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Enhanced Protein Production by *Saccaromyces cerevisae* by Exploitation of Pretreated Sugarcane Bagasse as Inexpensive Substrate

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Abstract

Sugarcane is backbone of Indian agriculture. Commonly, sugarcane is used for production of sugar and during this process a large amount of bagasse is generated. This bagasse is either burnt in the sugar mills for energy requirement or used as landfill. Some sugar mills are also using it for the purpose of electricity generation. However, this lignocellulosic waste could be converted in the value added Single Cell Protein (SCP) after pretreating the substrate with alkali. It was observed that pretreatment with 2% NaOH, increased the reducing sugar (10.81 mg/g) content in the medium. After inoculation with *Saccharomyces cerevisiae*, for five days, increased the mineral content from 2 to more than 9 folds. The SCB in the medium was found to be adequate to support 5.21%, 2.76%, 6.01%, 50.13% and 7.2% production of crude protein, true protein, lipid, crude fiber and crude ash.

Introduction

Proteins are associated with every aspect of animal life. The deficiency of protein in animals may lead to various health complications. The major source of animal intake of protein is through its diet. Most of the countries of the world, including some parts of India, are facing major malnutrition challenge due to the depletion of proteins and nutrition's from the foods of humans and animals. The search of unconventional source of protein is being investigated since mid twentieth century [1]. The inexpensive source of lignocellulosic feedstock is efficient and cost effective medium for production of Single Cell Proteins (SCP). Moreover, SCP production is more beneficial as compared to any other industrial agriculture uses.

Sugarcane occupies an important position in agrarian economy of India. The area under sugarcane is hovering around 4.4 million hectares and with an average productivity of 68 tonnes/ha. In more than 90 countries, it is being cultivated on about 25 million hectares, with more than 77 tonnes/ha of average productivity. Each tonne of raw cane production is associated with the generation of 130 kg dry weight of bagasse after juice extraction. It accounts nearly 279 MMT tones of biomass residues (bagasse and cane leaf matter) generation. Among the various crop residues, Sugarcane Bagasse (SCB) is very good staple sources of dry and green fodders, respectively for animals especially ruminants [2]. SCB, a fibrous waste after juice processing in sugar factory, is intensively being investigated for production of fuel [3]. However, the technological innovations have not lead to economical stable model for ethanol production from SCB. At present, the bagasse is being used inefficiently to meet internal power requirement for cane processing.

SCB comprises 40-42% cellulose, 24-28 % hemicelluloses, and 10-12 % lignin [4]. This could be used as substrate for the production of SCP, a high commercial product for animals. Moreover, due to high lignin and low N content SCB is not easily digestible and palatable [5]. Further, substrates with high nitrogen are prone to prevent digestibility among rumen [6]. However, it can be a potential source of energy for ruminants if exploited with suitable processing techniques [7]. To improve the nutritive value of crop residues, it is important to breakdown the linkages among cellulose and lignin by pretreating the biomass. The pretreatment process should be less energy consuming and cheap.

The aim of this study was to study the change in mineral content of SCB with microbial pretreatment method and to evaluate convertibility of SCB in SCP. Pretreatment of lignocelluloses is very important step, in order to increase the surface area of feedstock, removal of lignin and increasing the availability of fermentable sugar for optimum growth of the microorganism.

Yeast and yeast derivatives are natural diet additives that have been shown as effective growth enhancers and immunostimulants [8-10]. It is also good sources of vitamins and proteins. *Saccharomyces cerevisiae* has been at the top among all the microorganisms tested for SCP production. Other than the *Saccharomyces* the yeasts used as sources of SCP are *Pichia, Candida*, *Kluyveromyces* , *Torulopsis* , *Hansenula* , *Koloechera* etc. Many filamentous fungal species are also used as protein-rich food.

Materials and Methods

Microorganism

Saccharomyces cerevisiae was used for production of protein enriched animal feed. The yeast was maintained on Yeast Extract Peptone Dextrose Agar slants (in gram per liter): glucose, 20.0; peptone 20.0; yeast extract 10.0; and agar, 20.0 at pH 5.5, temperature at 30 °C.

Pretreatment of SCB

SCB was collected from Jaggery Unit, IISR, Lucknow. It was dried in a solar drier till it reached at constant weight. Dried SCB was pretreated with alkaline (2% NaOH, submerged for overnight) and acid (10% H_2SO_4, submerged for overnight) pretreatment at room temperature.

