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PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

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ABSTRACT

Herbal waste of processed medicinal plants of a local pharmaceutical industry was pretreated by steam explosion (160^0C for 0.5 – 2.0 h), and the process was accelerated by the use of Conc. H_2SO_4 (0.5 – 2.0%, 160^0C for 0.5 – 2.0 h), NaOH (0.5 – 2.0%, 160^0C for 0.5 – 2.0 h). Significant increase in the content of reducing sugars was found after acid/alkali pretreatment of steam exploded herbal biomass. Maximum Reducing sugars obtained was 145.9 mg/g of herbal waste with 1.5% of H_2SO_4 at 160^0C for 1.5 h. Bulk of herbal waste disposed off by pharma industry can act as secondary substrate as a source of fermentable sugars.

KEYWORDS: Saccharification, Pretreatment, Herbal waste

INTRODUCTION

Our industrial partner Ayurvet Ltd. formerly known as Dabur Ayurvet Ltd. (Baddi, Solan, H.P.) is one of India's leading animal care company. The company's portfolio includes herbal healthcare and nutritional products catering to a wide range of animal species. Ayurvet industry procures different medicinal plants and plant parts from different part of the country and whole biomass is dried before final industrial operation. The industrial operation includes grinding and mixing of various herbs and extraction with hot water. The extract obtained is filtered and processed for various commercial formulations and the residue left after extraction is known as herbal waste. During preparation of herbal products, industry comes up with enormous bulk of herbal biomass as waste. The waste is of diverse nature and composed of leftovers of medicinal plant and fruit parts. Although this waste is biodegradable, still industry has a problem in its disposal as in routine they get tonnes of this waste. Currently, most herbal waste is dried and used as low-value cattle feed. However a substantial part of herbal waste finds no suitable place for disposal. In a joint project we are trying to assess the potential of herbal waste as a secondary substrate for extraction of left over sugars. There is no report to the best of our knowledge on saccharification of diverse herbal waste

PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

(amalgamation of 7 plant parts) of industry. In our work, we have tried different pretreatment methods to get maximum released sugars from industrial herbal waste.

MATERIALS AND METHODS: -

Substrate

Herbal waste was dried and then ground mechanically to make it granular. The ground substrate was then sieved and stored at room temperature till further use.

Analytical methods

Composition of the herbal waste i.e. cellulose, hemicelluloses and lignin content was determined using Goering and Vansoest [1] and reducing sugars were determined by DNS method[2].

Extraction with Plain Water

Extraction with plain water was done using herbal industrial waste along with distilled water at shaking conditions 150 rpm for 2h.

Pretreatment of Herbal Industrial Waste

Ground waste was subjected to various physico-chemical pretreatments including steam hydrolysis, acid and alkali hydrolysis. Steam pretreatment was performed at various temperatures at 121^{o}C, 140^{o}C and 160^{o}C for 0.5h, 1h, 1.5h and 2h followed by sudden depressurization by fully opening the steam exhaust valve of autoclave. Acid and alkali hydrolysis were performed to accelerate the process at160^{o}C for different time intervals (for 0.5h, 1h, 1.5h and 2h). Sodium hydroxide (alkali pretreatment) and concentrated Sulphuric acid (acid pretreatment) from 0.5%. 1%. 1.5% and 2% w/v each were used respectively.

RESULTS

Industrial Operation and Quantity of Herbal Waste

The type of herbal waste used in this study was a complex mixture of all dicot species (*Punica granatum, Symplocus racemosa, Andrographis panicula, Salmalia malabarica, Woodfordia fruticosa, Berberis aristata, Aegle marmelos*).

Table 1: - Constituents of the Industrial Herbal Waste

Herbal waste Constituents	Percentage Content
Punica granatum (fruit)	24%
Symplocus racemosa (bark)	12%
Andrographis panicula (leaves)	10%
Salmalia malabarica (roots, stem, leaf)	3.5%
Woodfordia fruticosa (flowers)	14%
Berberis aristata (bark , roots)	13.5%
Aegle marmelos (fruit)	23%

The percentage of various herbs (Table 1) is only an approximate value because of varying constituents obtained after extraction of different polyherbal formulations. After the industrial operation, waste

PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

material in form of wet biomass is disposed off as such. Generally, industrial operation ends up with 1-2 tonnes of herbal waste daily which is point of concern for environment. Waste is variable and composition changes with every batch due to different product formulations in the industry. So, we have not specifically analyzed the cellulose, hemicelluloses, lignin content of each batch of the herbal waste coming out from the industry. Significant variations of carbohydrate composition were found even in same batch of the herbal waste. Hence, the waste has been found very heterogeneous in nature. However, the cellulose (%), hemicellulose (%) and lignin (%) were found in the range of 22-30, 20-30 and 10-18 on dry weight basis respectively. Remaining 20-30% was attributed as the amalgamation of pectin, crude fat, protein and organic solvents extractives; however the same was not determined practically in Laboratory.

