Value added products from waste biomass and its application

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Submitted for the partial fulfilment for the degree of

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INDIA

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A thesis submitted in partial fulfilment of the requirements of the degree of Master of Bioprocess Engineering

Examination Committee:

Certificate

This is to certify that this thesis work entitled "**Value added products from waste biomass and its application**" submitted by **Mr. Sanket Roy** is a bonafide thesis work carried out under my supervision and guidance and fulfilling the nature and standard required for the partial fulfilment of the degree of **Master of Bioprocess Engineering**. The work embodied in this thesis has not been submitted elsewhere for a degree.

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Abstract

Rice husk is a prime agricultural derivative of rice cultivation in countries such as India where rice is cultivated and consumed as a staple food. In the current hunt for renewable resources for energy generation and production of products with high market value in a cost-efficient manner, rice husk seems to be a major contender. Starting from biofuel to the production of value-added products alike activated carbon and flavouring agents, rice husk can play an important role as lignocellulosic biomass. As a source of nature's most dominant biopolymer, rice husk constitutes cellulose, lignin and hemicellulose as the major building blocks along with silica and phytochemicals. In order to disintegrate the structure of lignocellulose followed by extraction of these compounds, different pretreatment methods have been utilised. Initially the literature survey emphasises the constituents of rice husk along with different pretreatment methods to reduce lignocellulosic biomass as a mean to extract cellulose, lignin and hemicellulose. Moreover, the synthesis of cellulose, lignin, hemicellulose, silica and phytochemicals and their usage in manufacturing of profuse value-added products such as bioethanol, biofuels, vanillin, xylitol, xylan, furfural etc. has also been addressed.

In the first part of the research, effect of ultrasonication assisted acid-alkaline pretreatment on rice husk has been studied to obtain effective removal of cellulose, lignin and hemicellulose. Alongside that, synthesis of microcrystalline cellulose and silica nanoparticle has also been performed. Besides that bioethanol synthesis using *Sacchromyces cerevisiae* along with furfural has been done from rice husk derived cellulose.

In the later part of the study, alkaline treated rice husk derived cellulose was used to remove wastewater pollutant malachite green from an aqueous solution. Effect of different parameters along with contact time were observed such as adsorbent dose, MG concentration, initial pH of the solution and process temperature. The isotherm, kinetics and thermodynamic parameters were calculated. In other words, the journey of rice husk from being a landfilling agricultural waste to the synthesis of numerous value-added products has been studied.

Keywords: Lignocellulosic Biomass, Rice Husk, Biofuel, Bioethanol, Waste management, Value-added Products

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Signature Of Candidate..

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Chapter 1:

1.1. Introduction:-

1.1.1. Rice Husk: Composition and potential in “Waste-to-wealth”

Rice husk (*Oryza sativa*) is a common biomass by-product of rice cultivation. It accounts for around 23% of total produced rice, resulting in an annual production of 545 million tonnes of husk, with China accounting for 50%, India being the second. When discarded as garbage, rice husk is difficult to break down and takes up a lot of space due to the low density in landfills. A significant part of this agricultural waste is utilised as cattle feed but mostly thrown away for disposal and animal bedding materials (Fig. 1). Nowadays, the majority of rice husk is burned for energy generation, reducing the volume to be disposed of (Junqueira et al. 2009; Yam and Mak, 2014). Although the ash residue after burning contains a high amount of silica, it poses a significant threat of pollution (Zhang et al. 2015). Lignocellulose (LC) is a naturally occurring polymer that contains lignin, cellulose, and hemicellulose. Rice husk being a lignocellulose (LC), is comprised of cellulose (~33 %), hemicelluloses (~20 %), lignin (~28 %), silica (~3% %), solubles (~3 %), and moisture (~7.5 %) (Ludueña et al. 2011). Both the by-products of rice cultivation, straw and husk can be utilised as substrate in order to generate invaluable products such as bioenergy, biofuel, biomaterials and biochemicals via chemical, biochemical, and thermochemical processes.

The exterior surface of rice husk comprises a significant proportion of inorganic silica (2-5 wt %), whereas the interior layer is mostly made up of lignin (~28 %) and cellulose (~33) with many vessels for conducting transfer of nutrients and water (xylem and phloem). Throughout the development of rice and rice straw, these vascular bundles serve as routes for the delivery of water and nutrients. Rice husk has been identified as a viable precursor for the synthesis of a family of crucial materials (Shen, 2017; Moayedi et al. 2019), like silica, silicon, silicon carbide, and permeable carbons (Azat et al. 2019; Shi et al. 2019).

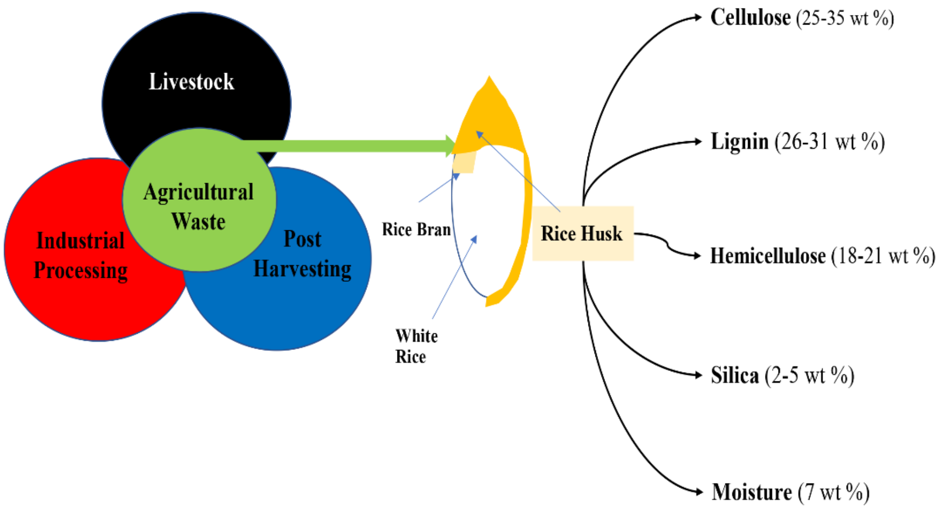


Fig: 1 Composition of Rice husk

1.1.2. Scope and Utilisation

Bioethanol and other eco-friendly substitutes to petroleum-derived fuels are being researched in order to minimise the world's reliance on fossil fuels (Gray et al. 2006; Balat and Balat 2008). Significant efforts have been taken to boost ethanol production from LC biomass in order to make this option economically viable and competitive with petrol (gasoline). On an industrial scale, bioethanol is presently generated from maize grains (starch) and sugarcane (sucrose), both of which compete for arable land and food production. Except that these food crops are genetically engineered to increase output in relatively short periods of time, and as the world's population grows, the use of these food resources for ethanol production will exacerbate the already-expanding food and energy crises (de Fraiture et al. 2008). As a result, lignocellulosic waste is viewed as an appealing resource for future ethanol supply due to its high availability, economical, and lack of conflict with human food sources (Tilman et al. 2006).

Moreover, when silica from RHs is extracted appropriately, it may be used to make a variety of silicon-based products like as silicon carbide, silicon nitride, silicon tetrachloride, silicon, and zeolite (Wang et al. 2011; Krishnarao and Godkhindi, 1992; Rahman and Riley, 1989; Seo et al. 2003; Wang et al. 2012; Sun and Gong, 2001). These materials are widely used in semiconductors, polishing materials, dyes, luminescence , biomedicine, and other fields. Recently, cellulose has emerged as an appealing sustainable resource, with applications ranging from gas permeation barrier films to sensors, and nanocrystals of cellulose (Yang et al. 2011; Poplin et al. 2007; Cai et al. 2008). However, the extraction of RH cellulose is a time-consuming and difficult operation. Ionic liquids (ILs) have been shown to successfully dissolve cellulose and lignin (Zhu et al. 2006) because the ions in the aforementioned liquid may behave as hydrogen bond acceptors, allowing the lignocellulose to be isolated from RHs (Swatloski et al. 2002; Liu et al. 2012). As a result, ILs are an excellent working medium for directly extracting lignocellulose from RH biomass and dissolving the organic components to produce a homogenous solution.

Considering the prior scenario with regards to the utilization of Rice Husk, the current bibliographical review shed some light on the prospect of rice husk utilisation, different treatment methods in order to synthesize various value-added products and their usage. This review aims to elucidate the journey of rice husk from agricultural waste to the source of various commercially viable products.