Inoculum Preparation and Fermentation

Inoculum (25 ml) was prepared in 100 ml Erlenmeyer flask with the following media composition (in gram per liter): glucose, 20.0; peptone 20.0; and yeast extract 10.0 at pH 5.0 and incubated for 24 h (150 rpm) at 30°C. After 24 h, the cells were recovered by centrifugation. The fermentation medium (100 ml) was prepared in 250 ml Erlenmeyer flask containing left over SCB with the supplementation of 0.2% of yeast extracts as a nitrogen source. The pH of the medium was adjusted to 5.0 using 1.0 M acetic acid by inoculating with 10 % of 24 h inoculum and incubated at RT at 150 rpm for 24 h [11].

Production of SCP

The ability of the organism to produce cell mass and protein from left over SCB (after harvesting of alcohol) as a sole carbon and energy source was examined in optimized salt medium containing (g/l): KH_2PO_4 0.25, $CaCl_2$, 0.025, KCl 0.5, $MgSO_4$, 0.025, yeast extract 0.3 and urea 4.22. The flasks were incubated at 30°C for 5 days.

Mineral Analysis

Sample (0.5 g) of SCP and control (SCB) was transferred to 50 mL beakers, 7.5 mL of nitric acid and 2.5 mL of hydrochloric acid were added. The samples were digested by heating on a hot plate, and about 20 mL of sterile distilled water was added and allowed to cool. The samples were filtered using Whatman No. 1 filter paper and the filtrates were made up to 100 mL with distilled water. The absorbance of the samples were taken using an atomic absorption spectroscopy and compared with prepared standard for each sample.

Determination of Nutrient Content

The nutrient analysis was done using the standard methods of Association of Analytical Chemists [12], for the determination of moisture content, ash content, crude protein, true protein, concentration of cell and lipid. Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

Culture samples (100 ml) were centrifuged (7000 g at 10°C for 10 min) to remove substrate. The substrate was washed twice with saline and dried. The culture broth (100 ml portion) was also centrifuged (10,000 g, 10 min). The cell mass was washed twice with saline, suspended in 10 ml distilled water and dried at 70°C. The remaining 100 ml sample, containing cell mass and unutilized substrate was dried (called dried biomass). The dried biomass was analysed for crude protein and true protein [13,14]. Micro-Kjeldahl's nitrogen of dry biomass was multiplied with 6.25 to calculate crude protein (Association of Analytical Communities, AOAC, 1990). For determination of true protein, 0.5 g homogenized and dry biomass was treated with 20 ml (5% v/v) trichloroacetic acid for 5 min with shaking and then placed at 90°C (in an oven) for 15 min and shaken occasionally. It was filtered while hot and the residue was rinsed with hot water thrice and dried to a constant weight. Its nitrogen content was determined with micro-Kjeldahl' method and true protein was calculated as above.

Statistical analysis

Statistical analysis was done by use of simple correlation coefficient.

Results and Discussion

Comparison between alkali and acid treatment

The bagasse after treatment with 2% NaOH exhibited maximum release of reducing (10.81 mg/g) and non-reducing (28.07 mg/g) sugar in the medium (Figure 1). The pretreatment process is cost effective and reliable way to disrupt the cellulose– hemicelluloses–lignin complex, and to achieve the increased digestibility of polysaccharides [15]. Alkali pretreatment method was reported to cause less sugar degradation than acid pretreatment, and exhibits lesser hemicellulose and cellulose loss than acid or hydrothermal processes [16]. After the addition of alkali, internal surface of cellulose increases due to and degree of polymerization decreases which leads to disruption of lignin structure.

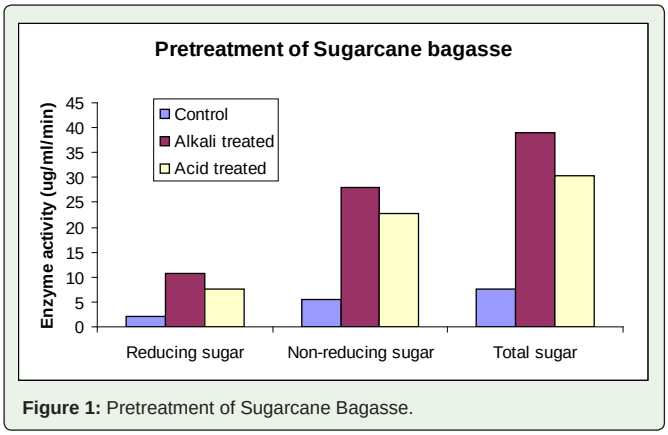

Figure 1: Pretreatment of Sugarcane Bagasse.

Table 1: Results for mineral analysis.

Mineral	SCB	SCP
Mg (ppm)	0.482±0.034	4.496±0.021
Zn (ppm)	-	0.308±0.032
Fe (ppm)	1.730±0.086	3.306±0.044
Ca (ppm)	8.730±0.348	17.418±0.431
Mn (ppm)	-	0.312±0.020

Table 2: Effect of pre-treatment on proximate composition.

