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Process Optimization of Production of Reducing Sugars from Spent Compost of Paddy Straw Mushroom

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Abstract—*Screening for raw material was done by comparative evaluation for the reducing sugar and cellulose content of SMS of different mushrooms. Spent substrate of paddy straw mushroom was selected as raw material owing to its high residual sugar content (83.2 µg/ml). The delignification of spent substrate was optimized using 4×4×4 CRD of Time, Temperature and Concentration of alkali by Tukey Test using SAS software. The correlation data by statistical analysis demonstrates that the major effect is of temperature and concentration of alkali with the responses being less dependent on time of processing. Delignification treatment was optimized at 0.75M NaOH at 60 ºC for 30 min. This was followed by treatment with enzyme cellulase to hydrolyze cellulose to simple reducing sugars to ensure maximum supply of reducing sugars.*

Keywords*: spent mushroom substrate, delignification, enzymatic hydrolysis.*

1. INTRODUCTION

With increase in acceptance of mushroom as next generation food, mushroom cultivation is on a rise. This increase leads to proportional increase in spent mushroom substrate. Handling and disposing of spent mushroom substrate (SMS) is envisaged to be a problem of future. Alongside with depleting levels of fuel and energy sources available for mankind new bio sustainable energy sources are being researched upon. Utilization of SMS for biofuel production will serve the dual purpose of waste management from mushroom industry and production of environment friendly fuel. Lau et al. [1] concluded that production of 1 kg of mushrooms will generate 5 kg of spent residual material. As per Singh et al. [2] an average farm discards about 24 tonnes of SMS per month. Approximately 254000 T and more than 800000 T of SMS is generated each year in Ireland and Netherlands respectively [3]. SMS has been used for various applications such as bioremediation, crop production, reusing as casing soil, animal feed, vermicompost, pest management and an alternative fuel.

SMS being lingo-cellulosic in nature could be a source of reducing sugars for producing biofuels and other value-added bio-materials [4, 5]. Removal of lignin and digestion of cellulosic component to reducing sugars is a mandatory step to realize these applications of SMS. Sulfuric acid has been used to pretreat SMS by Qiao et al. [6] and Kapu et al. [7]. The sulfuric acid pretreatment method involves high process temperature, which leads to high energy input and may also cause degradation of useful sugars and formation of fermentation inhibitors [8]. Sodium hydroxide, ammonia and lime have been widely employed as alkaline pretreatment agents in lingo-cellulosic biomass and have been proved to be efficient at mild conditions [9, 10]. Enzymatic treatment has also been tried by various researchers as Zhu et al. [11] by using cellulose and xylanase enzymes.

The present study is based on utilization of SMS for production of reducing sugars that can act as a quick substrate for fermentation to produce butanol. Two step pathway is optimized for maximization of reducing sugars production as a) lignin removal (by heat-alkali treatment) by CRD using *tukey* test; b) digestion to simple sugars (by hydrolyzing enzymes) using response surface methodology.

2. MATERIALS AND METHODS

2.1 Prescreening of SMS of different mushrooms

SMS of button (*Agaricus bisporus*), oyster (*Pluerotus ostreatus*), paddy straw (*Volvariella volvacea*) and shitake mushroom (*Lentinula edodes*) was dried and analyzed for total sugar (Phenol sulphuric acid method), reducing sugar (Nelson somogyii method) and lignin content. Sample with maximum sugar content and least lignin content was selected for further processing.

2.2 Heat alkali treatment

Treatment parameters (temperature, time and NaOH concentration) were varied according to 4×4×4 completely randomized design. NaOH (0.25, 0.5, 0.75 and 1M) at temperatures of 50,60,70 and 80 ºC inastatic water bath for 30, 60, 90 and 120 min were employed to pretreat dried milled SMS samples atasolid loading of10% (w/v).

After alkali pretreatment, the samples were adjusted to room temperature and filtered through whatmann paper 1. Pretreated solids were washed with deionized water repeatedly until the filtrate obtained neutral pH. This was followed by drying at at105 ºC for repeat analysis of total sugars, reducing sugars and lignin.