Extraction of reducing sugars using plain water

Plain water extraction yielded an amount of soluble reducing sugar which was exceedingly low i.e. 11.25mg/g of the partially digested initial biomass which indirectly proved the efficiency of the industrial operation. However, Industrial operation did not target the complex biomass i.e. cellulosic/hemicellulosic/lignin matter of the herbs which actually made the bulk of the herbal waste. With this fact different physic-chemical pretreatment methods were tried to get increased sugar yield from herbal biomass.

Extraction Using Steam Hydrolysis

Figure 1 depicts the effect of steam explosion on herbal waste. With increase in autoclaving time from 0.5 to 2 h at160^{o}C, maximum of 17.25 mg/g of the reducing sugars obtained in the liquid fraction. Temperature beyond 160^{o}C could not be increased due to technical limitations. The concentration of reducing sugars obtained with treatment of various temperatures as well as for different time periods were significantly different based on 2-way ANOVA analysis (Figure 1).

PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

Steam explosion of herbal waste

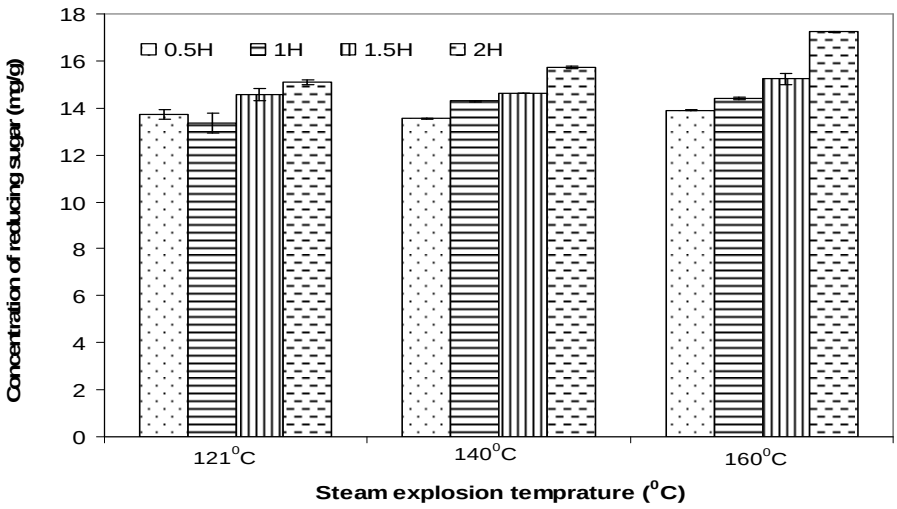

Figure 1 Experiments were done in triplicates. The vertical bar represents ± standard deviation. The LSD value is 0.84 at p = 0.05. The means of treatment at 121^{0}C and at 160^{0}C differ significantly between each other as the difference between them (1.062) is more than LSD value (0.84). All other mean differences are not statistically different. F-value between times is 300.24; between temperatures is 99.95 and the interaction between times x temperatures is 21.38; which are statistically significant at p < 0.001 based on 2-way ANOVA analysis.

Pretreatment of Steam Exploded Biomass using Acid and Alkali

Different percentages ranging from 0.5%, 1%, 1.5% and 2% both of acid (Conc. H_2SO_4) and alkali (NaOH) at 160^{o}C for different time intervals from 0.5h, 1h, 1.5h and 2h were tried. Maximum reducing sugar i.e. 144.75 mg/g of the initial biomass was obtained with 1.5% H_2SO_4 acid steam explosion at 160^{o}C for 1.5h. Fig 2 shows the effect of acid hydrolysis which was almost 8.3 fold increase in reducing sugars in comparison to steam explosion alone.

PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

Acid pretreatment of steam exploded herbal waste with variable concentration of H_2SO_4 at 160°C for different time intervals

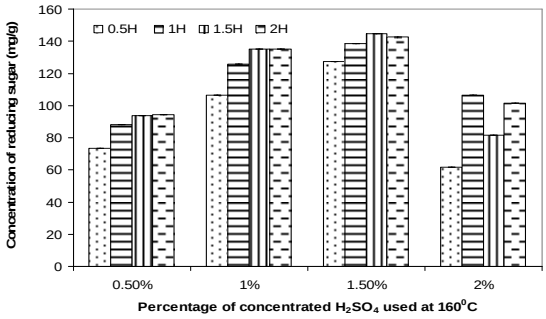

Figure 2 Experiments were done in triplicates. The LSD value is 17.51 at p = 0.05. The means of treatment at 1.0% and 1.5% of H_2SO_4 with different time period does not differ significantly between each other as the difference between them (12.78) is less than LSD value (17.51). All other mean differences are statistically significant at $p < 0.05$. F-value between treatments is 1157.62; between time periods is 237.46 and the interaction between treatments x time periods is 98.28; which are statistically significant at $p < 0.001$ based on 2-way ANOVA analysis.

Reducing sugar at a tune of 132.37 mg/g of the initial biomass was obtained with 2% NaOH at 160°C for 2h (Fig 3).

Alkali pretreatment of steam exploded herbal waste with variable conc. of Sodium hydroxide at 160°C for different time intervals

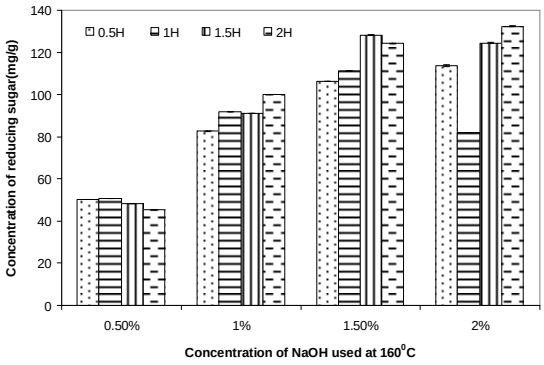

Figure 3 Experiments were done in triplicates. The LSD value is 23.31 at p = 0.05. The means of treatment at 0.5% and ≥ 1.0% of NaOH with different time period differ significantly between each other as the difference between them (≥ 42.81) is more than LSD value (23.31). All other mean differences are not statistically significant at $p < 0.05$. F-value between treatments is 51.08 and between time periods is 3.20; which are statistically significant at $p < 0.001$ and $p < 0.05$ respectively. The F-value of interaction between treatments x time periods is 1.75 and is not statistically significant based on 2-way ANOVA analysis.

PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

Concentration of alkali and acid was set towards higher side due to quite heterogeneous nature of the waste. No further increase in reducing sugar content was seen even after further increase of alkali/acid percentage (data not shown) vis-à-vis it also makes the process more cost intensive and polluting along with degradation of sugars. The concentration of reducing sugar obtained after acid hydrolysis and alkali hydrolysis is statistically significant using 2-way ANOVA analysis (Figure 2 & 3).

DISCUSSION

In contrast to our findings, other workers like Sharma et al [3] had observed significant increase in level of reducing sugar after pretreatment with steam hydrolysis method. Release of reducing sugar depends upon the nature of substrate used along with physical parameters. In most of the studies, virgin substrates like sunflower stalks, wheat bran etc. have been used, where as in our case a substrate used was the leftover of a mixture of processed herbs. So we tried to accelerate the process of reducing sugars extraction using acid and alkali treatment of steam exploded herbal waste.

Similar to our studies, Ruiz et al [4] and Zimbardi et al [5] got approximately 16.8% increase in sugar recovery in a synergistic action of acid and steam hydrolysis with corn stover. Ayurvet being a reputed animal pharma company that recently wins the TERI environmental excellence award for leadership efforts towards environmental management and sustainable initiatives is also at it's best in industrial operations leaving little available sugars in the disposed off biomass which is proved in our results as well. Low concentration of sugars may not be suitable for fermentation studies, however so many pharma companies working in our area disposing off herbal waste of variable nature and quantity may not be at their best as far as industrial extraction of sugars depends leaving substantial amount of sugars in the wet biomass which may act as an excellent substrate for bioethanol/biogas studies in future because it minimizes the potential conflict between land use for food production and energy feedstock production[6]. The concerted approach and improved processes are required to get hold of remaining fermentable sugars from the herbal waste of industries.

However, the viability of this technology on industrial scale depends upon multiple factors like yield of the reducing sugars, nature of the waste, cost analysis of the process, environmental policies etc. At present we are in process of characterization of the available sugars of Ayurvet industry.

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PRETREATMENT AND SACCHARIFICATION OF STEAM EXPLODED WASTE OF A PHARMA INDUSTRY

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