	Crude protein (%)	Lipid (%)	Crude fiber (%)	Crude ash (%)	Moisture (%)
SCP	5.21±0.04	6.02±0.32	50.13±1.21	7.20±0.06	14.32±0.90
SCB	2.5±0.24	0.5±0.4	67.4±1.50	3.3±0.04	7.5±0.48

Mineral analysis of SCP

Table 1 shows significant ($p < 0.05$) increase in amount of Mg, Zn, Fe, Ca and Mn (4.496 ppm, 0.308 ppm, 3.306 ppm, 17.418 ppm and 0.312 ppm, respectively) in the single cell protein produced as compared to control. In SCB the amount of Zn and Mn was not detectable. The content of Fe and Ca double folded in SCP while Mg content increases by more than 9 times. Some researchers have reported the increase in the mineral content in SCP. This could be explained due to the increase in biomass, the mineral content also increased. The result is also in concurrence of the increased ash content in SCP.

Proximate analysis and kinetics of SCP

In Table 2, the results of proximate analysis reveal that there was significant ($p < 0.05$) amount of crude protein. The SCB in the medium was found to be adequate to support 5.21%, 2.76%, 6.01%, 50.13% and 7.2% production of crude protein, true protein, lipid, crude fiber and crude ash. Significant ($p < 0.05$) amount of moisture content (14.32%) was also observed. The high ash content indicates mineral content to be high. The increase in crude protein in SCP produced from pretreated SCB was much higher than the earlier reports [17-19]. The increase in crude protein in SCP probably resulted due to increased accessibility of fermentable sugars in medium which leads to build biomass and increase in the protein content. There were many reports of increased protein content after nitrogenous supplementation such as urea, Ammonium Sulphate [20] and Sodium Nitrate. However, crude fibre content decreases by 25%. The decrease in fiber could be attributed due to exponential growth of yeast and conversion of oligosaccharides in its monomer form.

There was enhanced substrate metabolism, resulting in build up of high cell mass (maximum cell mass (X) is equal to 50.2 g/l. The *S. cerevisiae* exhibited a very short lag phase and linear log phase in its growth curve. The Substrate consumption was completed after four days of incubation. The Crude protein was released in the medium after one day of incubation, which did not increase much with the time (23.8 to 28.3 g/l) (Figure 2). The shorter lag phase of the yeast could be attributed due to abundance of monosaccharides in the medium. Therefore, the production of SCP using SCB after pretreatment is a viable option as it increases the protein and mineral content and the fermentation period was also very short.

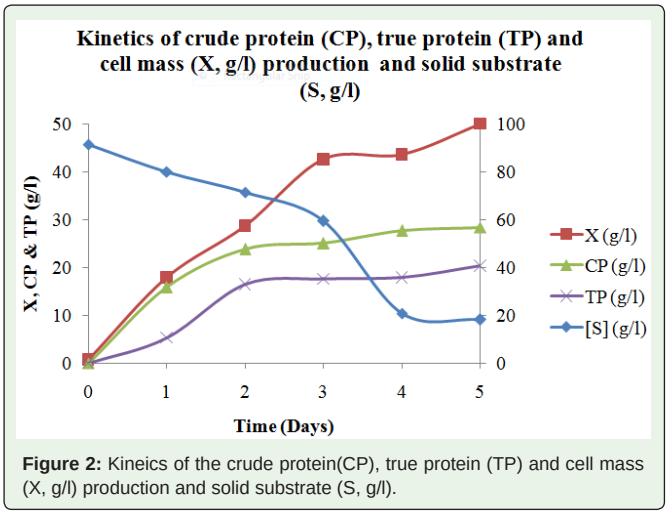

Figure 2: Kineics of the crude protein(CP), true protein (TP) and cell mass (X, g/l) production and solid substrate (S, g/l).

The work suggests that a large scale trial for the production of SCP using pretreated SCB should be considered.

Experimental

In the experimental design, effect of pretreatment on protein production was examined. It was compared to the control (Un-treated sugarcane bagasse). The pretreated bagasse was inoculated by *Saccharomyces cerevisiae*. This yeast has been widely used to produce proteins to be used as animal nutrition supplement [21]. The statistical analysis was done by correlation coefficient. The experiments were performed in triplicate and the results shown are mean values.

Conclusion

Based on the findings, it could be concluded that the sugarcane bagasse is a very good source of single cell protein production. The lignocellulosic nature of bagasse creates hindrance in accessing the microorganism to fully utilize the oligosaccharides. The pretreatment of bagasse removed lignin and hemicelluloses from the cellulose and increased the porosity of the materials. With increased ability to ferment the medium, the yeast was able to produce a mineral rich supplement which was found to be adequate to support 5.21%, 2.76%, 6.01%, 50.13% and 7.2% production of crude protein, true protein, lipid, crude fiber and crude ash.

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