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SOLID STATE FERMENTATION PRODUCTION OF FUNGAL CELLULASE ENZYMES AND THEIR APPLICATION IN BIOETHANOL PRODUCTION

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Abstract : Due to low biogas outputs, anaerobic digestion of bio sludge has yet to be adopted in pulp mills. Biogas yields have been improved by enzymatic pre-treatment of bio sludge, although the results have been mixed. Previous research has failed to account for the COD contribution from enzyme solutions, which is a major flaw. The purpose of this research was to look into the effects of enzymatic pre-treatment on the anaerobic digestibility of pulp mill bio sludge. Four of the six enzymes studied improved bio sludge anaerobic digestibility. With protease from *B. licheniformis*, a maximum improvement of 26% in biogas output was reported at the conclusion of the BMP. There was no link between enzyme activity on conventional substrates and/or bio sludge and the influence of enzymes on the environment. Yield of biogas Enzymes have the potential to improve bio sludge anaerobic digestibility, but further research is needed to determine ideal conditions and potential synergies with other pre-treatments.

Index Terms - : Anaerobic digestion, Bio-sludge, Enzymes, Pre-treatment ,Biogas.

I. INTRODUCTION

Cellulases are a class of hydrolytic enzymes that can depolymerize cellulose and break it down into smaller molecules. Cellulases are mostly produced by fungi, however some bacterial strains have been shown to produce them as well. (Priyanka P. and colleagues, 2017).

Trichoderma, Humicola, Penicillium, and *Aspergillus* are the most commonly studied cellulolytic fungi (Gupta et al., 2015). Basidiomycetes, such as *Chrysosporium sp*., are also widely dispersed and well-known for their ability to degrade cellulose (Thomas et al., 2013).
Due to lower penetrating ability and inability to use a wide range of substrates for cellulase production, bacterial cellulase was also researched, though in smaller numbers than fungal cellulase. The well-known cellulase-producing bacterial species are *Bacillus sp*., *Cellulomonas*, and *Clostridium* (Thomas et al., 2014).

In a 2019 study titled "Isolation of Cellulose Degrading Fungi from Decaying Banana Pseudostem and Strelitzia alba," **Legodi et al.** discovered that all *Trichoderma* and *Aspergillus* strains produced significant levels of all three enzymes required for complete cellulosic material hydrolysis (exoglucanase, endoglucanase, and -glucosidase). *Trichoderma* strains had larger amounts of ercellulase and endoglucanases, while *A. fumigatus* strains had higher levels of -glucosidase. The interaction of starting pH and incubation temperature on the microorganisms seems to have a significant impact on the production of all cellulases components. The efficiency of these fungal strains' cellulases in hydrolyzing agricultural lignocellulose wastes, such as banana pseudostem, for the manufacture of bioethanol will be tested.

Khattab et al. observed that *Penicillium chrysogenum* has potential cellulase production characteristics to use agricultural wastes as a substrate for producing cellulase enzyme, which could be an effective additive to improve ruminant diet digestion and utilisation in their 2019 study "Production Optimization of Fungal Cellulase and its Impact on Ruminal Degradability and Fermentation of Diet."

Vimal et al. observed that cellulose producing bacteria from areas rich in cellulosic biomass as well as optimise the fermentation conditions in order to achieve the maximum production of cellulase by the isolated microorganisms in their 2016 study "Isolation and identification of cellulose degrading bacteria and optimization of the cellulase production." Based on their capacity to thrive in

CMC coated plates, three bacteria were identified from paper industry waste. The ability of the strains to generate distinct zones in the presence of Grams iodine validated their potential cellulase generating activities. The isolates were recognised as *Bacillus cereus* after molecular identification using the 16s r RNA sequence.

The need to use renewable resources to meet future fuel demand has heightened interest in cellulose, the world's most plentiful and renewable material. Our current research looked on the superiority of *Aspergillus niger* over the other fungal cultures tested in terms of producing extracellular cellulases. The data show that the technique for producing cellulose from coconut cake trash was successful, since it produced a significant amount of this enzyme under laboratory settings. Furthermore, while the evolutionary operation factorial-design process may be useful in maximising enzyme yield, all parameters were optimised one at a time. Cellulase enzymes' high activity and stability will be useful in a variety of industrial and biotechnological applications.