1.2. Review of Literature

1.2.1. Methods of Pre-treatment to synthesize value-added products:

Lignocellulosic biomass such as Rice Husk is resistant to biochemical degradation due to the presence of cellulossse crystalline structures, degree of lignification and complex structure of the cell wall. This phenomenon is known as biomass recalcitrance(Guerriero et al. 2016).

In order to amend the structural integrity of the biomass and reveal the lignocellulosic fractions for easier degradation, various pretreatment methods are used. These can be classified in two major classes viz. biological and non-biological. Non-biological (also known as non-microbial) pre-treatment is further classified into three regimes viz. physical, chemical and physicochemical while biological pretreatment involves the usage of microorganisms for the degradation of lignocellulosic biomass (Fig. 2).

1.2.1.1 Non-Biological pretreatment methods:

1.2.1.1.1 Physical Pretreatment:

a. **Mechanical extrusion**: The most common method of pre-treating lignocellulosic biomass where the raw material is undergone heating at around 300°C under convective mixing. This process is primarily used in order to produce gaseous products and activated carbons from pre-treated biomass (Shafizadeh and Bradbury 1979). In this process, the crystalline structure of the cellulose matrix is disrupted due to the high heat in the barrel and constant shearing force by the rotation of screw blades. Mechanical extrusion is used in the interest of defibrillation and fibre shortening along with the increment of overall carbohydrate availability for enzymatic degradation (Karunanithy et al. 2008). Studies have clearly indicated the importance of mechanical extrusion in the fractionation of cellulose and hemicellulose from lignocellulosic biomass. Nevertheless, due to the utilization of high energy to generate heat, mechanical extrusion is not a cost-effective method for industrial scale, hence this process is integrated with other pretreatment methods for better yield (Zhu and Pan 2010) (Fig. 2).

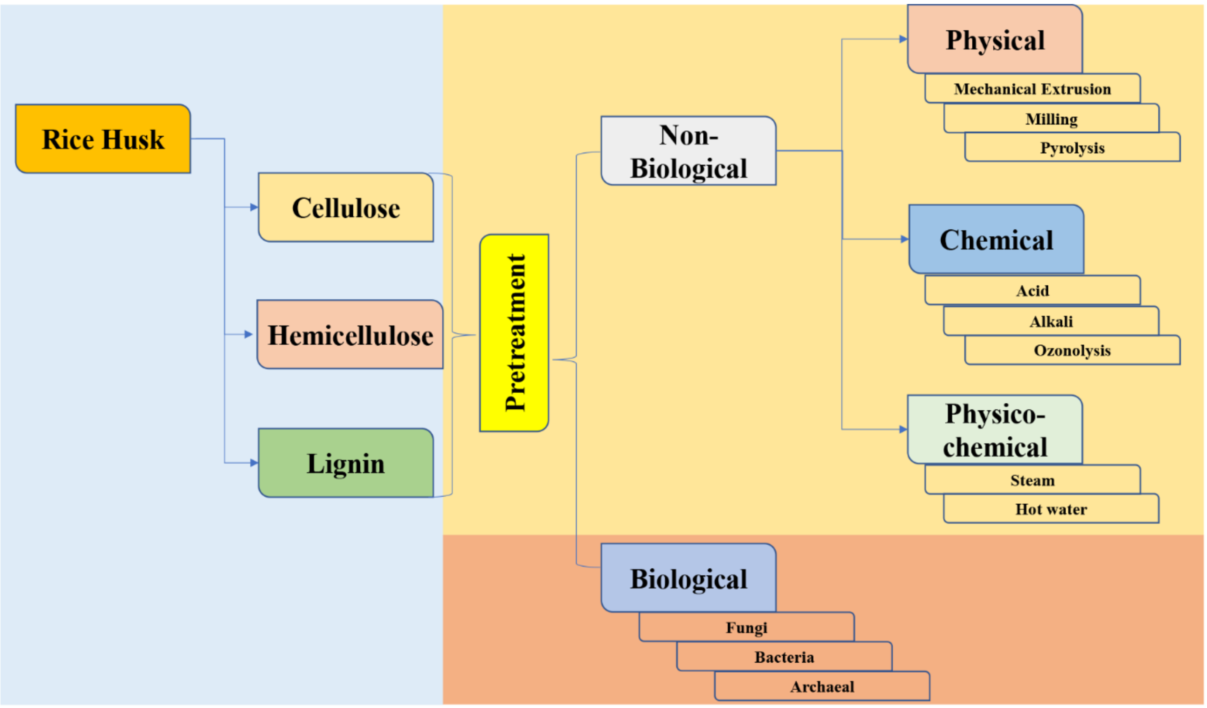


Fig.2: Pretreatment methods of Lignocellulosic biomass

b. **Mechanical Grinding (also known as Milling):** For the purpose of decreasing the crystallinity of cellulose, mechanical grinding is used. It majorly incorporates grinding, chipping and milling techniques. The size of the biomass can be reduced to 10-30mm due to chipping whereas the biomass size can be further reduced (up to 0.2mm) using grinding and milling. Although, it has been seen that, lowering the size of the biomass particle below 0.4mm does not have any crucial impact on the rate and output of hydrolysis (Chang et al. 1997). The decrease of mass and heat transfer limitations and reduction of particle size along with cellulose crystallinity are associated with chipping and milling, respectively. The enhancement of biomass surface area, the absolute polymerization level and the eventual reduction in the degree of cellulose crystallinity are contingent upon the type and duration of milling along with the type of lignocellulosic biomass used. In addition to that, various other milling techniques such as hammer milling, two-roll milling, colloid milling, and vibrator milling are also utilised to enhance the degradation of the LC material (Taherzadeh and Karimi 2008). Among these methods, vibrator ball milling is more potent in decreasing cellulose crystallinity and enhancing the digestibility of lignocellulosic biomass while due to the low consumption of energy, wet disk milling is a favoured mechanical pre-treatment. Although in a study conducted by da Silva et al. 2010, pre-treatment using ball milling has a significantly high yield of xylose and glucose hydrolysis yields over wet disk milling. Kim et al. 2013 found that attrition and planetary milling were further effective in the reduction of biomass size in comparison to ball milling, while planetary milling resulted in the most amount of glucose and galactose in comparison to other methods (Fig. 2).

c. **Microwave:** Due to the simplicity, cost-effectiveness, minimal energy demand, high heating capacity in shorter duration and efficient degradation of cellulose structural organisation, microwave irradiation is vastly used in the pretreatment of lignocellulosic biomass degradation. While performing microwave irradiation, the introduction of mild-alkali reagent can catalyse the reaction significantly resulting in higher yield (Hu and Wen 2008). The intensity of the microwave (power) can have an crucial role in the structure and yield of the product, but time doesn’t have a significant impact on the yield (Lu et al. 2011).

d. **Pyrolysis:** In biorefinery processes, pyrolysis has emerged as an important pre-treatment method for lignocellulosic biomass (Fig. 2). This is mostly used in the production of biofuel production from lignocellulosic waste instead of bioethanol production. In pyrolysis, a high temperature (500-800°C) is applied to the biomass in the absence of oxidizing agent. During this process, cellulose is directly converted into charcoal, pyrolysis oil and gaseous substances after decomposing (Kilzer and Broido 1965). Pyrolysis can be divided into two classes viz. slow pyrolysis and fast pyrolysis based on the rate of temperature increase. The amount of yield is vastly dependent on the pyrolysis conditions, biomass characteristics and reaction parameters. Notably, research has shown that pyrolysis exhibits greater efficiency at lower temperatures in the presence of oxygen (Kumar et al. 2009). Pyrolysis can be coupled with chemical pre-treatment to synthesize reducing sugar from cellulose. Fan et al. 1987 found almost 85% conversion from cellulose to reducing sugar after mild sulfuric acid (1N) hydrolysis on the pyrolyzed-pre-treated biomass.

e. **Pulsed-electric field:** In the pulsed-electric field, an immediate flow of high voltage (5-10kV/cm) is applied to the biomass resulting in creating a pore in the cellular periphery and exposing cellulose to degrading agents to synthesize reducing sugars. PEF is efficient due to the consumption of low energy as the exposure time is short (100µs). Along with that, in ambient conditions, it can be performed. PEF is utilised in the production of methane from sludge and manure during anaerobic digestion. This method is also used to extract phenols, proteins and other high-value components from agricultural biomass (Salerno et al. 2009; Yu et al. 2016).

