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PHYSICAL CHARACTERIZATION OF AGRICULTURAL BIOMASS AND EXTRACTION OF CELLULOSE FOR BIOFUEL PRODUCTION

B.Rabi Prasad[1*], Acharya Barsha [2], Sahu Monali[2a,] PanigrahiAnwesha [2b], PanigrahiSamikshya[2c]

[1]Department of Biotechnology, SoET , GIET University, Gunupur, Odisha, India,

[2] Department of Lifescience, School of Sciences , GIET University, Gunupur, Odisha, India

Abstract

Biofuel is the ultimate solution for the gasoline crisis that could have an impact on the next generation is biofuel. The creation of biofuel will also significantly reduce the need for and reliance on fossil fuels.The field of research is becoming increasingly interested in recent developments in the manufacture of bio-fuels from agricultural waste.The current study focuses on many processes that are involved, including the use of agricultural wastes for the manufacture of biofuels. The physical characterization of biomass is vital for selection and design of an efficient pretreatemt method for biofuel production. Cellulose is the major component of lignocellulosic biomass, its optimum extraction by using a suitable pretreatment method is necessary. In this study, we performed both chemical and biological methods of pretreatment, showing significant delignification. However, in order to make the pretreatment economical, eco-friendly and sustainable, optimization of biological method is required for large scale implementation.

Key words: Proximate analysis, Ultimate analysis, Pretreatment, LCB, Fossil fuel, Bioenergy

I. INTRODUCTION

The global energy consumption largely depend upon the fossil fuel, according to Holechek*et al*. (**2022**), it accounts around 81% of total energy consumption[1].Use of fossil fuel leads to depletion of fossil fuel reservoir, release of greenhouse gases and global warming. Fossil fuel emits carbon, nitrogen, sulphate as well as other oxides which results acid rain that cause soil infertility and effects many creatures like plants, human, animal as well as non-living things like monuments. Since 1970, overall greenhouse gas emissions from industrial processes have increased by roughly 78% while carbon emissions from fossil fuels have climbed by about 90% globally (**Ceylan S and Topçu Y2014**). The extraction of fossil fuel is much menace, difficult and mining of coal destroys the landscape. Fossil fuel is the non-renewable and unsustainable energy source. It is become necessary to find an alternative renewable energy resource (**Hamelinck*etal.*2005**). Bioenergy is one of the renewable energy derived from biomass such as plant, algae, agricultural and forest waste, biofuel is an alternative renewable bioenergy. The Indian Council of Agricultural Research estimates that India produces roughly 350 million tonnes of agricultural waste annually. This opens a wide scope in production of bioenergy as well as in the solid organic waste management. The biofuel derived from sugar, starchy crops, vegetable oil or animal fat is called as first-generation biofuel which affects the food chain. The second generation of biofuel, however, is made from lignocellulosic biomass, which includes waste from the food sector,agricultural, energy crops, grasses, and forestry. The cell wall of biomass primarily composed of 40–60 % cellulose, 20–40% hemicellulose, and 10-24% of lignin as main components (**Putro*et al* 2016**). The main factors influencing the composition of lignocellulosic biomass are the kind of biomass, the habitat, and the climate (**Jeffries 1994**).Cellulose and hemicelluloses are used for production of biofuel but their crystallinity structure with lignin prevents their large-scale use in biofuel production (**Horn *et al*.2012, Zhang*et al*.2007, Zavrel*et al*.2009**). The biofuel production is a three-step process such as pre-treatment, hydrolysis and fermentation. Pretreatment is the primary step that separates cellulose and hemicelluloses from lignin and thereby increases the accessibility of cellulose for hydrolysis. Pretreatment is performed by using one of the following methods like physical, chemical, physiochemical, and biological.

Homogeneity is a natural characteristic of biomass that determine the feasibility and viability of the recovery of products from biomass. In essence, products of value from biomass are to be recovered through the two major conversion pathways. The characteristics of biomass are also important for selecting a conversion method, and it is therefore essential to assess the fundamental physicological properties of biomass which will enable us to understand their suitability as feedstock in bioconversion processes; these factors shall be an integral part of effective use of biomass(**Adapa*et al*.2009, Asthana 2009**).