Paper and Pulp Industry

The world's largest industries are paper and pulp. In 2013, the world's total paper production was expected to be 403 million tonnes (Bajpai, 2015). Special mechanical forces were used to grind and refine agro-industrial wood, resulting in a pulp with a high degree of acceptable stiffness and bulk quantity. Biomechanical grinding and refining with cellulases and hemicellulases is more cost-effective, saving 20-40% of energy and giving paper sheets more strength (Imran et al., 2019). The pulp and paper sector, which includes items such as office and catalogue paper, glossy paper, tissue, and paper-based packaging, uses approximately 40% of all industrial wood traded globally, according to the World Wildlife Fund (WWF) (Jayasekara and Ratnayake, 2019). Cellulases in conjunction with xylanases or cellulases alone are helpful in the deinking of many forms of paper waste (Imran et al., 2019).

Paper and pulp are natural resources that can be replenished. As a result, two popular concepts in this market are recycling and reuse. This is usually accomplished through the use of microbial cellulases. Cellulases are used in a variety of ways in this business. From the 1980s to the present, the potential applications have expanded into a wide range of fields. Deinking, pulping, bioremediation of industrial wastes, bleaching, and fibre improvement are only a few examples (Jayasekara and Ratnayake, 2019).

Textile Industry

The most often utilised enzymes in the textile industry are cellulases. Biostoning of denim garments (jeans) and biopolishing of cotton and other cellulosic fabrics are both done with cellulases (Bera et al., 2015). Traditional stonewashing of denim clothes involves amylase-mediated starch coating removal (desizing) and pumice stone treatment (abrasion) in huge washing machines (Menon and Rao, 2012). Cellulases are used in the biostoning of denim clothes to provide softness and a faded appearance, replacing the traditional use of pumice stones in the industry (Ray and Behera, 2017).

In the textile industry, fungal cellulases from Trichoderma reesei are the most commonly used enzyme. Actinomycetes of the genera *Streptomyces* and *Thermobifid*a, as well as other genera *Pseudomonas* and *Sphingomonas* bacteria are two examples of enzyme-producing bacteria.be utilised for textile dye decolorization and degradation (**Jayasekara and Ratnayake**,**2019**).

Waste management

Waste management can benefit from the usage of cellulase. Cellulases, for example, are used to convert cellulosic municipal solid wastes into useful chemicals and energy. Cellulases have the advantage of reducing the environmental impact of cellulose waste and pushing the conversion of pollutants to alternate sources of energy and chemicals, so reducing our growing reliance on fossil fuels (Ahmed, 2018).

Animal Feed Indusrty

Cellulases are used in animal feed production to improve the digestibility of cereal-based foods and increase nutritional contents for better forage quality. There have been instances of *Trichoderma cellulase* being used effectively as a feed supplement to improve feed conversion ratios and digestibility of cereal-based foods (Jayasekara and Ratnayake, 2019). Crude fibre is a measure of the amount of fibrous, poorly digested material in the feed in terms of feeding value. Cellulases are utilised to increase the nutritional quality of forages in this setting **(Brokner et al., 2012).**

Microbial fermentation has also been utilised to eliminate antinutritional factors such as agalactosides, glycinin, and -conglycinin are nonstarch polysaccharides found in animal feed. **Opazo (2012)** cultivated mesophilic, aerobic, and cellulolytic bacteria (from the genera Streptomyces, Cohnella, and Cellulosimicrobium) and tested their capacity to decrease soybean meal agalactosides and nonstarch polysaccharides using SSF. According to **Ray and Behera (2017),** SSF reduced total nonstarch polysaccharides by roughly 24%, stachyose by 83 percent, and raffinose by 69 percent while increasing protein content.

Laundry and Detergent Industry

Cellulases, particularly EGIII and CBH I forms, are widely employed in detergents for cleaning textiles, which is a relatively new development in the detergent business **(Khattak et al., 2012)**. EG III variations, particularly those from *T. reesei*, have been reported to be appropriate for usage in detergents by several groups of researchers **(Ray and Behera, 2017)**. Cellulases from fungi such as *Trichoderma sp*. (*T. longibrachiatum, T. reesei, T. viride, and T. harzianum*), *Aspergillus niger*, *Humicola (H. insolens and H.*

griseathermoidea), and *Bacillus sp*. have been extensively explored for use in detergents thus far. The most suited additions to ordinary detergents are alkaline cellulases. It's due to their ability to remove dirt and soil particles from the fabric's interfibrillar regions. The cellulases dissolve the rough projections of the cellulases. Attached to the cloth are cellulose fibres or cellulose aggregates. The fabric gains a higher sheen and smoothness as a result of this **(Jayasekara and Ratnayake, 2019)**.