1.2.1.1.2 Chemical Pretreatment:

a. **Acid Pretreatment:** Acid pre-treatment is the most frequently utilised chemical pretreatment method of agricultural lignocellulosic waste materials but due to its corrosive nature and production of inhibitory substances such as phenolics, aldehydes and 5-hydroxymethylfurfural, it needs special reaction conditions and reactor setups (Saha et al. 2005). Irrespective of that, pretreatment of lignocellulose using acids is the preliminary process of treatment in industries (Fig. 2). This involves two types of treatments viz. 1. Acid pretreatment for a shorter time (1-5 mins) at high temperature (>180°C) and 2. With lower temperature (<120°C) for a longer period (30-90 min) respectively. Concentrated acid pre-treatment of lignocellulosic biomass can lead to the synthesis of various inhibitory products but can also be recovered after pretreatment for use making the process economically viable. Different acids have been used in order to break down the complex structure of lignocellulose and produce reduced products. H2SO4 (Sulfuric acid) is the most common acid used for biomass pretreatment in a diluted condition. It has been found that a combined method of 1.5% H2SO4 followed by enzymatic treatment has resulted in 22.93% and 19.71% yield of reducing sugars from rye straw and Bermuda grass respectively. Pre-treatment using H2SO4 has resulted in a yield of more than 90% reducing sugar when coupled with aqueous ammonia. It can be identified that this method can be effective in the removal of lignin and hemicellulose from lignocellulosic biomass. Pretreatment of rice straw and wheat using acid yielded 287 and 565 mg/g of sugar respectively with less hydroxymethyl furfural and furfural synthesis (Saha et al. 2005). Regarding the cost-effectivity of sulfuric acid, pre-treatment of lignocellulose using this has enormous advantages along with some disadvantages such as contamination of inhibitory products (Lee and Jeffries 2011). In order to deal with that, some other acids have been utilised to break the structural integrity of lignocellulosic biomass such as rice husk. Apart from sulfuric acid, oxalic and maleic acids having higher pKa values can hydrolyse lignocellulosic substrates over a bigger span of temperature and pH (Lee and Jeffries 2011). Along with that, oxalic acid is far less toxic to yeast and other microorganism that are responsible for the extraction of value-added products from lignocellulosic waste materials.

b. **Alkali Pretreatment:** In contrary to the high temperature required for acid pre-treatment, alkaline pre-treatment methods can be conducted at normal ambient temperature (Fig. 2). Hydroxyl forms of potassium, sodium, calcium, and ammonium salts are the most common forms of alkali used in the pretreatment of lignocellulosic biomass such as rice (Kumar and Wyman 2009). Among these, NaOH (Sodium hydroxide) was identified as the most effective for the breakdown of the complex structure. It generally breaks down the ester and glycoside side chains leading to the change in the structure of lignin, swelling of cellulose, crystallisation of cellulose and hemicellulose liquefication (Ibrahim et al. 2011; McIntosh and Vancov 2010; Sills and Gossett 2011). It has been found that at 20°C and a reaction time of 144 hours, 1.5% NaOH can release 60% lignin and 80% hemicellulose from the lignocellulosic biomass in order to separate cellulose (Sun et al. 1995). Production of biogas has also increased by almost 37% after the treatment of sodium hydroxide in comparison to the raw material (Zhu et al. 2010). Compared with the acidic pretreatment, the solubility of hemicellulose and lignin is very low in alkali pretreatment. Due to this, the surface area of the cellulose can be increased along with the decrement of the degree of polymerization and crystal structure followed by lignin structure disruption (Taherzadeh and Karimi 2008). In alkali pretreatment, generally, ambient temperature is sufficient to conduct the pretreatment but in order to conduct the treatment for a long time, heat can be required. Also, after pre-treatment, a neutralisation step is necessary in order to remove inhibitors (Brodeur et al. 2011). The low cost of lime pre-treatment and its simple recovery method makes it suitable for large industrial-scale pre-treatment. Although it has certain drawbacks such as high downstream processing costs and the use of excess water.

c. **Ozonolysis:**

For the purpose of reduction of lignin content in lignocellulosic biomass, ozone treatment is used (Fig. 2). It inconsequentially influences hemicellulose and cellulose

(Kumar et al. 2009). Pretreatment of wheat straw using ozone resulted in the removal of 60% lignin along with 5 increments in enzymatic hydrolysis. Also, in the case of poplar sawdust, the lignin percentage was decreased to 8% and the yield of sugar has increased up to 57% (Vidal and Molinier 1988). Moreover, ozonolysis does not require any external heat or pressure. Along with that, the production of inhibitory compounds is limited in this method, making this environment friendly. Although, the moisture present in the biomass can negatively regulate lignin oxidation. Alongside, for the treatment, the amount of Ozone required is very high. Hence the cost related to the production of Ozone makes it a non-favourable industrial method for lignocellulose pretreatment (Quesada et al. 1999).

d. **Physico-chemical Pretreatment:** Under physicochemical pretreatment methods, the explosion of steam is one of the most abundantly used methods for lignocellulose breakdown (Agbor et al. 2011). It is commonly combined with mechanical (pressure) and chemical (autohydrolysis of hemicellulose acetyl groups) to generate high pressure (0.8-4.8MPa) saturated steam at a high temperature (160-260°C) for a shorter time causing the release of hemicellulose due to hydrolysis (Rabemanolontsoa and Saka 2016). Alike steam explosion, hot compressed water treatment uses high temperature and pressure to hydrolyse the lignocellulosic biomass but here hot water is used instead of steam (Yang and Wyman 2004).

   Other than these two, in wet oxidation, biomass is treated at high temperature using air or oxygen in a combination with water or hydrogen peroxide while under the CO2 explosion method entails passing supercritical CO2 via a high-pressure tube having the LC biomass in order to facilitates its breakdown (Varga et al. 2003; Kim and Hong 2001).

e. **Biological pretreatment:** When compared with the conventional physical pretreatment methods, the usage of microorganisms with the intent to reduce the lignocellulosic biomass via biological pretreatment is more environmentally friendly and efficient (Fig. 2). It requires less energy and cost as the abundancy of cellulolytic and hemicellulolytic microbes in nature is significant and these microorganisms are specific to a specific category of biomass (Vats et al. 2013). Biological organisms such as White, Brown, and soft-rot fungi are used to facilitate the degradation of lignin and hemicellulose along with a trace amount of cellulose as these organisms contain genes responsible for the production of peroxidase and laccases (Sánchez 2009; Kumar et al. 2009). These enzymes are accountable for the degradation of lignin. *Ceriporia lacerate, Phanerochaete chrysosporium and Ceriporiosis subvermispora* are some examples of soft-rot fungi responsible for lignin degradation. Alongside, *Ganoderma resinaceum, Irpex lacteus and Trametes versicolor* are some of the basidiomycetes which can help in the breakdown of several lignocellulosic feedstocks via delignification (Kumar et al. 2009). It has been identified that microbial treatment of wheat straw for 10 days using fungal isolate RCK-1 has increased the number of fermentable sugars and decreased the amount of fermentation inhibitors (Sun and Cheng 2002). Even though the pretreatment using biological organisms has high significance and conversion rate, the process seeks a longer time because of the the low rate of hydrolysis of lignocellulosic biomass.

1.2.2. Different Value Added products from lignocellulosic biomass

1.2.2.1. Cellulose from lignocellulosic biomass and usage :

The most abundant renewable polymer in the world, cellulose, serves as a vital constituent of the cell wall of higher plants and marine organisms (Hu et al. 2019; Liu et al. 2020). β-1,4-glycosidic bond acts as the linkage between D-glucopyranosyl units in order to form the chemically conserved structure of the polymer. In the structure, the abundance of hydroxyl groups in the C2, C3 and C6 of glucose helps in the formation of hydrogen linkage within and between the monomers of cellulose fibre. Hence hydroxyl group and associated hydrogen bonds play an important role in the crystallinity, structural integrity and physicochemical behaviour of cellulose (Moon et al. 2011; Wang et al. 2020). From the structural analysis of the cell wall of plant species, it is concluded that though the length of cellulose fibre depends on the source of the compound, as a single chain cellulose does not exist in nature. By combining multiple cellulose fibres more complex structures viz., elementary fibrils, microfibrils, and macro fibrils developed inside the biological organisms. Furthermore, these structures combine with hemicellulose and lignin to form cell walls (Sorieul et al. 2016). The extraction of cellulose from raw biomass can be done by using different pre-treatment methods discussed earlier in this chapter. During these processes, different stress is applied to the biomass that breakdown the bonds and separates different components. After breakdown cellulose can be isolated and washed for further applications.