II. MATERIALS AND METHODS

2.1 Sample collection and preparation

Biomass samples collected from the agricultural fields of the Baragarh District of Odisha, such as rice straw (Oryza sativa), wheat straw (Triticum aestivum), bagasse (Saccharum officinarum), maize straw and corn cob (Zea mays).The samples were cleaned, sun dried, and then oven dried for six hours at 650C. Using a Jaisinghani 2HP SS pulverizer, dried samples of lignocellulosic biomass were processed into powder with a particle size of 1 mm **(Saifullah, 2019)** in**Fig 1**.

Fig. 1 Preparation of biomass sample from agricultural waste

2.2 Physical characterization of sample

Physical characterization of biomass sample is necessary to determine the caloric value and this information is essential for evaluating the different uses of biomass, notably its potential to produce energy.The physical characterization includes the estimation of amounts of fixed carbon (FC), volatile matter (VM), ash content (A), and moisture content (MC) by following standard methods (**APHA 2005**). For the generation of biofuel, biomass with a larger proportion of cellulose, volatile matter and a lower proportion of ash, moisture content is preferred (**de Lima *et al*.2021**). According to**Martinez et al**. (**2019**),fixed carbon (FC) wascalculated as the difference between 100 and the sum of VMand ash. These experiments were conducted using a muffle furnace (SISCO 220-230V) was used.

Biomass samples are generally hydrophilic in nature, the moisture content influence the storage of biomass and reduce net energy density by mass. The percentage of moisture content can be derived by the formula given below.

(%) of MC $= \left(\frac{\text{wt of sample - wt of oven dried sample}}{\text{wt of oven dried sample}} \right) \times 100$

The measurement of non-water gases produced during the heating of a biomass sample is known as volatile matter, it is determined as a mass loss. 1 g of oven dried biomass was placed in a crucible with a lid, and it was heated for 7 minutes at 925±10^0 C. The crucible was then put in a desiccator and left to cool to room temperature.The following equation can be used to get the volatile matter percentage.

(%) of **Volatile matter** = M (%) – MC (%)

Since, **(%)** of **M =** $\left[\frac{A - B}{A} \right] \times 100$

Where, A is the sample's initial weight, B is the sample's weight after heating, and M is weight loss.

The total ash content is the quantity of biomass that is still present after the sample has been burned. Using a muffle furnace (SISCO 220-230V), the ash content of the biomass samples was determined. A crucible containing a 1g oven-dried biomass sample was placed in the muffle furnace at 575±10°C for 3 hours. After that, the crucible was put in a desiccator to cool to room temperature. The ash content percentage was calculated using the formula given below.

(**%)** of **Ash =** $\frac{\text{Ashed weight - Crucible weight}}{\text{Sample weight}}$

The solid combustible residue known as fixed carbon is what remains after a sample has been heated at 900 °C for 7 minutes and the volatile materials have been driven out. The formula shown below was used to determine the amount of fixed carbon.

(%) of **FC**= $100 - \{(\%)MC + (\%)VM + (\%)Ash\}$

2.3 Chemical Pretreatment and Assessment of Biomass

One of the most effective ways to improve the biodegradability of cellulose by eliminating lignin and/or hemicelluloses, as well as to reduce the degree of polymerization (DP) and crystallinity of the cellulosic component in lignocellulose, is chemical pretreatment (**Valery et al**. **2011**).In order to breakdown lignin, hemicelluloses, and cellulose from lignocellulosic wastes, chemicals such as oxidising agents, alkali, acids, and salts can be utilized (**Behera *et al*.2014**).