2.3 Enzymatic hydrolysis of cellulose

The dried alkali treated SMS is treated with enzyme (*cellulase*) at a solid loading of 5% (w/v). The solution is kept in a shaker incubator at RPM 50. Enzyme concentration, temperature and time of treatment are varied as per Design expert. Optimization is done using Response Surface Methodology (RSM) by design expert software.

3. RESULTS AND DISCUSSION

3.1 Prescreening of SMS of different mushrooms

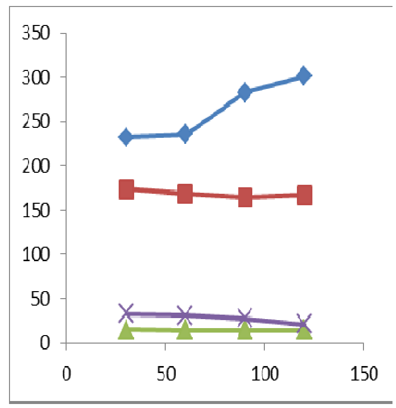
Selection of SMS from different mushrooms was done on basis of maximum availability of fermentable sugars. Content of total sugars, reducing sugars, cellulose and lignin in SMS of different mushrooms is shown in Table 1. As the amount to sugars available in SMS of *V. volvacea* ismaximum, spent compost of paddy straw mushroom is selected as raw material for processing into fermentable sugars.

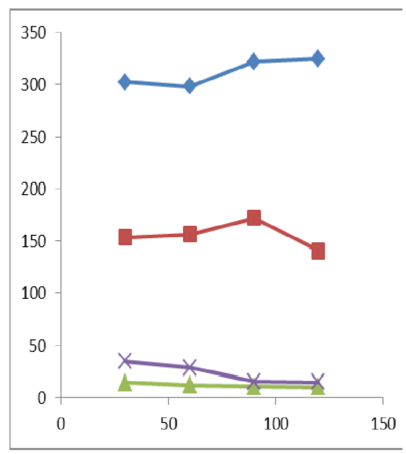
Table 1: Total sugars, reducing sugars, cellulose and lignin in SMS of different mushrooms.

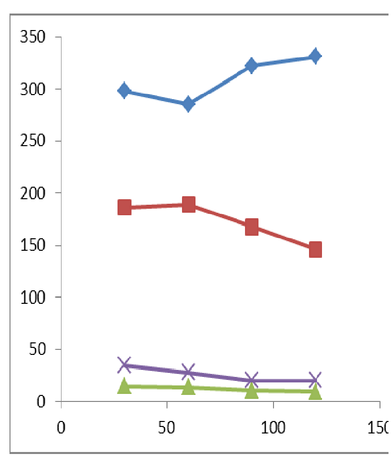
S. No	SMS	Total Sugars (µg/ml)	Reducing Sugars (µg/ml)	Cellulose (%)	Lignin (%)
1	Agaricus bisporus	72	45	16	20
2	Volvariella volvacea	83.2	50	6	32
3	Pleurotus ostreatus	74	23	20	22
4	Calocybe indica	86	13.5	6	28
5	Lentinula edodes	71	28	4	18

3.2 Optimization of alkali heat treatment

Delignification is a necessary step in processing of SMS to produce fermentable sugars as lignin interferes with fermentation. Effect of alkali (NaOH) heat treatment for delignification of SMS from *V. volvacea* is shown in Fig. 1-4. The delignification of spent substrate was optimized using 4×4×4 CRD of time, temperature and concentration of alkali by *Tukey Test* using SAS software. Delignification treatment was optimized at 0.75M NaOH at 60 ºC for 30 min.







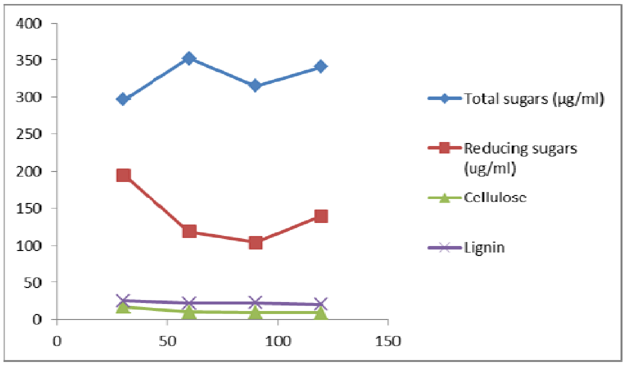


Fig 1.Effect of alkali heat treatment (0.25 M) on SMS of *V. volvacea* at 50, 60, 70 and 80 ºCwrt time (min).

