Optimization of Agricultural Waste Hydrolysis using *Nepenthes mirabilis* Digestive Fluids

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Abstract—Waste management in the 21st century has been motivated by the rapid population growth and industrialization, climate change, generation of different waste, scarcity of resources and stringent environmental legislation. Waste management in South Africa in particular, is motivated by the need to develop green technologies for energy production and ensure compliance with national imperatives. It has been ascertained that the agricultural industry in South Africa largely contributes to the nation's total waste- an immense threat for surface and underground water bodies. This paper places a focus on a waste management strategy that utilizes the organic fraction of agro-waste (referred to as agro-waste/ agricultural waste/ lignocellulosic waste) as a raw material for biofuel generation using a cocktail of enzymes naturally produced by the *Nepenthes mirabilis* plant (pitcher plant) coupled with other pretreatment techniques. It highlights a proposed optimization approach for the technique using cofactors that naturally enhance the activities of previously isolated *N. mirabilis* enzymes.

Keywords—Biovalorisation, Co-factors, Delignification, Lignocellulosic biomass.

I. INTRODUCTION

Agricultural waste varies in physical, chemical, and microbial composition depending on the source. The greatest threats agricultural waste accumulation poses are landfill dumping, underground, surface water and soil sources contamination by virtue of their pollution potential. The generated agro-waste consists of different carbohydrate sources in varying amounts with great bio-valorisation potential. For example, citrus fruits for the beverage industry reached a worldwide production of 68x106 tonnes (t) in 2012. Approximately 30-40% of the fruits accumulate as citrus waste in the form of seeds and peels with a high cellulose and low lignin content respectively and are sold has dried feed. Another example includes the potential use of palm oil residues that amounts to 70% of the crop yield for cellulosic fibres [1].

Irrespective of the source type, the abundant lignin, cellulose and hemicellulose in agricultural waste material remain a great carbohydrate source for bio-valorisation. Considering that

agriculture is a common practice worldwide, it is thus safe to positively predict the sustainable production of biofuel from these crop residues [2].

A. The Need for Bio-valorisation of Agro-wastes

For decades, incineration has been the auto-remedy for agricultural waste management. The challenges with this waste strategy range from the illogical amount of energy required for incineration to the unacceptable amount of toxins released into the environment. With the stringent environmental protection legislations established, comes an even greater need for more eco-friendly strategies to agricultural waste management. When agro-waste undergoes pretreatment, lignin, the macromolecule responsible for the rigidity and recalcitrance of the plant is destabilised. This exposes the plant's hemicellulose and cellulose to hydrolysis that can further be utilised for biogas and bio-alcohol production. For example Oksman et al. [3] and Tsukamoto et al. [4] reported a suitable and profitable pathway to using beverage industry by-products by transforming fermentable sugars into bioethanol and cellulose nanocrsytals. Dlangamandla et al. [5] also reported the transformation of agricultural waste hydrolysates into butanol and ethanol. This integrated technology cuts down the amount of waste generated from agricultural activities while achieving a sizeable benefit for industries and reducing production costs [2].

II. OVERVIEW

A. Agricultural Waste

Agricultural waste is described as any residue obtained from first processing of farming products such as crops, meat, vegetables, fruits, and poultry. It encompasses both organic and inorganic wastes generated form the agricultural industry, i.e. livestock breeding, forestry, farming, grazing land, etc. These wastes accumulate in the form of slurries or sludge, solid state, and liquids rich in pathogens, organic and inorganic chemicals. Agricultural wastes can be characterised on the basis of the activities that generate them. A brief summary is given in Table I.

B. Physico-chemical Properties of Some Agro-wastes

Plant cell walls are composed of mainly cellulose, hemicellulose, and lignin. Agricultural lignocellulosic material contains approximately 40-50% cellulose, 20-30% hemicellulose, and 10-25% lignin with a great potential for biofuel generation [6]. The relative proportions of these polysaccharides (Table II) in a plant is an important factor to consider for pretreatment, i.e. a lignin-rich plant will resist

saccharification by virtue of its increased recalcitrance, while a cellulose-rich plant will require minimum energy for hydrolysis. Furthermore, choosing the ideal lignocellulosic biomass for subsequent bio-energy production is equally dependent on the ratio content of these polysaccharides [7].

TABLE I: A FEW AGRICULTURAL ACTIVITIES AND THEIR RELATED WASTES [8].

Agricultural activity	Type of wastes
Crop production and harvest	Straw, stover
Fruit and vegetable processing	Biological sludge, trimmings, peels, leaves, stems, soil, seeds and pits
Sugar processing	Biological sludge, pulp, lime mud
Animal production	Blood, bones, feather, litter, manures, liquid effluents
Dairy product processing	Biological sludge
Rice production	Bran, straw, hull
Leather tanning	Fleshings, hair, raw and tanned trimmings, lime and chrome sludge, grease

TABLE II: CHEMICAL COMPOSITION OF SOME AGRO-WASTE USED IN VALUE ADDED PRODUCT GENERATION (ANGADAM ET AL., 2019)

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Biomass in general	35–50	20–35	10–30
Different grass mixes	28.8 - 36.0	17.9 -24.7	13.4 -17.5
Wheat straw	40.2	38.8	17.0
Grasses	25–40	25–50	10–30
Corn stover	38–40	28	7–21

III. MATERIALS, METHODS AND ASSOCIATED LITERATURE

A. Pretreatment of Agricultural Waste

The natural tendency of lignocellulosic biomass to resist microbial and/or enzymatic digestion is termed recalcitrance. For this study, to achieve saccharification of cellulose, the biomass will be exposed to physical, chemical, and thermal pre-treatment. The pre-treatment methods render the tightly lignin-bound cellulose and hemicellulose accessible for hydrolysis [9]. Physical pre-treatment methods include grinding to reduce particle size and increase surface area for the lignocellulosic biomass [10] while hot water pre-treatment (HWP) techniques allows rapid delignification of biomass, eliminates the need for invasive chemicals usage, and is independent of the biomass particle size [11].