Because of the abundance of cellulose, it has various applications in construction, wastewater treatment, pharmacology and nanomaterial research. Carboxymethyl cellulose (CMC) is a well-known compound used for drilling. Drilling is a technique involved in the oil exploration process (Aftab et al. 2017). The most commonly used drilling fluid, oil-based drilling fluid causes safety and environmental issues. To eliminate these problems CMC is used in water-based drilling and act as an additive. It has an important role to maintain the fluidity of the medium in high temperatures and pressure. The Commercial CMC is capable of reducing filter loss due to its high viscosity and low viscosity grades. (Jha et al. 2015). Another compound derived from cellulose is Polyanionic cellulose (PAC) with similar characteristics to CMC but with high purity and degree of substitution. From recent studies, it is clear that PAC is more efficient than CMC for performing the drilling process (Bennion et al. 1998). Another significant cellulose derivative used for drilling is hydroxyethyl cellulose (HEC) not used directly as drilling fluid but due to its molecular weight act as a viscosifier (Caenn and Chillingar, 1996). In the last few years, many patents are proposed for the fluid loss control capacity of HEC. In the 1950s, Hurley developed a carboxymethyl hydroxyethyl cellulose (CMHEC) based cement slurry that can reduce fluid loss as well as adjust the time for thickening (Hurley, 1959). Frederick manufactured a high-temperature-resistant cement slurry that contains 52.5% HEC (Frederick et al. 1964).

Apart from the application in the drilling and cementing industry, the depolymerization of cellulose opens numerous possibilities to develop new products and potential monomers. The depolymerization of cellulose can be done by the hydrolysis of β-1,4- glycosidic bond and producing glucose. Various processes viz., enzymatic degradation (Zhang et al. 2005), degradation in supercritical water (Sasaki et al. 2000) or acid-catalysed process (Climent et al. 2011) are used to depolymerise cellulose. Glucose, the most relevant monomer created after the depolymerization process can undergo fermentation and produce ethanol (Sun et al. 2002). Furthermore, some other products viz., itaconic (Abe and Takagi, 1991), lactic (Kong et al. 2010), glucaric (Pamuk et al. 2001) and succinic acid (Delhomme et al. 2009) are also can be produced by chemical methods.

Another derivative of cellulose is cellulose-based nanocomposite. The application of this substance is from the paper and packaging industry to medical applications. The use of cellulose nanocomposite in the paper and packaging industry is very significant to replace synthetic polymers (Lavoine et al. 2012). It also acts as a gas and water barrier with a toxicity-free nature. For this reason, it is highly accepted in food packaging (Nair et al. 2014). Nanocellulose composites are also a potential candidate to use in the electronic industry to enhance conductivity and flexibility (Razaq et al. 2012). Apart from these, another major application of the composite is in the field of biomedical research. Because of their properties as modifiable surface chemistry, and high surface area to volume ratio, it is used in the development of hydrogel that is capable in drug and gene delivery (Sampath et al. 2017). studies also reported that the electrospun poly (e-caprolactone)/nano cellulose composite has scaffold properties and can be used in tissue engineering (Si et al. 2016).

Further processing of cellulose viz., cooking, bleaching can develop microcrystalline cellulose (MCC). This compound is a purified and partially de-polymerized version of cellulose. Cellulose fibre has mainly two parts viz., crystalline part and amorphous paracrystalline part (Yang et al. 2019). During the acid hydrolysis of cellulose, the paracrystalline area hydrolysed results in the formation of more crystalline MCC. MCC is used as a filler and binder in the medical and food industries. Also it has applications as a suspension stabilizer, viscosity regulator and emulsifier in colloidal form (Trache et al. 2016). Some characteristics of MCC viz., water insolubility, lightness, non-toxicity, crystallinity, biodegradability etc. make this product more accepted at the research and industrial level.

Various techniques are used to isolate MCC from the rest of the biomass. Mainly physical, chemical, biological and combined pre-treatment is used to extract cellulose from lignocellulosic biomass (Agbor et al. 2011; Pandey et al. 2015). Furthermore, the production of MCC demands maintaining extreme purity. Hence, the extracted cellulose undergoes cooking, acid hydrolysis, neutralization and bleaching (Thoorens et al. 2015).

Another variant of these extremely pure cellulose derivatives is nanocrystalline cellulose (NCC). Unlike MCC, NCC is more pure, crystalline and fine particles. They are rigid, rod-like crystals with diameters ranging from 10-few hundred nm (Peng et al. 2011). The main process to produce NCC from native cellulose is acid hydrolysis. As mentioned before, cellulose fibres contain two main parts an amorphous region and another crystalline region. By conducting acid hydrolysis, the amorphous regions break down releasing the individual crystals (Rånby, 1951).

1.2.2.2 Hemicellulose from lignocellulosic biomass and usage :

Hemicellulose is a polymer containing various sugars, including pentoses (such as xylose, arabinose), hexoses (glucose, mannose, galactose) and sugar acids. Depending on the structure, hemicelluloses are classified into four types, viz., Mannans, Xylans, Xyloglucans and mixed-linkage β-glucans. Unlike cellulose, it is not chemically homogeneous. Hardwood hemicellulose contains mostly xylans, on the other hand, softwood contains glucomannans. Xylan in particular, is composed of xylose monomers interlinked through β-(1,4)-linkages (Rennie and Scheller, 2014). Though the compounds of the backbone are frequently accompanied by various carbohydrate monomers and oligosaccharides like xylose, glucose, arabinose, and glucuronic acid (Heinze and Liebert, 2012).

The xyloglucan backbone is made up of D-glucopyranosyl residues, which are further replaced by several small-chain polysaccharides(also termed oligosaccharides), such as galactosyl, arabinopyranosyl, and arabinofuranosyl. By substituting additional mono- or disaccharide chains for these side chains in some plant species, xyloglucan becomes a complex hemicellulose that varies between different stages of plant cell growth (Pauly and Keegstra, 2016).

Mannans are hemicellulosic heteroglycans with complex structural characteristics that help plants maintain their cell wall structure and storage (Ebringerov´and Hrom´adkov´a, 2005). They have a hydrogen bonding connection to cellulose, and this connection is comparable to that of other significant hemicelluloses. Glucomannans, galactomannans, homomannans, and galactoglucomannans are the four structural subtypes of mannans, respectively. While the mannose backbone in galactoglucomannans and glucomannans exhibits interruptions due to the non-repeated glucose patterns, the mannose backbone in homomannans and galactomannans is solely comprised of mannose residues linked by β-1,4-linkages. Additionally, it has been suggested that galactose residues connected to mannosyl backbone residues hinder polymer self-aggregation (Qaseem et al. 2021).

Hemicellulose extracted from lignocellulosic biomass has been utilised to synthesize biofuels after bioconversion using microbes. In one such study, pentose degrading organisms called *Pichia stipitis* and *Candida shehatae* have been used in order to produce bioethanol from hemicellulose derived from wheat straw (Koti et al. 2016). Alongside, sodium xylene sulfonate was utilised to produce biobutanol from hemicellulose (Qi et al. 2019). *Candida guilliermondii* has been found to be producing xylitol (0.071g/g) using rice straw hemicellulose hydrolysate (Roberto et al. 1996). In a study conducted by Luo et al. 2019, it has been found that valorisation of hemicellulose and hemicellulose-derived products isolated from lignocellulosic biomass can produce furfural directly .

1.2.2.3 **Lignin from lignocellulosic biomass and usage:**

Lignin is one of the major building blocks of the cell wall. The structure of lignin differs based on the source of extraction. Natural lignin consists of three monomers, coniferyl alcohol (G-lignin), p-coumaryl alcohol (H-lignin), and sinapyl alcohol (S-lignin), stabilised by most prevalent chemical linkage that stabilizes this structure is the β-O-4 ether bond (Rinaldi et al. 2016; Wang et al. 2017). In softwood, the percentage of the bond is 45-50% and in hardwood, it is 60-62% (Rinaldi et al. 2016). The benzene ring of G- and H- lignin has more reactive sites, making them capable of chemical treatment and other reactions, thus softwood and grass lignin are more promising as they contain a high amount of the mentioned type (Kalami et al. 2018). It was found that the lower molecular weight and reactivity favours the production of lignin-phenol-formaldehyde resins (Lourençon et al. 2020; Stücker et al. 2016).