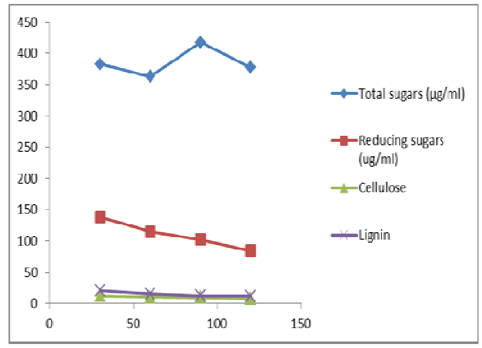
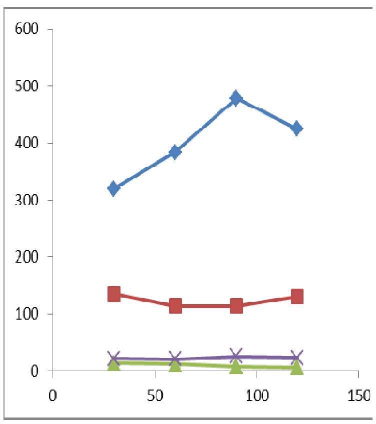
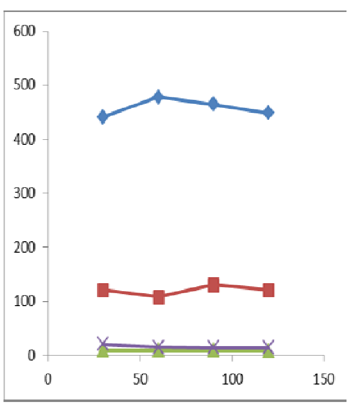
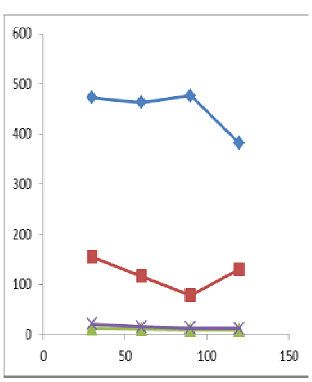
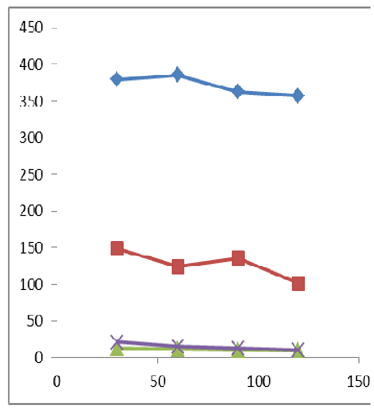


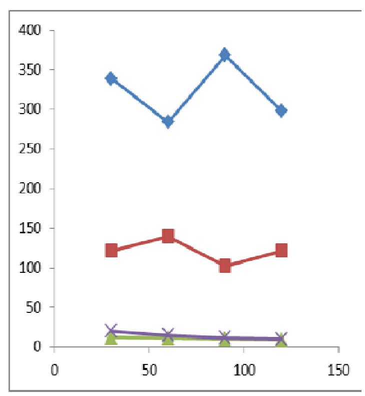
Fig 2.Effect of alkali heat treatment (0.5 M) on SMS of *V. volvacea* at 50, 60, 70 and 80 ºC wrt time (min).

