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Nitrogen Source Pretreatment of Ragi Straw for Fermentable Sugar Production using *Bacillus Pumilus*

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ABSTRACT

Biochemical conversion of lingo cellulosic biomass to ethanol provides a sustainable energy production system. Ragi straw is a fast growing domestic feedstock for Bio-ethanol production. In the present study ragi straw (*Eleusine coracana* L) was supplemented with different nitrogen Source prior to enzymatic hydrolysis by Bacillus pumilus isolated from marine sources process to produce fermentable sugars using different concentrations of the nitrogen source in the range of 0.5%, 1.0%, 1.5 % and 2.0%.The presence of nitrogen sources had an impact on the growth of the organism and supported enzymatic hydrolysis to degrade complex sugars to simple sugars, which could be further used for Bio-ethanol production. Ammonium nitrate proved to be the best nitrogen source at 0.5% concentration to support enzymatic hydrolysis of ragi straw which could be utilized for ethanol production as ragi straw is abundantly available.

1. INTRODUCTION

Lignocellulosic biomass, including forestry residue, agricultural residue, yard waste, wood products, animal and human wastes, etc., is a renewable resource that stores energy from sunlight in its chemical bonds [1]. Lignocellulosic biomass typically contains 50%-80% (dry basis) carbohydrates that are polymers of 5C and 6C sugar units. Most carbohydrates can be processed either chemically or biologically to yield bio-fuels such as ethanol. The prerequisite in the utilization of lignocellulose for ethanol production is to efficiently yield a fermentable hydrolyzate rich in glucose from the cellulose content present in the feedstock. Employment of enzymes for the hydrolysis of lignocellulose is considered the prospectively most viable strategy to offer advantages over other chemical conversion routes of higher yields, minimal by product formation, low energy requirements, mild operating conditions, and environment friendly processing [2,3]. Many agricultural wastes contain starch and/or cellulose which are rich in terms of carbohydrate contents. Complex nature of these wastes may adversely affect the biodegradability. Starch containing solid wastes is easier to process for carbohydrate and hydrogen gas formation. Starch can be hydrolyzed to glucose and maltose by acid or enzymatic hydrolysis followed by conversion of carbohydrates to organic acids and then to hydrogen gas. Cellulose containing agricultural wastes requires further pre-treatment. Agricultural wastes should be ground and then de-lignified by mechanical or chemical means before fermentation. Cellulose and hemicellulose content of such wastes can be hydrolyzed to carbohydrates which are further processed for organic acid and hydrogen gas production. It was reported that there is an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of agricultural wastes [4]. Ragi is a millet grown in India as a subsistence crop and is predominantly used by rural population for consumption [5,6] and India is a major cultivator of finger millet with a total cultivated area of 15870 km^2. The state of Karnataka is the leading producer of finger millet, accounting for 58 % of India's Ragi production (Govt of India - Ministry of Agriculture Report on Ragi harvest).

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2. MATERIALS AND METHODOLOGY

2.1 Isolation and identification of microorganism

The microorganisms were isolated from the marine sediment samples collected from the coastal areas of Cuddalore and identified using basic microbiological techniques [7] and molecular analysis. The microorganism was cultured in marine agar medium and checked for its cellulose [8,9,10], lignin [11] and xylan [12,13] degrading capacity. The microorganism which had the ability to degrade all the three substrates was found to be *Bacillus pumilus*. The screening of the organism on chemically purified substrates exhibited the capability to degrade the complex structures into smaller and simpler molecules, thus this was further used for the degradation of an economical and abundantly available agro waste like ragi straw which is grown in the southern parts of India throughout the year.

2.2 Optimization of nitrogen source

Five different Nitrogen sources were selected for the study namely, ammonium nitrate, ammonium sulphate, urea, sodium nitrate and potassium nitrate. Different concentrations (0.5%, 1.0%, 1.5% and 2.0%) of each source were taken into flasks. 5 g of ragi straw substrate was ground/cut into small pieces and was added into flasks containing 50ml water with different concentrations of nitrogen sources - 0.5%, 1.0%, 1.5% and 2.0%. The flasks were then autoclaved at 121^0C for 15 minutes at 15 psi. Once the flasks had cooled, they were inoculated with *Bacillus pumilus*.