Several methods are used to isolate lignin from different lignocellulosic biomass. The main process to extract lignin is acid-alkali treatment. It was found that the chemical extraction process leads to more variation in lignin structure. This lignin is called technical lignin and lacks the characteristics of the naturally available lignin (Karthäuser et al. 2021). In the alkaline treatment method, due to the elevated temperature and alkaline environment, kraft lignin changes its structure which makes them less reactive than naturally available lignin and decreases the yield (Crestini et al. 2017). To enhance the yield of extraction different solvents are also used, such as alkaline hydrogen peroxide. This process can be performed at a low temperature of 70°C with a relatively high yield (Zhang et al. 2015). Under the sulfide method, the lignocellulosic biomass is treated in the acid medium. This lignin is called lignosulfonates and has a higher molecular weight than kraft lignin. Due to the sulfonation in the α-position, lignosulfonates are soluble in water (Kai et al. 2016). For non-wood species, the Soda pulping method is used. In this process no sulfur is used, hence lower sulfur content is the main dissimilarity among the soda pulping lignin and kraft lignin (Wang et al. 2020; Kai et al. 2016). This structure of lignin develops an idea that cleaves the lignin in such a way that valuable products can be gained. It can be achieved by solubilizing lignin in an organic solvent. Lignin that is cleaved and soluble in an organic solvent is known as organosolv lignin (Paone et al. 2018). Organosolv lignin is more like natural lignin in comparison to other technical lignin depending upon the severity of the extraction (Kai et al. 2016).

Various compounds can be produced as the byproducts of lignin. Phenolic compounds one of the most important derivatives of lignin are used to produce biopolymers, drugs, herbicides, pharmaceuticals, cosmetics, sunscreen and hair oil. One of the most used flavouring agents vanillin (3-methoxy-4-hydroxybenzaldehyde) is a bioproduct of lignin. Lignin-derived vanillin is a premium product and is priced at $ 100-200 per kilogram. One of the major processes to produce vanillin is acid extraction and oxidation of lignin. Another product derived from lignin is carbon fibre, a strong and light material with several applications mainly in the automotive industry (Brodin et al. 2009).

1.2.2.4 Silica and its usage:

Rice husk acts as a protective layer of the rice seed. The abundant availability of rice husk as an agricultural residue in major rice cultivating countries can be undoubtedly used as a rich source of Silica after combustion. It can contain almost 95% silica after combustion and is further utilised as fertiliser and can be accompanied by concrete fabrication. The economic significance of the rice husk ash as the rich raw material of silica, has immense importance and suffices the trouble of ash disposal. In order to extract silica, Leaching has been used on a vast scale. Leaching can be defined as the extraction of a substance of interest from a solid mixture using a liquid as a separation medium, as the component diffuses into the liquid media from its natural solid form.

In intend to extract silica from rice husk, leaching can play a significant role. Under conventional extraction procedure, pyrolyzed rice husk ash is digested using NaOH to extract silica as sodium silicate (Na2SiO3). Production of silica is more favourable in rice husk ash compared to rice husk raw. The silica present in the rice husk experiences modifications in structural conformation based on the burning conditions such as temperature and time. At 550-800°C, the structure of the silica produced is generally amorphous whereas crystalline silica is synthesized beyond the temperature. The nature of the silica synthesized can have different functions due to the structural dissimilarities (Chandrasekhar et al. 2006).

Due to the high expense of the conventional method of silica production, rice husk can be a viable raw material as silicon with significant purity and crystalline structure can be utilised for the preparation of battery materials. Alongside that, Aminated SiO2 (ASiO2) (produced from rice husk using 3-aminopropylthiethoxysilane) has been utilised significantly for the removal of various contaminants from the environment. Bakdash et al. 2020 have identified ASiO2 as a great adsorbant of Carbon dioxide. It also can remove CH4, hydrogen and Nitrogen from the air. Along with that, it has also been seen that the silica isolated from rice husk can also have great heavy metal(loid) adsorption capacity. At 28°C, silica has been found to be removing 96.6% of Pb2+ and 80.9% Cu2+. The removal efficiency is Pb2+ >Zn2+ >Mn2+ >Cu2+ .

In addition to that, it has been observed that rice husk-derived silica can absorb harmful dyes like Safranine, Rhodamine B etc (Gan et al. 2013).

1.2.2.5 Other value-added products and their usage:

Apart from cellulose, lignin, hemicellulose, and silica, different bioactive phytochemicals have been extracted from rice husks due to their capabilities to prevent oxidative stress damage. The relation between antioxidant activity and the production of phytochemicals, especially phenolics has been studied and identified that phenolics can play an important role in the prevention of oxidative stress damage and factor for anti-oxidant capacity. From rice husk, two phenolics named vanillic acid (14-26 µg/g) and p-coumaric acid (4.8-28.9 µg/g) have been extracted. In addition to that, ferulic, p-hydroxybenzoic, protocatechuic and chlorogenic acids were extracted in small quantities (Butsat and Siriamornpun 2010). In another study, caffeic acid has also been extracted from rice husk. In addition to that, the presence of flavonoids was also been found in the rice husk. In the aforementioned study, it was observed that the fractionation of rice husk containing phenolics and flavonoids does not have any cytotoxicity but it has inhibited the proliferation of HepG2 human liver cancer cells by 80% (Gao et al. 2018).

1.3. Aims and Objectives:

Aim: The study was intended to investigate the potential of Ultrasonication-assisted acid and alkaline pretreatment method in order to extract different value added products such as cellulose, hemicellulose, lignin, xylan, and furfural from lignocellulosic waste material rice husk followed by synthesis of bioethanol from the cellulose residue after saccharification by *Aspergillus* and fermentation using *Sacchromyces cerevisiae.* Alongside that, production of microcrystalline cellulose and silica nanoparticles have also been studied.

Objectives:

1. Study the Ultrasonication-assisted acid and alkali-pretreatment of rice husk to extract cellulose, hemicellulose, lignin, xylan, and furfural from rice husk.
2. Characterization and estimation of value-added products extracted from rice husk.
3. Synthesis of silica nanoparticle from rice husk and their characterizations.
4. Production of Bioethanol from rice husk derived cellulose after saccharification and fermentation.

1.4. Materials and Methods:

Collection of raw feedstock:

Rice Husk was collected from a local rice mill near Jadavpur, Kolkata.

Chemical Reagents:-

Concentrated Sulphuric Acid (Merck, India), Hydrochloric Acid (Merck, India), Sodium hydroxide (Merck, India), Agar-Agar (Himedia, India), Anthrone Reagent (Merck, India), Dinitrosalicylic Acid (Merck, India), Yeast Extract (Merck, India), Glucose (Himedia, India), Ammonium sulphate (Himedia, India), Peptone (Himedia, India), Dextrose(Himedia, India), Czapex dox (Merck, India) , Ethanol , Sodium Nitrate (Himedia, India), Magnesium Sulphate Heptahydrate (Himedia, India), Ferrous Sulphate (Himedia, India) , Potassium Chloride (Himedia, India), Dipotassium Hydrogen Phosphate (Himedia, India), Magnesium Chloride (Himedia, India), Magnesium Sulphate Heptahydrate (Himedia, India), Potassium Dihydrogen Phosphate (Himedia, India).

Pretreatments:

Physical Pretreatment:

In order to reduce the size of the rice husk particles, crystallinity of cellulose, and increase the surface availability alongside pores of cellulose , milling has been executed prior to the Ultrasonication-assisted acid and alkali pretreatment method.