2.3.1 Alkali treatment

The dried and powdered lignocelluloic material was treated with NaoH solution**(Zhao, 2011)**.5g of processed LCB material was treated with 100ml of each concentration of NaoH such as 1%, 2%, 3%, 4%, 5% and autoclaved at 15 psi for 15 minutes. The biomass was separated by filtration and washed repeatedly with tap water followed by distilled water until the pH of filtrate reached neutral**(Singh, 2019)**. Then the biomass residue was oven dried at 65°C for 3 hours and packed in air tight container for cellulose estimation.

2.3.2 Acid treatment

The dried biomass samples were treated with different concentrations of H_2SO_4solutions. 5g of dried biomass was treated with varying concentrations of sulphuric acid and autoclaved at 15 psi for 15 minutes**(Soni, 2023).** Then, the material was filtered and washed with tap water followed by distilled water until the filtrate pH reached neutral. The sample was kept in an air tight container for further analysis.

2.3.3 Cellulose estimation

The assessment of pretreated biomass was done through cellulose estimation, the estimation of cellulose of biomass samples were done by Anthrone assay (**Updegraff, 1969**). Taken 0.2g of sample and treated with 10ml of 67% (V/V) sulphuric acid and incubated for 1hr. Then, 0.1ml of this solution was mixd with 10 ml distilled water from which 2ml of sample solution was taken and mixed 3ml anthrone reagent. The mixture was boiled at 40°C for 15 minutes, allowed the solution for cool down at room temperature and O.D was measured at 630nm using Uv-Vis spetrcosopy (Systronics-119) (see **Fig.2**).

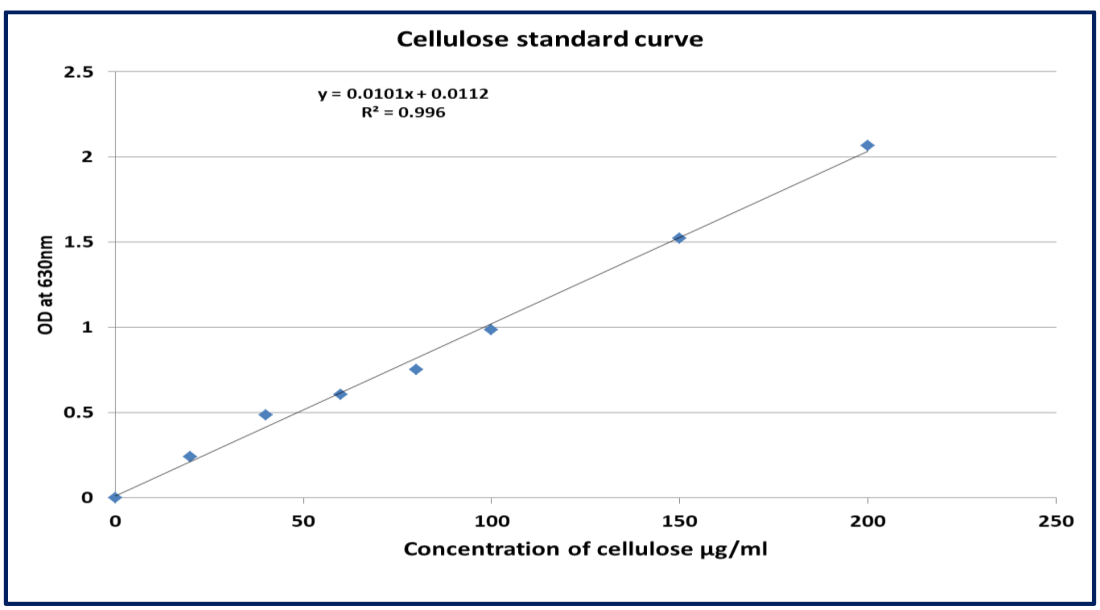


Fig.2 Cellulose standard curve

III. RESULTS AND DISCUSSION

The physical characterization presents a low content of moisture in wheat straw (5.9%) and high in corn cob (18%), biomass with moisture content ranging from 5% to 10 % is considered as suitable for biofuel production (4). However, volatile matter is high in corn straw (72.4%), ash (1.8%) and fixed carbon (9.2%) content is comparatively low in bagasse and corn cob respectively (Table-1).