Ladeira *et al.*, (2015) reported on the manufacture of cellulase by thermophilic *Bacillus sp.* SMIA-2 using sugarcane bagasse and corn steep liquor as substrates, and the detergent industry's potential. After 120 h and 168 h of culturing time, maximum avicelase (0.83 U/mL) and CMCase (0.29 U/mL) activity were achieved, respectively. Cellulase compatibility differed by laundry detergent, being more stable in the presence of Ultra Biz and less stable in the presence of Ariel. Furthermore, the enzyme remained stable in TritonX-100 and H2O2 inhibited sodium dodecyl sulphate and RENEX-95 **(Ray and Behera, 2017).**

Biomass Hydrolysis and Biofuel Production

Cellulases have lately been studied in the bioconversion of lignocellulosic wastes for the production of biofuel. Despite the abundance of cellulosic wastes, the cost effectiveness of this biodegradation method is a key drawback. Cellulases transform lignocellulosic material into fermentable sugars such as glucose and maltose, which are utilised as substrates to make bioethanol and other products. Although certain microbes have been found to have the potential to convert biomass directly to different alcohols, they are not employed as a commercially viable source. To convert lignocellulosic material into bioethanol, this technology uses a multi-step procedure. The hemicellulose and lignin fractions are improved throughout the pretreatment process, allowing for more efficient processing. The residues are next hydrolyzed at 50°C to yield fermentable sugars, followed by a final step of fermentation.

Reed has the potential to be used as a biobutanol and cellulase feedstock **(Srirangan *et al.*, 2012)**. Using Clostridium acetobutylicum under SSF**, Zhu *et al.* (2015)** successfully produced cellulase and biobutanol from reed. Without the use of commercial cellulase, Organsolv pretreatment can efficiently produce reed hydrolysate that can be turned to biobutanol. Furthermore, C. acetobutylicum fermented the hydrolysate medium and produced 14.24 g/L biobutanol and acetone/butanol/ethanol (ABE) with a yield of 0.33 g/g **(Ray and Behera, 2017).**

Wine and Beverage Industry

Cellulase enzymes, in combination with glucanase, can increase the quality and yields of fermented products like wine and drinks. Cellulase, pectinases, glucanases, and hemicellulases, for example, are employed in wine production to increase colour extraction, skin maceration, must clarity, filtering, and finally wine quality and stability. By hydrolyzing glycosylated precursors into aglycones and glucose, -glucosidases can improve the scent of wines **(Ahmed, 2018).**

Other Application

Cellulases have also been utilised in agriculture to hydrolyze the cell walls of plant pathogens, thereby preventing plant infection and illness. Many cellulolytic fungi, such as *Trichoderma sp., Geocladium sp., Chaetomium sp.*, and *Penicillium sp*., are recognised to play an important role in agriculture by improving seed germination, plant development and flowering, root system improvement, and crop yields **(Behera *et al.*, 2016).**

In addition, cellulases have been employed to improve soil quality **(Phitsuwan *et al.*, 2013).** In addition, cellulases are employed in food processing to increase the extraction of fruit and vegetable juices **(Sharada *et al.*, 2014).**

Cellulases, in combination with macerating enzymes, have been proven to boost olive oil extraction under cold processing conditions, as well as its antioxidant and vitamin E content **(Sharma *et al*., 2015).** Furthermore, because humans have a hard time digesting cellulose fibre, using a digestive enzyme product containing cellulases, such as Digestin, can aid with digestive issues like malabsorption **(Gurung *et al.*, 2013, Sharada *et al.*, 2014)**. Finally**, Li *et al.* (2012)** investigated the possibility of using cellulases enzymes in chemical analysis, such as diagnostic and food analysis.

Conclusion

As determined by BMP tests, enzymes can improve the anaerobic digestibility of biosludge. In these investigations, we discovered that pretreatment with B. licheniformis protease resulted in a maximum improvement of 26% after 62 days of biosludge digestion. With the exception of protease BCE 2078, all enzymes tested increased biogas output. Proteases from B. licheniformis and A. oryzae, a new glycosidase (SCO6604), and lysozyme from chicken egg white were among those tested.

Working under ideal conditions is recommended in order to assess the enzymes' optimum potential in improving anaerobic digestion and biogas production. As a result, more research into improving the enzymatic treatment conditions could be undertaken.
In this study, COD solubilization was not identified as a mechanism for improving anaerobic digestibility of biosludge; therefore, a dual treatment that first solubilizes COD and then uses enzymes to hydrolyze the solubilized COD is suggested to improve the impact of enzymatic treatment on biosludge.

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