Ultrasonication assisted chemical pretreatment:

An efficient chemical pretreatment process has been encountered in intend to disrupt the lignocellulosic structure of rice husk and extraction of different value-added products such as cellulose, hemicellulose, lignin, xylan, and furfural. Three different concentrations (2%, 4%, and 6% W/V) of Alkali (Sodium Hydroxide) and Acid (Sulphuric acid) have been used for the pretreatment of rice husk. In order to understand the effect of sonication in removal of lignin and hemicellulose from the lignocellulosic biomass and structural change in cellulose, ultrasonication has been encountered after chemical pretreatment for 30 minutes and mixed afterwards. In intend to separate the cellulose from the liquid mixture, filtration was used. The residue containing the major amount of cellulose was neutralised via thorough washing followed by drying in a hot air oven at 70°C. The filtrate was mixed with ethanol

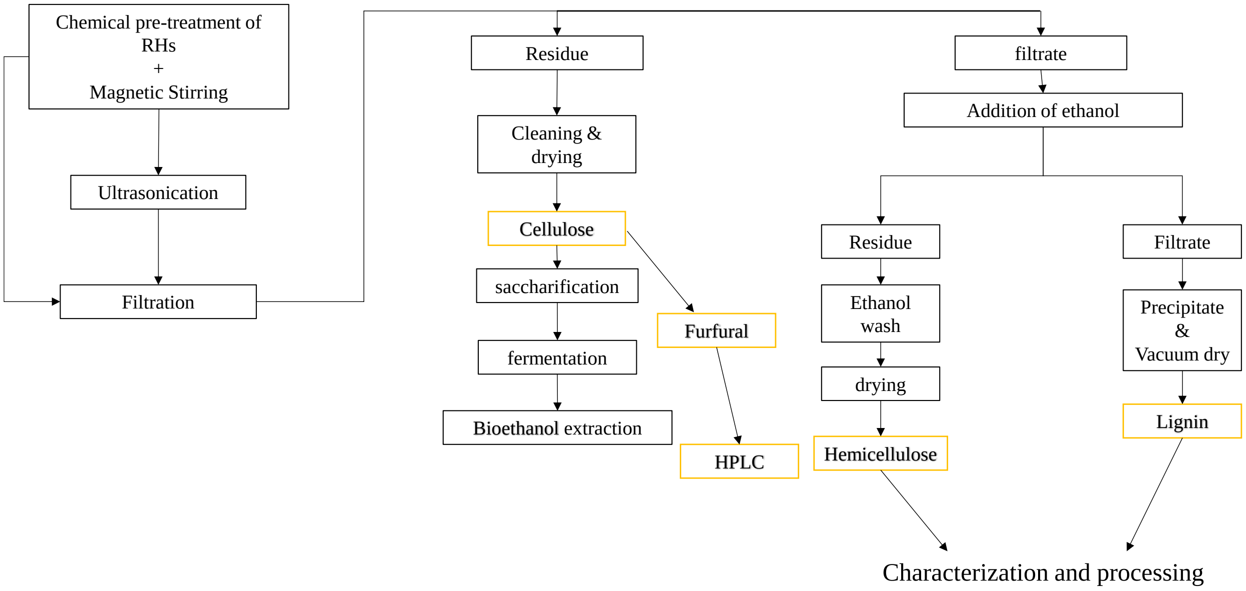


Fig. 3 Plan of Work

in order to precipitate hemicellulose. After filtration of the ethanol-filtrate mixture, the residue has been separated and washed with ethanol followed by drying. The filtrate containing lignin has been precipitated after gradual addition of HCl with stirring. The precipitated lignin has been vacuum dried. As a by product of this pretreatment method, xylan and furfural has also been found to be present in the filtrate.

Determination of Cellulose content in Rice Husk after pretreatment:-

Cellulose Estimation:-

Anthrone test was performed for the estimation of cellulose content extracted from rice husl. In this test the carbohydrate gets dehydrated when reacted with concentrated sulphuric acid (H2SO4) forms furfural. This furfural which is formed in the previous step reacts with anthrone reagent to give bluish green coloured complex. This coloured complex intensity is measured by spectrophotometer at 630nm (ThermoFisher, USA).

Chemical Reagents:-

1. Pretreated (acid, alkaline, Ultrasonication-assisted acid, Ultrasonication-assisted alkaline) Rice Husk.

2. Anthrone Reagents: 100ml of ice cold H_2SO_4(95% conc.) was take in which 200mg of anthrone was dissolved to prepare the anthrone reagent solution. The solution was prepared fresh and refrigerated for 2hrs before use.
3. 67% sulphuric acid (H_2SO_4).

Standard curve preparation:-

For the standard curve preparation cellulose (10 mg) was dissolved in 100 ml of double distilled water. From the stock solution aliquots of 0.25, 0.5, 0.75 and 1 ml were taken. Further 10 ml of anthrone reagent was added and the solution was heated in water bath for 15 minutes. Finally the absorbance was measured at 630nm and the standard graph was plotted.

Experiment:-

1. 0.1 gram of each pretreated samples were taken in different test tubes.
2. 10ml of 67% H_2SO_4 was added to each test tubes and was kept for 1hr at room temperature.
3. 1ml from the previously prepared solution was taken and was further diluted to 100ml by adding distil water.
4. 1ml from the above stock solution was taken to which 10ml of Anthrone reagent was added and thoroughly mixed.
5. The test tubes were heated in the water bath at boiling for 15 minutes.
6. The above solutions in the test tubes were cooled and finally the absorbance was measured at 630nm.

Synthesis of Furfural

Furfural is one of the most important value-added product and lignocellulosic biomass such as rice husk can be a source of it. In order to synthesize furfural from the cellulose extracted from rice husk after ultrasonication assisted acid-alkali pretreatment, H_2SO_4 was used. In a hydrothermal reaction, 50mL of 1.5% H_2SO_4 was taken and mixed with 1g of rice husk derived cellulose. The reaction was taken place at 185°C for 10 mins. After the reaction ends, the solution was cooled down and the supernatant was considered for quantification of Furfural.

Synthesis of Silica from Rice husk:

In order to synthesize silica nanoparticles from rice husk, physical and chemical treatment on rice husk has been performed. 100mg of dried rice husk has gone under pyrolysis in a muffle furnace at 800°C to make ash. A solution of 12% NaOH (W/V) and mixed with the ash in a 7:1 ratio followed by stirring at 100°C for 90min. After stirring filtration has been done and the liquid part is taken. And stirred for another one hour. The solution was further taken and acidified with concentrated H_2SO_4 . There was a precipitation of Silica which was further filtered out followed by washing with water and dried at 120°C for 2 hr.

Synthesis of Microcrystalline Cellulose:

Initially 20gm of dried rice husk was taken and mixed with 250mL of 1M NaOH solution and stirred at 80°C for 90 minutes. After that, the alkaline solution was refluxed with 300ml of Hydrogen Peroxide at 80°C for 60 minutes. Followed to that, the modified rice husk was separated and treated with 150mL of 2M HNO3 solution at room temperature for 30minutes. Then the treated rice husk was washed with water until the pH becomes neutral. The treated rice husk was further dried and grinded for the production of microcrystalline cellulose.

Characterization of value-added products from rice husk:

Elemental Analysis:

Rice husk is a lignocellulosic biomass consisting of cellulose as a major component. In order to identify the elemental carbon, hydrogen, nitrogen and sulphur concentration present in rice husk, elemental analysis was performed using an element analzer (Elementar, Vario). Sulfanilamide has been used as a reference standard after normalising with air.

Thermogravimetric Analysis:

Rice husk was analysed for thermogravimetric analysis in a moisture free air atmosphere with heating rate of 40°C/min with sample weight having 10mg using TGA 4000, PerkinElmer instrument. The predefined temperature increase has been maintained from ambient temperature to 850°C.

Fourier transform infrared spectroscopy (FTIR) analysis:

In order to identify the functional groups, present in the cellulose, hemicellulose, and lignin extracted from rice husk after ultrasonication-assisted acid-alkali pretreatment, Fourier transform infrared spectroscopy (FTIR) analysis has been done using Spectrum Two FT-IR spectrometer, Perkin-Elmer Inc.,USA . The wavenumber scan has been done on the range of 4000-450 cm^{-1} to identify the chemical bonds and functional groups present in the sample as each group has a specific energy absorption band.

X-ray diffraction analysis

In order to identify the crystal lattice of rice husk-derived cellulose, the X-ray diffraction analysis was performed by a diffractometer from Malvern, Pananalytical, UK, operating at 40kV and current flow of 40mA over an intensity range of (2θ) of 5-80°.

Scanning Electron Microscopy

The surface ultrastructure of cellulose, lignin, hemicellulose , microcrystalline cellulose and silica extracted from rice husk has been observed using Scanning Electron Microscopy (INSPECT-F50, FEI, Czech Republic and Zeiss EVO LS10, Carl Zeiss, Germany). As a step of sample preparation, the samples were coated with a thin layer of gold (Au) under high vacuum conditions. The chemical composition of silica has also been identified using Energy-dispersive X-ray (EDX).