Irrespective of the aforementioned pros, Angadam et al [11] affirm that Dilute Acid pre-treatment (DAP: 0.4%-2% H_2SO_4 at 160-220°C) is advantageous over HWP as the latter produces a higher yield of fermentable sugars as compared to DAP. However, the authors report that both techniques result in the formation of inhibitory compounds at different conditions, e.g. HWP result in the production of furfural and phenolic compounds at elevated temperatures that impound on downstream processes, while DAP may result in the formation of soluble lignin from xylose degradation.

Both DAP and HWP serve as entry processes for Cellulases Pre-treatment (CP). Cellulases consist of a combination of endoglucanases, cellobiohydrolases (exoglucanases), and β-glucosidases [12]. Angadam et al [11] observed that pre-treating lignocellulosic biomass with mild acid prior to cellulases digestion resulted in an increase of 33% total readily dissolvable sugars as compared to the untreated biomass.

However, in spite of this impressive effect of dilute acid pre-treatment, the author reports formation of toxicants when subsequently treated with cellulases as opposed to sole-cellulases pre-treatment.

B. Nepenthes mirabilis: A Plausible Green Solution

Tropical pitcher plants, commonly known as Nepenthes spp., is the name given to the group of carnivorous plants belonging to the family Nepenthaceae. Naturally occurring in many tropical regions of South Eastern Asia, these plants have evolved physiological mechanisms that allow them to prey on insects and other small animals to thrive in their naturally low-nutrient habitats. These survival mechanisms are in the form of engulfing organs into which preys are trapped and utilised as primary food sources.

Carnivorous plants have captured the undeterred interest of scientists in the field of botany by virtue of their evolutionary defense and survival mechanisms, intriguing structure, drought tolerance, and nutritional strategy [13]. An example of its survival mechanism is the symbiotic relationship between the carnivorous plant and its endophytes present within the monkey cup that confer the plant survival properties. These endophytes include several fungal and bacterial species that asymptotically colonise the plant organs and are established as the natural microbial flora of this preventing secondary succession.

C. Physico-chemical Composition of the N. mirabilis Digestive Fluids

Dlangamandla et al [5] describes the physico-chemical properties of the pitcher plant's digestive fluids. To determine electrolyte strength and the ability of the digestive fluids to facilitate oxidation reactions, conductivity and redox potential of the fluids were measured using a multi-parameter meter. These characteristics are listed in Table III.

TABLE III: PHYSICO-CHEMICAL COMPOSITION OF THE *N. MIRABILIS* DIGESTIVE FLUIDS [5].

Characteristic/Parameter	Results
pH	1.80-2.2
Specific gravity	0.67-0.82
Redox potential	510-526 mV
Conductivity	3.5-5.89 mS/cm

D. Enzymes of the Digestive Fluid

The pitcher fluid contains digestive enzymes that extract nutrients from entrapped insects. Table IV shows a non-exhaustive list of prominent isolated enzymes of the carnivorous plant from different literature sources and basic substrates.

TABLE IV: ENZYMES IN NEPENTHES MIRABILIS PLANTS' DIGESTIVE FLUIDS [5, 11]

Enzyme	Substrates
Chitinases	Chitin
Phosphatases	Phosphomonoester
Proteases	Proteins
Nucleases	Nucleic Acids
Peroxidases	H_2O_2
Glucanases	Glucans
Lipases	Lipids

IV. RESULTS AND DISCUSSION

A. The use of Cofactors for Optimisation of Agro-waste Hydrolysis

Countless literature studies encourage pre-treatment of agricultural waste to facilitate and optimize downstream processes. An amalgamated pre-treatment technology ultimately results in higher product yield and simultaneously, form inhibitory compounds. It thus becomes paramount to introduce fundamental concepts that present possible solutions to ensuring product yield contain little to no inhibitory compounds that may halt important catalytic reactions. The use of a stream of co-factors that will bind to the digestive fluids' enzymes may not only result in an increase in product formation, but also in a decrease in furfurals and phenolic compounds.

Wilkie et al [14] reported that anaerobic digestion of Napier grass supplemented with nitrogen and phosphorus decreased methane (CH_4) production and increased volatile fatty acids (VFAs) production. Moreover, the author also reports a 40% increase and a significant decrease in CH_4 and VFAs respectively when a stream of molybdenum, sulphate, selenium, cobalt and nickel were added daily. Using the fundamental knowledge of the relationship between co-factors and co-enzymes, it is plausible to hypothesize positive results in terms of optimization. Moreover, the daunting task will lay in determining appropriate nutrients/cofactors for isolated enzymes.

V. CONCLUSION

The use of a stream of nutrients for agro-waste hydrolysis using the carnivorous plant's digestive fluids is a promising and innovative approach to waste management process optimization. To date, no study has directly reported this and thus challenges pertaining to process parameters such as optimum ratio of enzyme: cofactor quantities and even appropriate nutrients for isolated enzymes may prove a daunting task.

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