The substrate was subjected to enzymatic hydrolysis at Room Temperature and neutral pH. The amount of sugar released and lignin oxidised was measured for 8 consecutive weeks. Readings were taken for reducing sugar estimation using DNS (Miller 1959) and lignin degradation assay using veratryl alcohol [14].

3. RESULTS AND DISCUSSION

About 128 bacterial isolates were obtained from Cuddalore sediment samples and subjected to screening for lingo cellulolytic enzymatic hydrolysis. The microorganism exhibiting maximal hydrolysis was identified as *Bacillus pumilus*, which makes the microorganism important for degrading agro waste into renewable energy.

The possibility of developing effective and inexpensive pre treatment techniques by modifying the pulping processes has been considered [15]. Ragi straw is one such agro waste which contains high amount of ligno cellulosic biomass which can be converted into energy like bioethanol using microorganisms similarly [16] carried out optimisation of waste like paddy straw, wheat straw and sugarcane bagasse using *Trichoderma reesei*. [17] carried out experiment on production of glucose, using enzymatic hydrolysis of waste cellulose fibre by the cellulose complex from *Trichoderma reesei* after pre treatment with H_3PO_4 (0.25%) which resulted 80 per cent degradation of substrate. Similar study was done by [18] wherein he concluded that *Phanerochete chrysosporium* was best suited for saccharification as it utilizes the substrate resulting in rapid breakdown of agro waste. [19] examined *Trichoderma viridae* and *Aspergillus niger* for their ability to produce fermentable sugars from cellulosic waste like wheat straw at 25°to 30°C within 3 days. For the better degradation of the biomass the substrate has to be pre treated with some of the chemical and physical treatments in which nitrogen source is an essential parameter which helps in the luxuriant growth of the microorganisms.

In the present study all the five nitrogen sources were utilised by the microbe for the growth and the organism showed cellulose degradation maximum with ammonium nitrate (Figure 1), ammonium sulphate (Figure 2) and sodium nitrate(Figure 5) at 0.5% followed urea (Figure 3) and ammonium phosphate (Figure 4) at 1.0%. The lignin degradation was maximally observed at 2.0% for all the nitrogen sources. Ammonium nitrate proved to be the best source of nitrogen for the degradation of ragi straw by *Bacillus pumillus* where ammonium sulphate at 0.05% was found to be optimal nitrogen concentration. The highest degradation of cellulose and lignin was observed in 4th week.

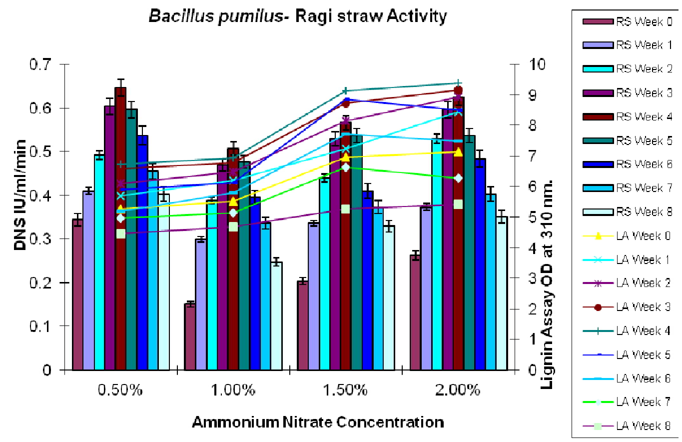

Fig. 1: Optimisation of Ammonium nitrate concentration for Lignocellulosic degradation by *Bacillus pumilus* on Ragi straw.