Table 1: Physiochemical Characterization of LCB

Sl.No	Biomass	Moisture (%)	Volatile matter (%)	Ash (%)	Fixed carbon (%)
1	Rice straw	6.1±0.36	60±1.26	19.4±0.26	14.7±0.3
2	Wheat straw	5.9±0.25	56±1.23	16±0.24	16±0.20
3	Bagasse	13.8±0.26	71.5±1.26	1.8±0.28	13.2±0.48
4	Corn straw	14.3±0.26	72.4±0.82	3.2±0.22	9.6±0.56
5	Corn cob	18±0.28	72.3±0.26	3.5±0.21	9.2±0.45

The chemical pretreatment of biomass makes the material more accessible for enzymatic hydrolysis and fermentation**(Volynets, 2017)** . The results of both alkali (NaOH) and acid (H_2SO_4) pretreatment were analyzed through cellulose estimation using anthrone test by UV-Vis spectrophotometer**(Ganguly, 2021)**. Different concentrations of both alkali and acid (1%, 2%, 3%, 4% and 5%) treatment was performed for all the five biomass samples, the results indicates that the treatment with 2% concentrations of alkali and acid showed a significant delignification (see **Fig. 3and 4**) for all the samples. This adds advantage that chemical treatment with 2% of alkali or acid not only reduces use of large quantity of chemical solvents but also significantly reduces the formation of chemical inhibitors, use of distilled water for washing the pretreated biomass to make it neutral before hydrolysis. Moreover, it reduces the process cost and speed up the process, but in order to make the pretreatment process environmentally friendly and sustainable. It is necessary to look for a biological method of pretreatment.