Particle Size analysis of Silica:

To measure the average particle size of the silica particle synthesized from rice husk, Zetasizer Nano ZS Size Analyzer from Malvern Panalytical was used. 5mg of silica sample was mixed in deionised water followed by vortex and sonication for 10minutes in order to achieve even distribution of the nanoparticles.

Quantification of Furfural using HPLC

A Waters model 1525 binary HPLC pump , model U6K manual injector with Waters model 2489 UV/Visible detector were used to determine the amount of furfural synthesized from cellulose derived from rice husk. Sunfire C18 column was used for the separation of components and Breeze software was used for analysis. 20uL of filtered sample was injected manually and Acetonitrile-water was used isocratically at 2.0 mL/min as a mobile phase. Retention time and peaks were determined by Waters model 2489 UV/Visible detector system at 280nm.

Bioethanol Synthesis:

Biological Treatment Method for ethanol production:

After the pre-treatment of rice husk and estimating their cellulose content by Anthrone test the cellulose was used for bioethanol production. The bioethanol production process includes two different steps, viz., saccharification to breakdown the cellulose fibre to produce reducing sugar and fermentation for converting the reducing sugar to ethanol. Both the process was conducted by microbial treatment. For saccharification

Aspergillus sp. (Genbank: MH119104) was used because of their capability to produce cellulase enzyme. This enzyme reacts with cellulose and produce reducing sugar.

Inoculum preparation:

Aspergillus sp. was isolated from soil sample that was collected from Sundarban area of West Bengal, India. The identified and isolated fungal strain was further subcultured in czapekdox medium. Further the *Aspergillus sp.* strain was again subcultured in an agar plate and incubated at 35°C for 7 days. After the fungus fully sporulate, the plates were further treated with tween 80 (0.1%). As the tween 80 has a detergent property, the spores were dislocated and gently collected using pipet. The spore suspension was centrifuged and washed several times and finally stored in refrigerator to be used as inoculum.

Media composition for saccharification and recovery of microbial strains:

To conduct saccharification minimal media was used. The composition of the selected minimal media is described below:

Sodium Nitrate- 0.2%

Potassium Chloride- 0.05%

Magnesium Sulphate- 0.05%

Ferrous Sulphate- 0.001%

Dipotassium Hydrogen Phosphate- 0.1%

Peptone- 2%

Before inoculating the pre-treated sample for saccharification, the isolated *Aspergillus sp.* was subcultured in czapekdox medium and incubated at 35°C. The growth rate of the microorganism was monitored by UV-Visible spectrophotometer and the pre-treated sample was inoculated when the OD reaches to 1.

Experiment:

1g of pre-treated rice husk were taken into 250 ml Erlenmeyer flasks and then mixed with the selected minimal media. The mixture of minimal media and pre-treated sample was sterilized by autoclave at 121°C and 15psi pressure. After sterilization the media was inoculated by 3ml of sample and inoculated with 3ml of inoculum and incubated at 35°C for 6 days.

Estimation of total reducing sugar content by DNS method:

3,5- Dinitrosalicylic acid (DNSA) is a very commonly used compound in biochemical science to estimate the total reducing sugar content in any solution. Because of their structure the DNSA detects the presence of free carbonyl group present in the reducing sugar molecule. For this experiment the oxidation of the ketone functional group in case of fructose and aldehyde functional group for glucose is involved. During the reaction, the DNSA is reduced to form 3-amino-5-nitrosalicylic acid (ANSA) that develops a reddish-brown colour with the maximum absorbance at a wavelength of 540nm which can be analysed by using UV-Visible spectrophotometer.

DNS reagent preparation:

The DNS reagent was prepared by adding 1 gram of DNS, 200 mg of Crystalline Phenol and 50 mg of Sodium Sulphite in 100 ml of 1% NaOH solution by continuous stirring.

Sodium-Potassium tartratesolution was prepared by adding 40 gram of Rochelle salt in 100 ml of DI.

Standard stock and curve preparation:

To prepare the standard stock solution 100 mg of commercial dextrose powder was dissolved in 100 ml of DI. Further 1 ml of the sample was diluted to DI and the volume was made up to 100 ml to prepare 1 mg/ml solution. From that 0.1 mg/ml solution was prepared. 3ml of each solution was taken and 3ml of DNS solution was added to them. Next, they were heated for 10 min. After cooling the solutions appear as reddish-brown. 1 ml of 40% Rochelle salt was added to each of the solutions and mixed thoroughly. Finally, the absorbance was measured at 540nm using UV-Visible spectrophotometer (Perkin Elmer, Germany).

Experiment:

After the saccharification is complete, 1 ml of liquid media from each sample was collected and 2ml of distilled water was added to it. After making up the volume to 3ml, 3ml of the DNS reagent was added to each of the media sample. The test tubes were heated for 10 min. and after cooling 1ml of 40% Rochelle salt was added in each test tube. As the reddish-brown colour developed the absorbance was recorded at 540 nm using UV-Visible spectrophotometer (Perkin Elmer, Germany).

Fermentation:

For this step of bioethanol production *Saccharomyces cerevisiae.* (MTCC 170) was used. *Saccharomyces cerevisiae* is a very common species of Yeast and universally used for fermentation. This fungus is capable to break down polysaccharides specially hexose and convert them to alcohol by fermentation.

Media preparation and culture of *Saccharomyces cerevisiae.:*

To make a culture of *Saccharomyces cerevisiae.* YEPD media was used. The composition of that media is described below:

Yeast extract- 1%

Peptone- 2%

Dextrose- 2%

The media was mixed with 100 ml of DI in an Erlenmeyer flask and autoclaved at 121°C and 15 psi pressure to sterilize the media. After cooling down the *Saccharomyces cerevisiae* strain was added to it and incubated at 37°C for 2-3 days.

Experiment:

After the saccharification, the samples were again autoclaved at 121°C and 15 psi pressure to kill the fungal strain. After sterilization and cooling them down the same media with substrate was inoculated with 1ml of *Saccharomyces cerevisiae* and incubated at 37°C for 4days.

Estimation of ethanol by dichromate test:

Reagent preparation:

Acid dichromate solution: 125 ml of DI was taken in an Erlenmeyer flask and 70 ml of Sulphuric acid was added to it. After mixing them together and cooling the mixture under tap water, 0.75 gram of Potassium Dichromate was added to it. Further distilled water Further DI was mixed in the solution and volume was made up to 250 ml.

Starch indicator solution: 1 gram of starch was dissolved in 100 ml of recently boiled water.

Sodium thiosulfate solution: 7.44 gram of Sodium thiosulphate to 1 lit. of volumetric flask. After that the volume was made of to 1lit. and mixed well.

Potassium iodide solution: To prepare this solution 5 gram of KI was dissolved in 25 ml of water.

Experiment:

1 ml of fermentation broth (/standard ethanol solution) was added with 25mL of K2Cr2O7 solution in an Erlenmeyer flask followed by 9mL of distilled water to make up the volume to 35mL each. After that, the mixture was heated at 65°C for 25minutes in a water bath. Then the solution was cooled down to room temperature and 15mL of deionised water was added to make up the volume upto 50mL. To measure the concentration of ethanol present in the solution, absorbance was taken at a wavelength of 600nm in UV-VIS spectrophotometer (Shimadzu, Japan).

1.5.Results and Discussion:

Estimation of Cellulose:

Pretreatment of Rice Husk	**Conc. of cellulose in mg/g of extracted cellulose residue**
Raw Rice Husk	87.1
2% H_2SO_4 Non-sonicated	197.1
4% H_2SO_4 Non-sonicated	131.7
6% H_2SO_4 Non-sonicated	200.3
2% H_2SO_4 Sonicated	199.1
4% H_2SO_4 Sonicated	142.9
6% H_2SO_4 Sonicated	230.4
2% NaOH Non-sonicated	335.6
4% NaOH Non-sonicated	288.2
6% NaOH Non-sonicated	126.6
2% NaOH Sonicated	347.9
4% NaOH Sonicated	129.8
6% NaOH Sonicated	139.3

Table 1: Quantification of cellulose using Anthrone method

Anthrone method has been used to estimate the amount of cellulose present in the raw rice husk along with pretreatment derived cellulose residue. It has been found that, the amount of cellulose present in raw rice husk is 87.1 mg/G of rice husk. In intend to increase the quantity of cellulose after removal of lignin, hemicellulose and other components, ultrasonication assisted chemical pretreatment methods have been encountered. From the residue after pretreatment, it has been found that 2% NaOH pretreatment coupled with sonication has resulted in extraction of most amount of cellulose (347.9 mg/g of cellulose residue) whereas, 6% NaOH without sonication has resulted in least amount of cellulose (126.6 mg/g of cellulose residue. In case of Acidic pretreatment, 6% H2SO4 sonicated (230.4 mg/g of cellulose residue) samples were found to be the most amount of cellulose containing whereas, 4% H2SO4 non-sonicated (131.7 mg/g of cellulose residue) has been found to be the least cellulose containing sample.

