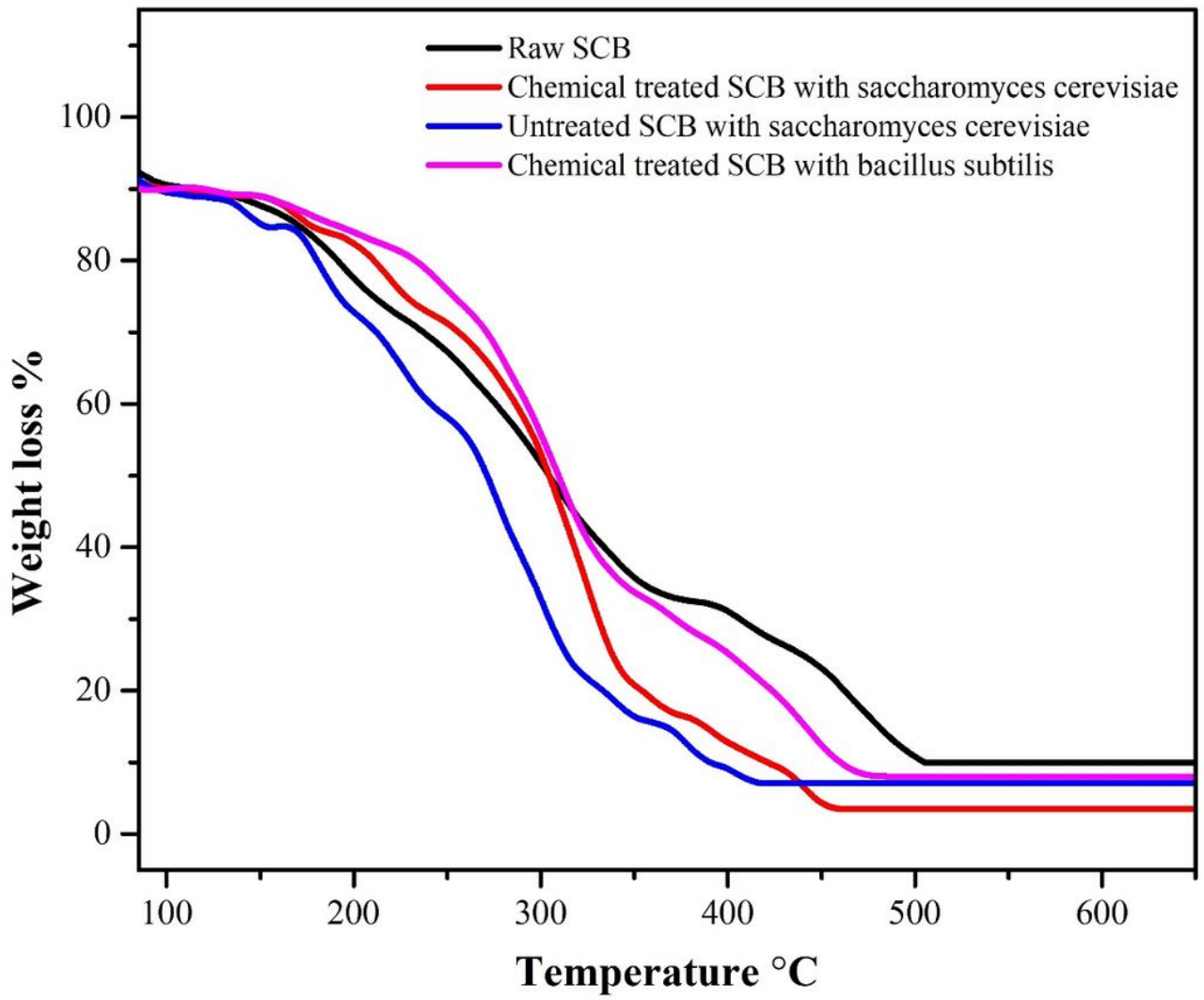

Figure 6

Thermogravimetric analysis of Raw Sugar Can Bagasse, chemically treated Sugar Cane Bagasse with Saccharomyces Cerevisiae, Untreated Sugar Cane Bagasse with Saccharomyces Cerevisiae, and chemical treated Sugar Cane Bagasse with Bacillus Subtilis.