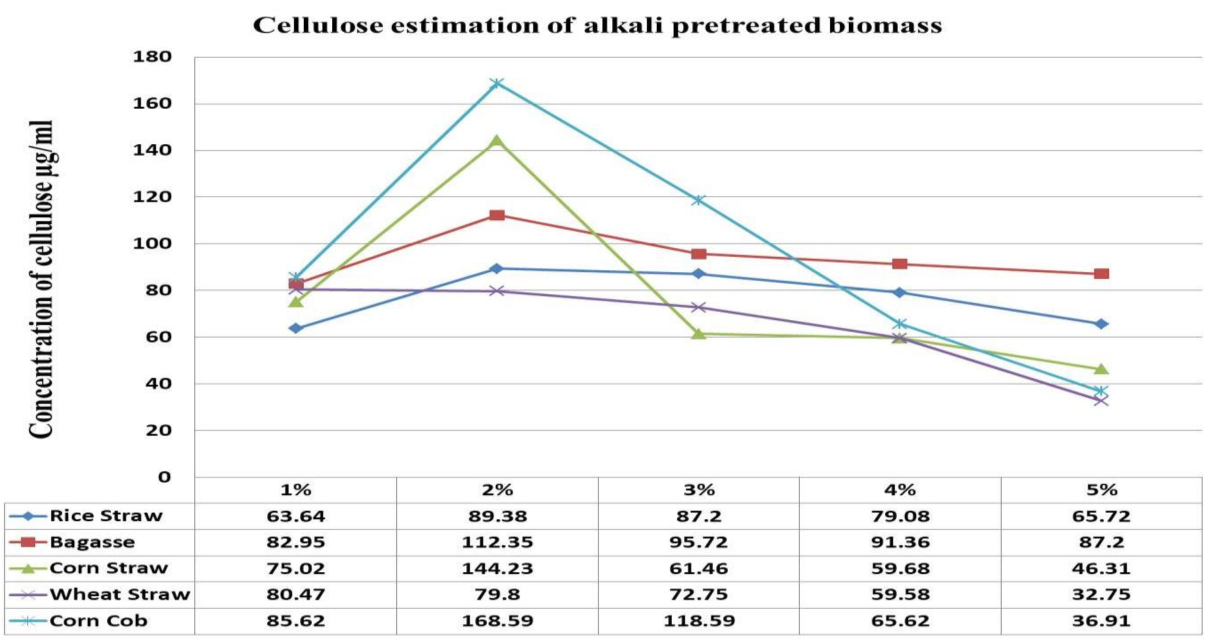
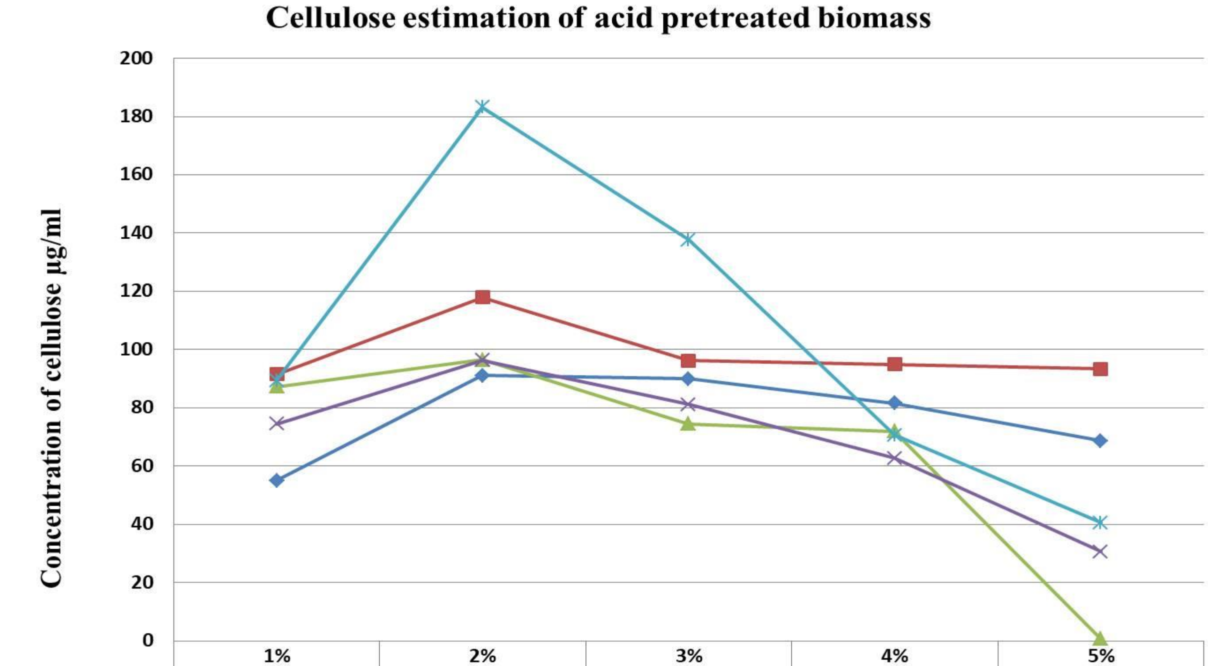


