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Ethanol Production by using Vegetable waste as substrate

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Vegetable waste, enzymatic hydrolysis, *Trichoderma atroviride*, bioethanol

Abstract

Ethanol is a forthcoming source of alternative energy. Various researches are targeting on bioethanol production from waste biomass. In this study, vegetable waste was explored as feedstock for cellulase production by *Trichoderma atroviride*. The obtained cellulase culture filtrate was applied for saccharification (15% w/v) of alkali treated vegetable waste, in 0.1M citrate buffer pH 4.8 in shaker water bath of 150 rpm. Maximum reducing sugars (593.92 ± 5.63 mg/g) was achieved after 72 hrs at 50°C. The amount of ethanol produced after fermentation was analyzed by gas chromatography and found to be highest for the vegetable waste with yield of 25.65 ± 0.55 g/L. These results indicate the promising future for generation of ethanol from cellulosic wastes on a large scale.

Introduction

The demand for alternative fuel sources is increasing due to the excessive consumption of petroleum based fuels (Demeke *et al*., 2013). Currently, ethanol is produced by fermentation process that uses crops such as sugar cane and corn but they have social concerns associated with the exploitation of potential food or feed resources (Ferreira *et al*., 2009). Therefore, the use of nonfood crops i.e. lignocellulosic biomass is creating interest worldwide for bioethanol production. The lignocellulosic biomass has the advantage of highly abundant, being economical in nature, reduced greenhouse gases emissions and does not have the socioeconomic issues regarding the use of food crops. All these factors make lignocellulosic biomass, one of the most promising technological approaches available for supplementing the current source of transportation fuel. Effective conversion of lignocellulosic biomass to bioethanol includes four subsequential steps: (1) pretreatment, (2) enzymatic hydrolysis (saccharification), (3) fermentation and (4) distillation (Cardona and S´anchez, 2007).

Vegetable waste can be excellent sources of sugars which can be used for the production of ethanol through saccharification followed by fermentation (Tsigie *et al*., 2013). Many pathways for the disposal or utilization of the wastes have been investigated throughout the years. These wastes generally end up in garbage dumps or biogas production. The generation of biofuels from these wastes provides an attractive solution towards both waste management and energy generation. These substances provide low cost and sustainable resource for production of many chemicals and organic fuels that can reduce greenhouse gas emissions, improve the economy, enhance energy security, disposal problems of solid wastes and improve air quality (Singh *et al*., 2012).

The study was focused on the potential use of vegetable waste as the raw material for second-generation ethanol production. The vegetable wastes samples were subjected to pretreatment and then saccharification was carried out by microbial cellulases. The liberated sugars (glucose) can then be utilized by fermented microbes and produced bioethanol was estimated and quantified.

Materials and Methods

Microorganism and Culture media

Trichoderma atroviride was originally isolated from soil samples collected from different regions of Haryana. *S. cerevisiae* were procured from MTCC (Microbial Type Culture Collection, Chandigarh). *T. atroviride* was cultured on Potato Dextrose Agar (PDA) and *S. cerevisiae* on Malt Yeast Extract Peptone Dextrose (MYPD) at 30˚C. These cultures were then preserved at 4˚C and subcultured monthly.

Raw material

Different types of vegetable wastes used for the study were directly procured from Saraswati girls' hostel canteen, M.D.University, Rohtak (Haryana). These wastes were washed twice with water and dried in a hot air oven at 60˚C for 24 hours. After that these wastes were powdered using a grinder and subsequently passed through 2 mm sieve. Then these were pretreated with 1% sodium hydroxide for 1 hour in an autoclave at 121°C. The alkali pretreated vegetable waste was allowed to cool, filtered and washed upto neutral pH. The solid residue was then separated from the liquid fraction by filtering through muslin cloth and then it was dried at 60°C. This solid residue was subsequently used as raw material (substrate) for enzymatic hydrolysis.

Enzymatic hydrolysis of vegetable waste

Enzymatic saccharification (hydrolysis) of alkali treated vegetable waste was carried out in reaction mixture containing 1 g treated vegetable waste in 0.1 M citrate buffer, pH 4.8 with cellulase loading of 10 FPU/g biomass from *Trichoderma atroviride*. The reaction mixture was incubated in an orbital shaker adjusted to 50°C and 150 rpm. The samples were withdrawn at an intervals 24 hrs. for glucose and total sugars were determined according to the dinitrosalicylic method (Miller, 1959). The optical densities of the samples were measured against the blank at 540 nm. The glucose concentration was then calculated using standard glucose curve.

Fermentation

After enzymatic hydrolysis, the hydrolysate was filtered using Whatman No 1 Filter Paper and the sugars solution was concentrated in a rotary vaccum to 60g/L glucose. The flasks were then aseptically inoculated with 10% v/v of 24 h old seed culture of *S. cerevisiae* and incubated at 30°C and 150 rpm for 32 hrs. The ethanol produced was determined by gas chromatography using flame ionization detector. Samples are withdrawn after an interval of 4 hrs for ethanol and residual glucose determination.