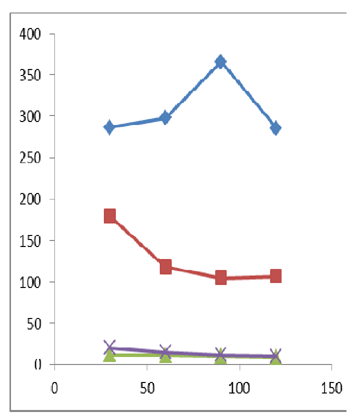












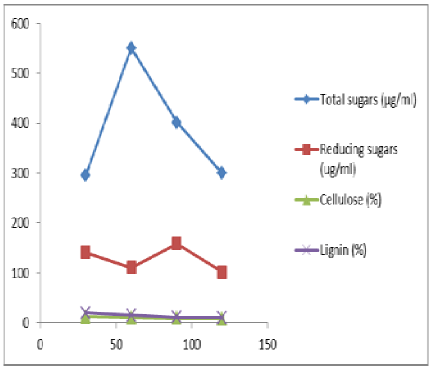
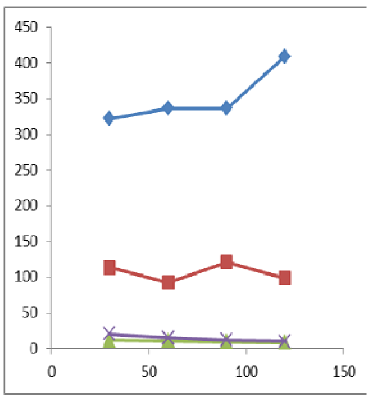
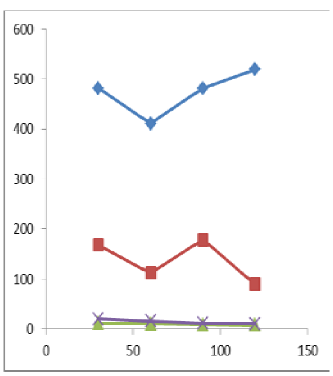
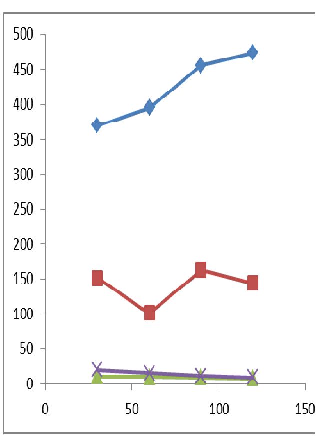


Fig 3.Effect of alkali heat treatment (0.75 M) on SMS of *V. volvacea* at 50, 60, 70 and 80 °C wrt time (min).







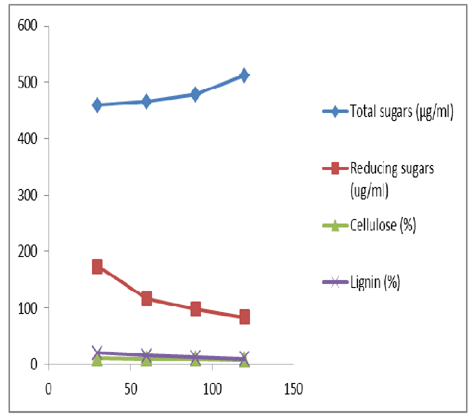


Fig 4.Effect of alkali heat treatment (1 M) on SMS of *V. volvacea* at 50, 60, 70 and 80 °C wrt time (min).

3.3 Enzymatic hydrolysis of cellulose

Delignified SMS was then treated with cellulose digesting enzyme (*cellulase* from *Aspergillus niger*). Time and temperature conditions for hydrolysis of cellulose were optimized using response surface methodology. Enzyme conc. varied from 4 to 8% in 5% substrate load basis, time varied from 48 to 72 h and temperature varied from 25 to 50 °C. Desirability for enzymatic hydrolysis of 0.784 was achieved at enzyme concentration of 7.8% for 72 h at 36.5 °C.

4. CONCLUSION

SMS can be a good source of fermentable sugars that can be production of many environmentally safe and biodegradable materials such as biofertilizers and biofuels. SMS of paddy straw (*V. volvacea*) mushroom had the highest residual level of reducing sugars. Delignification of SMS can be achieved by alkali heat method to a good extent without damaging the sugars as with acid treatment methods. The delignified SMS can be easily hydrolyzed by enzymes for release of fermentable sugars as the end product. The present study successfully optimized the delignification (alkali heat method) and hydrolysis (cellulase digestion) steps for production of fermentable sugars from SMS of paddy straw mushroom.

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