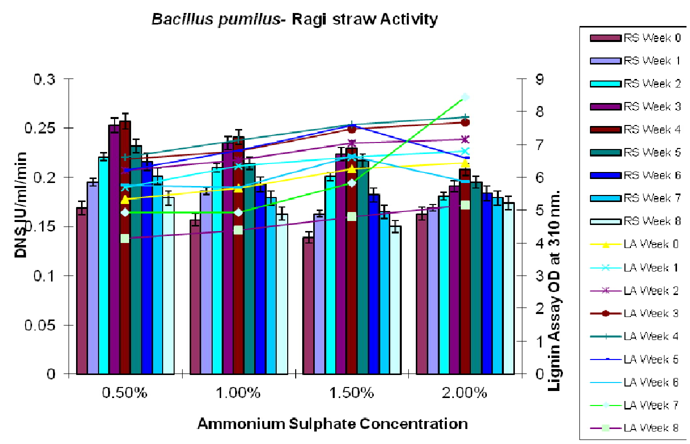

Fig. 2: Optimisation of Ammonium sulphate concentration for Lignocellulosic degradation by *Bacillus pumilus* on Ragi straw.

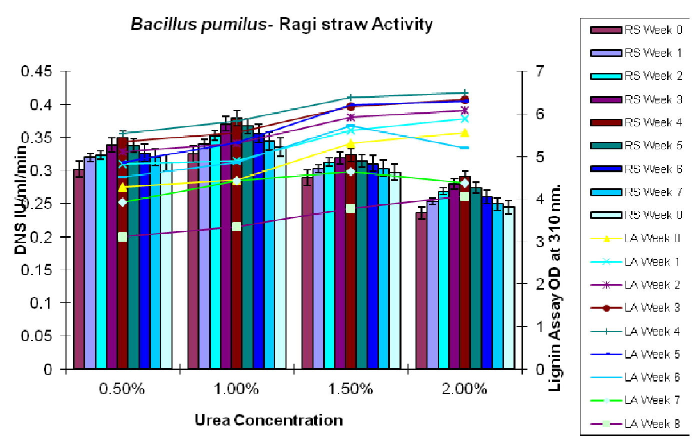

Fig. 3: Optimisation of urea concentration for Lignocellulosic degradation by *Bacillus pumilus* on Ragi straw.

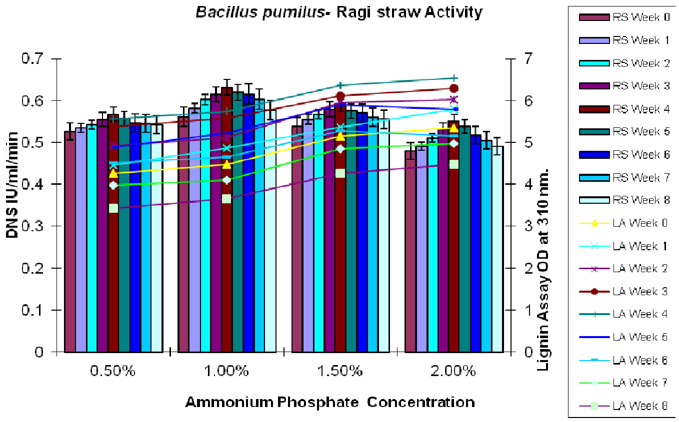

Fig. 4: Optimisation of Ammonium phosphate concentration for Lignocellulosic degradation by *Bacillus pumilus* on Ragi straw.

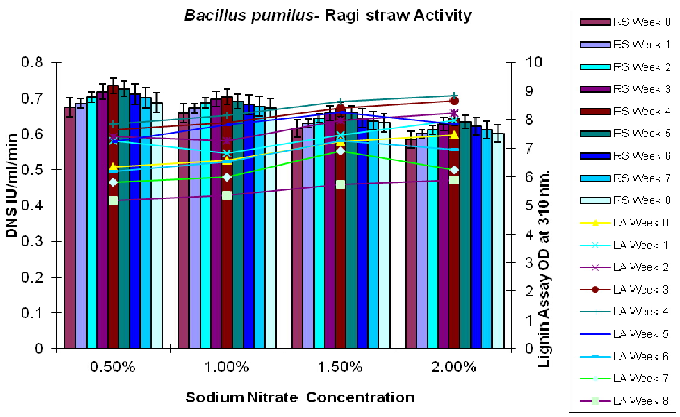

Fig. 5: Optimisation of Sodium nitrate concentration for Lignocellulosic degradation by *Bacillus pumilus* on Ragi straw.

CONCLUSION

No data available

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