Results and Discussion

Compositional analysis of vegetable waste

In the present study, five different types of vegetables such as carrot (*Daucus carota*), pea (*Pisum sativum*), potato (*Solanum tuberosum*), cabbage (*Brassica oleracea*) and cauliflower (*Brassica botrytis*) were investigated. The compositional analysis of vegetable waste sample revealed that the biomass contains cellulose (25.5 ± 3.27%), hemicellulose (16.7 ± 1.09%), lignin (5.1 ± 2.04%) as shown in Table 1. Pretreatment of lignocellulosic biomass is a crucial step for enhancing the enzymatic hydrolysis process (Okeke and Obi, 1995). The pretreatment method consists of breaking the lignocellulosic structure to expose the accessible area for the enzyme action (Soares *et al.*, 2011).Results indicated that the pretreatment of substrate with 1% NaOH increased the proportion of cellulose by 45.23±1.38%, and reduced the lignin content by 3.16±0.86% in the biomass. Alkali pretreatment of substrate reduces the lignin content of the agro-residues. It may cause saponification of inter-molecular ester bonds cross-linking xylan, hemicelluloses and lignin, thereby increase the porosity of the substrate (Sun and Cheng, 2002). Dilute NaOH pretreatment have been reported to improve the susceptibility of hemicelluloses to enzymatic hydrolysis by many researchers (Gokhale *et al.*, 1998).

Table 1 Compositional analysis of untreated and alkali pretreated vegetable waste

Components	Untreated vegetable waste (%)	Alkali (1% NaOH)
Cellulose	25.5 ± 3.27	45.23±1.38
Hemicellulose	16.7 ± 1.09	9.08±3.19
Lignin	5.1 ± 2.04	3.16±0.86

Enzymatic hydrolysis of alkali pretreated vegetable waste

Cellulase production from vegetable waste with fungi like *Trichoderma atroviride* through solid state fermentation is important because in this way production of cellulase can be increased. This an important enzyme required for breakdown of polysaccharides into monosaccharide, those can further converted into ethanol through fermentation process. Cellulase has a lot of industrial applications that include production of food and medicines and help to breakdown the waste plants materials for clean up the environment.

Enzymatic hydrolysis process is an essential process to enhance complete degradation of cellulose. In the present study, saccharification of vegetable waste was carried out by cellulase produced from *Trichoderma atroviride* using alkali pretreated vegetable waste under solid state fermentation. Addition of surfactants during hydrolysis process can modify the cellulose surface properties which increase in sugar yield. Various surfactants used in this study are Tween 20, Tween 80 and PEG 6000 as shown in fig. 1. Enzymatic hydrolysis of alkali pretreated vegetable waste under optimized conditions using enzyme loading of 10 FPU/g of cellulase enzyme of *T. atroviride* at 50°C and pH 4.8, resulted in maximum sugar release of 593.92 ± 5.63mg/g after 72 h of saccharification when Tween 80

was used as surfactant. It is reported that the addition of Tween 80 as surfactant plays an important role in preventing nonspecific binding of enzyme to lignin residues which allow more enzymes to be accessible for the conversion of substrate and results in a higher conversion rate (Ouyang *et al.*, 2011).Thus, vegetable waste was used further as the substrate in order to increase the fermentable sugars yield which can later be used for bioethanol production.

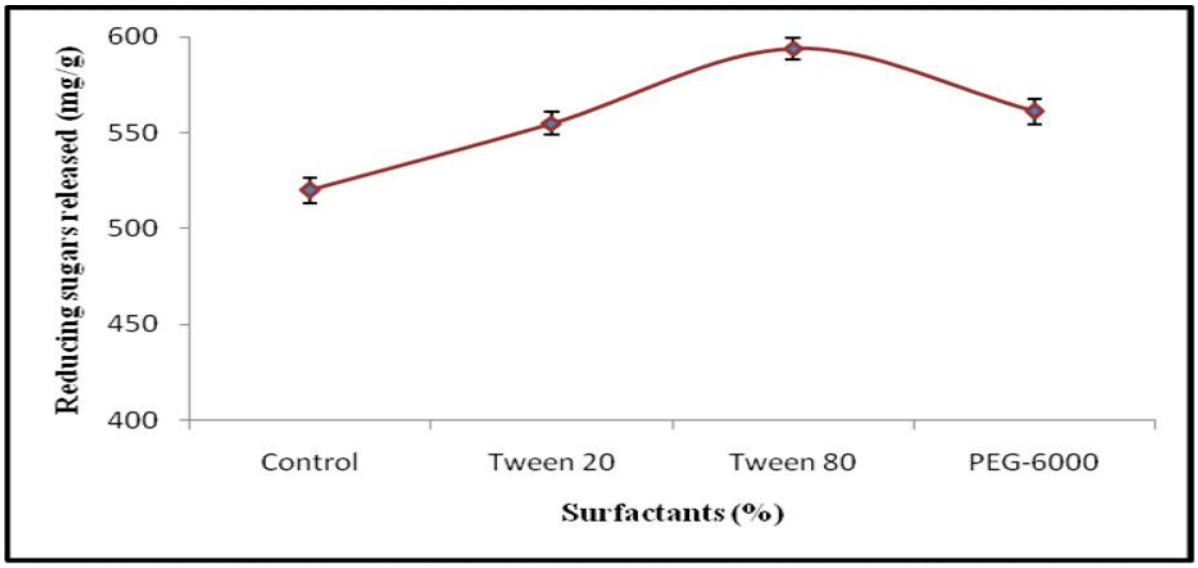

Fig.1 Effect of various surfactants on the enzymatic saccharification of alkali pretreated vegetable waste with partially purified cellulase from *Trichoderma atroviride*