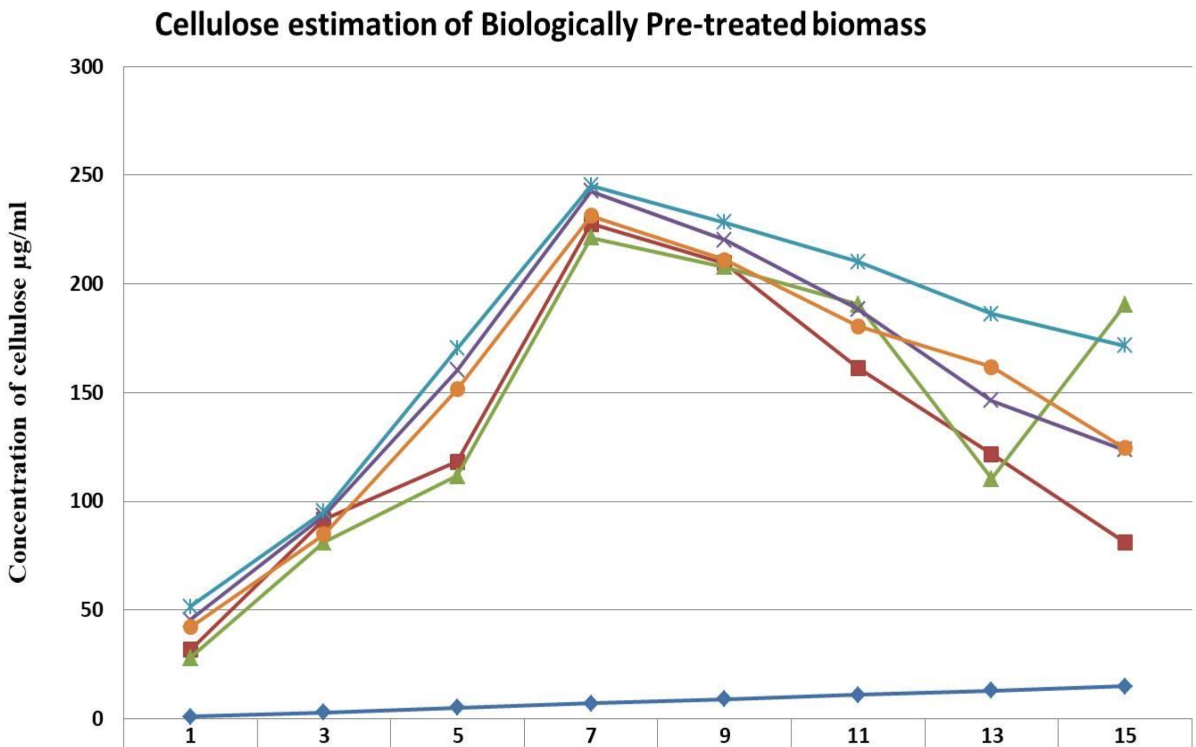
Fig.3 Cellulose estimation of alkali pretreated biomass



	1%	2%	3%	4%	5%
Rice Straw	55.12	91.06	89.98	81.66	68.69
Bagasse	91.56	118	96.21	95.02	93.34
Corn Straw	87.3	96.51	74.53	71.96	0.77
Wheat Straw	74.53	96.31	81.16	62.75	30.61
Corn Cob	89.38	183.24	137.7	70.67	40.57

Fig.4 Cellulose estimation of acid pretreated biomass

The biomass samples pretreated with bacteria, isolated from soil showed high rate of delignification, the result was accessed through cellulose estimation. The cellulose yield of all the biomass samples is more on 7th day of incubation and cellulose concentration is comparatively higher than the chemical pretreatment process (see **Fig.5**). This suggests that use of biological method for pretreatment is more efficient, environmental friendly, economical and not produce any toxic inhibitors. However, the incubation period makes the process lengthier and late. It is necessary to design a combination of chemical and biological pretreatment to make the process faster and environmental friendly.



Days	1	3	5	7	9	11	13	15
Rice straw	31.63	91.56	118.19	227.6	209.58	161.46	121.76	81.16
Bagasse	28	81.26	111.66	221.26	207.7	190.57	110.37	190.37
corn straw	45.32	93.34	160.27	242.65	220.27	188.39	146.31	123.64
corn cob	51.56	95.32	170.37	245.32	228.19	210.17	186.21	171.56
wheat straw	42.05	84.93	151.56	231.36	211.06	180.47	161.76	124.53

Fig.5 Cellulose estimation of biologically pretreated biomass

CONCLUSION

The results of proximate analysis like percentage of moisture, ash content and volatile matter indicates that the agricultural wastes such as bagasse, corn straw and corn cob afforded the highest potency and greatest prospect as a raw material in the production of biofuel. Similarly, the cellulose yield from biomass after pretreatment suggests that the biological method of pretreatment is efficient and sustainable. A critical step is to carefully select suitable microbial consortiums for efficient pretreatment of biomass. Moreover, the studies indicate that the co-culture of bacteria and fungus in combined bioprocessing would give better result.

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