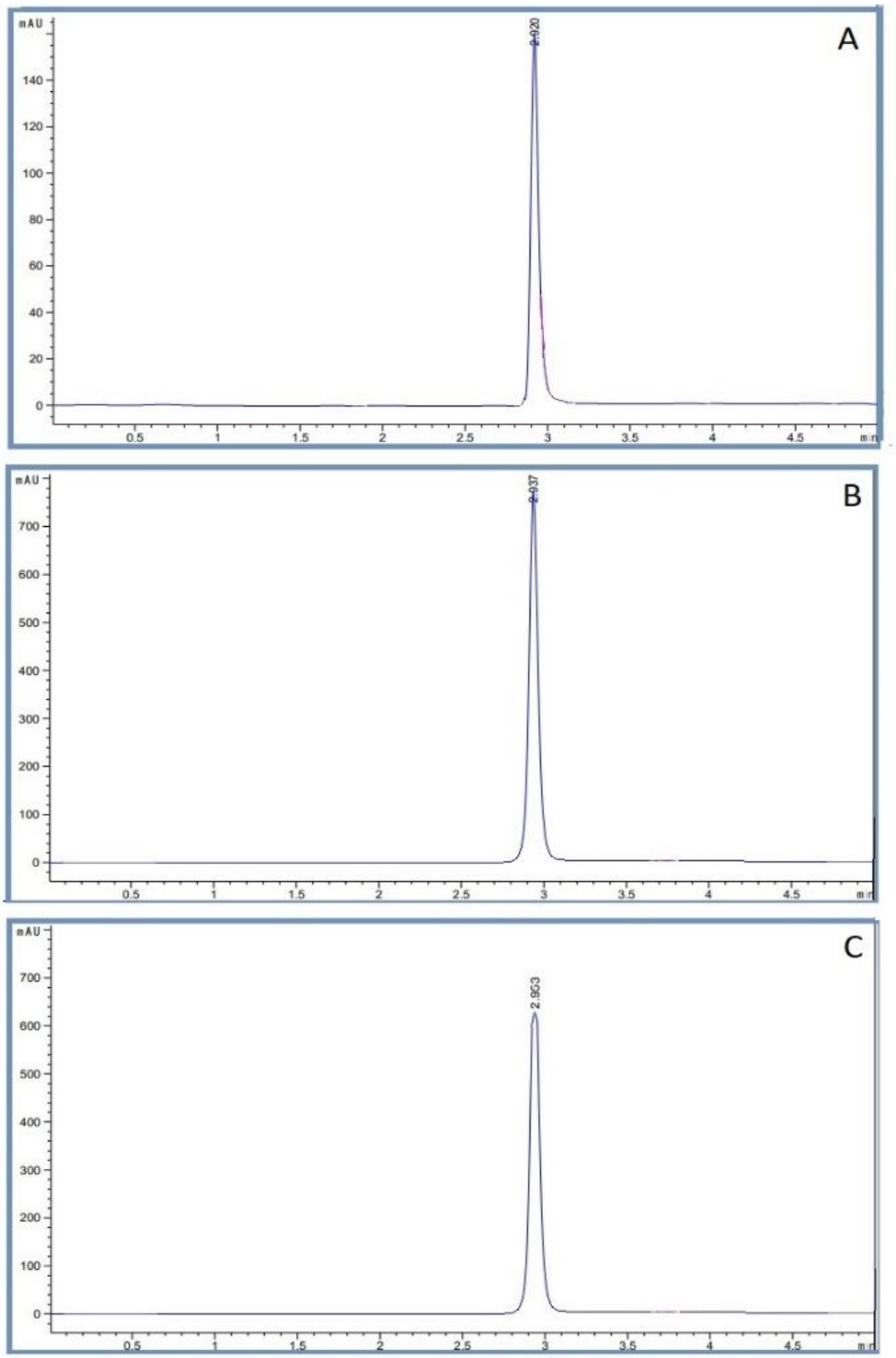

Figure 7

(A) Treated with Saccharomyces Cerevisiae, (B) Untreated with Saccharomyces Cerevisiae, (C) Treated with Bacillus Subtilis.

Figure 8

Scanning electron microscopy (SEM) images [A, B, C] Represents Alkaline Treated Sugar Cane Bagasse at 100µm, 50µm, 10µm Magnifications with Bacillus Subtilis, [D, E, F] Represents Alkaline Treated Sugar Cane Bagasse at 100µm, 50µm, 10µm Magnifications with Saccharomyces Cerevisiae and [G, H, I] Represents Un-Treated raw Sugar Cane Bagasse at 100µm, 50µm, 10µm Magnifications.