Ethanol production by *Saccharomyces cerevisiae*

The alkali pretreated vegetable waste was hydrolyzed enzymatically by cellulase isolated from *T. atroviride*. Then this enzymatic hydrolysate of alkali pretreated substrate was subjected to *S. cerevisiae* at a temperature of 30°C. Enzymatic hydrolysate of alkali pretreated vegetable waste with a reducing sugar concentration of 60 g/L was used for the fermentation process. The fermentation proceeded gradually for 0-32 h. It is clear from the fig. 2 that with increase in incubation period there was rise in the ethanol production up to 0-20 h along with the growth of yeast and decrease in the reducing sugar. After 20 h time period, ethanol production decreases with increase in incubation time. It might be due to the consumption of sugar molecules by microorganisms for ethanol production. The maximum ethanol concentration (25.65 ± 0.55 g/L) with 0.43 (g/g) ethanol yield and 1.28 g/L/h productivity was obtained at 20 h at 30°C. Similar ethanol production was also reported by Kim *et al.* (2011) who observed increase in ethanol yield from 0.31 g/g to 0.43 g/g when separate hydrolysis and fermentation process was used on cafeteria food waste.

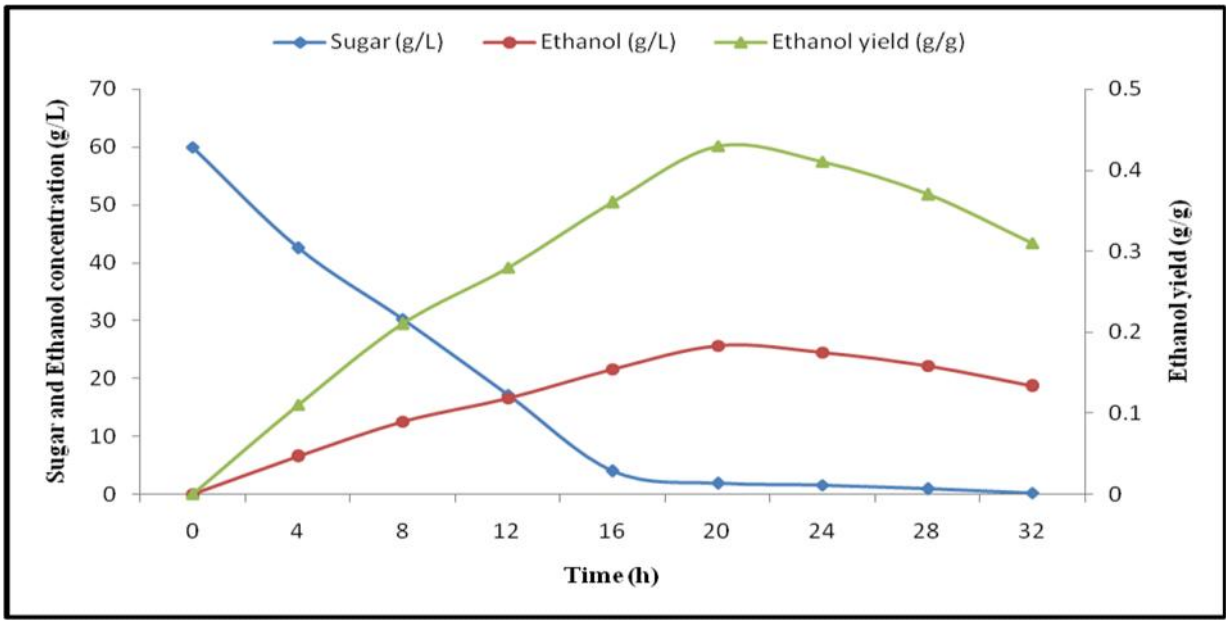

Fig. 2 Ethanol production using *Saccharomyces cerevisae* at 30°C, pH 5.0 and 150 rpm

Conclusions

In this study the fungus *T. atroviride* was exploited for cellulase production in flask experiments which was used for hydrolysis of alkali pretreated vegetable waste into glucose and then fermented to ethanol by *S. cerevisiae*. This study has explored the possibility of using vegetable waste for the purpose of bioethanol production with minimal energy consumption to provide aeration. These cellulosic substrates usually have a lot of lignin content which prevents easy access for the microorganisms for saccharification. Thus an alkali pretreatment method is necessary to delignify these wastes and to obtain higher reducing sugar yields and hence higher ethanol yields also. The ethanol production was studied in batch fermentation and the results showed that the batch fermentation produced 25.65 ± 0.55 g/L with 0.43 (g/g) ethanol yield and 1.28 g/L/h productivity.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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