Elementary Analysis:

The elementary analysis determines the carbon, hydrogen, nitrogen, and sulphur percentage present in the raw material. From Fig. 4, it can be understood that the raw material rice husk predominantly consists of Carbon (30.59%).

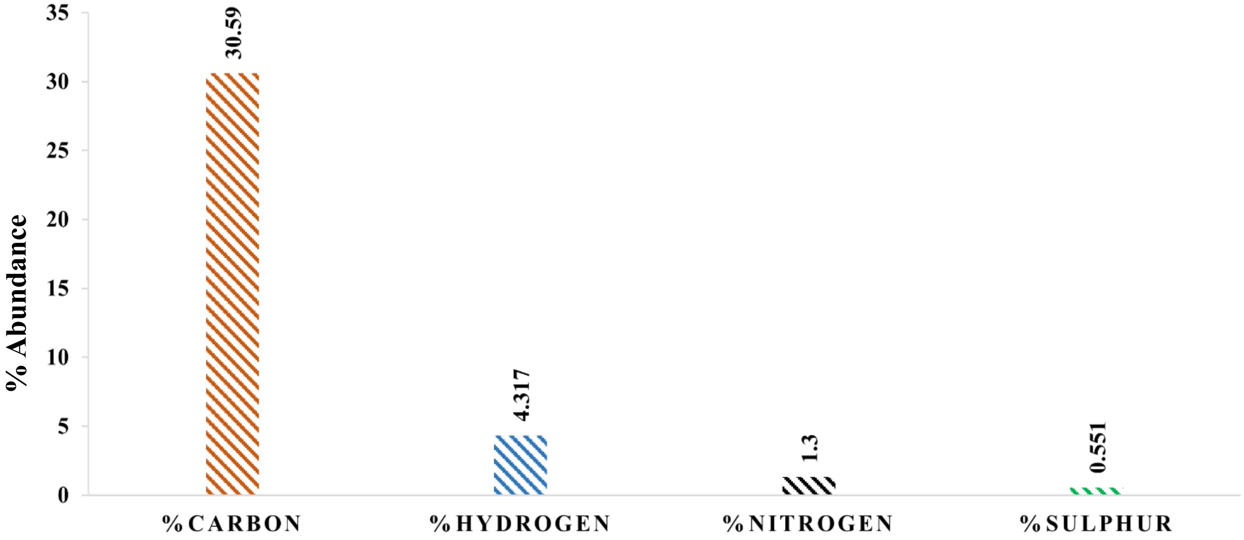


Fig. 4: Elemental analysis of Rice Husk

Along with that, the percentage distribution of Hydrogen and Nitrogen is 4.317 % and 1.3% respectively. Whereas a trace amount of sulphur (0.551)has been found in the analysis. The prevalence of carbon and scarcity of nitrogen can indicate the abundance of carbohydrates in their composition, leading to an increase in the chances of active sites present for efficient adsorption (Scaglioni et al. 2016) .

Thermogravimetric Analysis:

In order to understand the thermostability of rice husk, Thermogravimetric analysis has been performed. The change in weight has been analysed with varying temperature with a rate of 40°C/min from ambient temperature to 850°C. When the weight change has been plotted against the change in sample temperature, it has been found that, there is a slight loss of weight around 100-110°C due to the removal of moisture. Besides that, there was a slight loss of weight till 240°C followed by a major loss around 280-300°C which further completed by 380°C. This range of temperature (230-380°C) is known as active pyrolysis zone regulating the oxidation of volatile products synthesized sue to the active pyrolysis method. Further, there was a slight decrease in weight after 380°C indicating the initiation of passive zones (secondary zones), and at around 500° the zone gets terminated suggesting the oxidation of residual char.

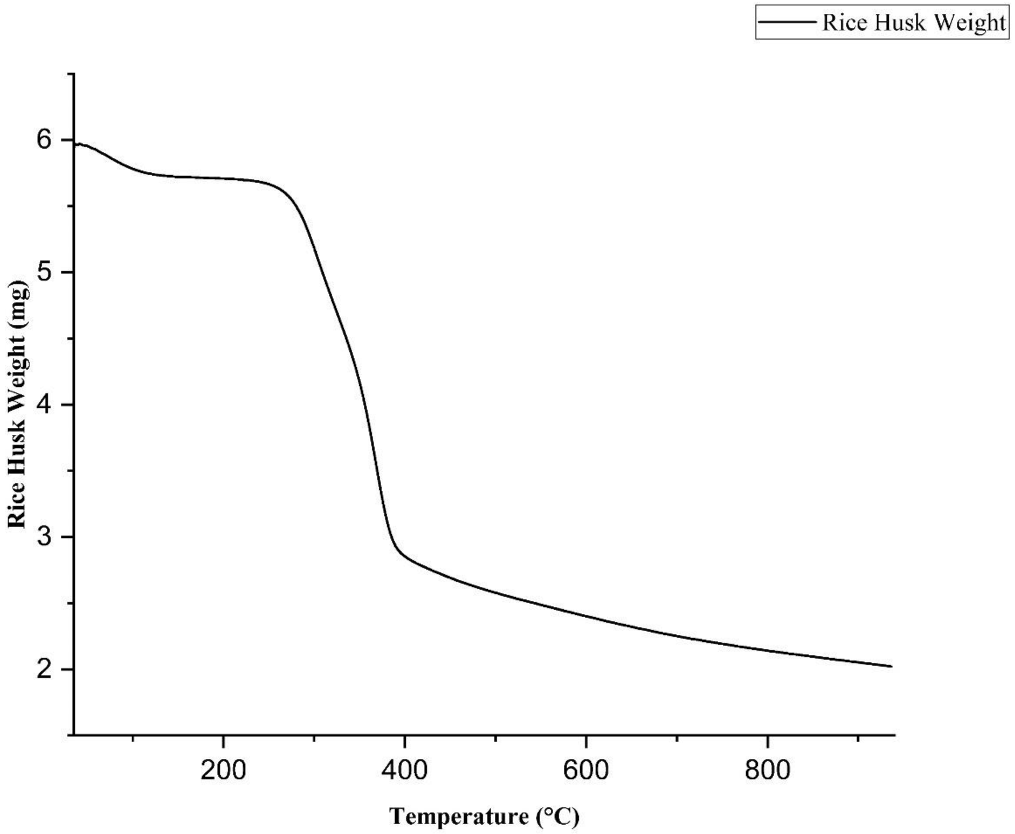


Fig. 5: Thermogravimetric analysis of Rice husk

Fourier Transform Infrared Spectroscopy:

Cellulose:

The Fourier transform infrared spectroscopy (FTIR) spectrum of the NaOH pretreated rice husk-derived cellulose (sonicated and non-sonicated) has depicted the major peaks around 3346cm^{-1} (-O-H group), 2900 cm^{-1} (C-H groups), 1638 cm^{-1} (C=O group), and 1596 cm^{-1}(C=C groups) (Fig. 6). Along with that, the presence of CH_2 and CH_3 groups has been indicated by peaks at 1418 cm^{-1} wavenumber.

On the other hand, the abundance of carboxyl-carbonate structure and aromatic CH stretching have been observed at 1367 cm$^{-1.}$ Alongside that, the presence of Si-O-Si and Si-H bonds have been depicted at 1032 cm^{-1} and 558 cm^{-1} respectively (Fig. 6). However, the presence of carbonyl group (CO) has been confirmed at peak 1159cm^{-1}. Among the sonicated and non-sonicated NaOH-treated rice husk-derived cellulose, the intensity of the peaks of all the functional groups is slightly higher in the sonicated sample (Fig. 6) (Daffalla et al. 2020).

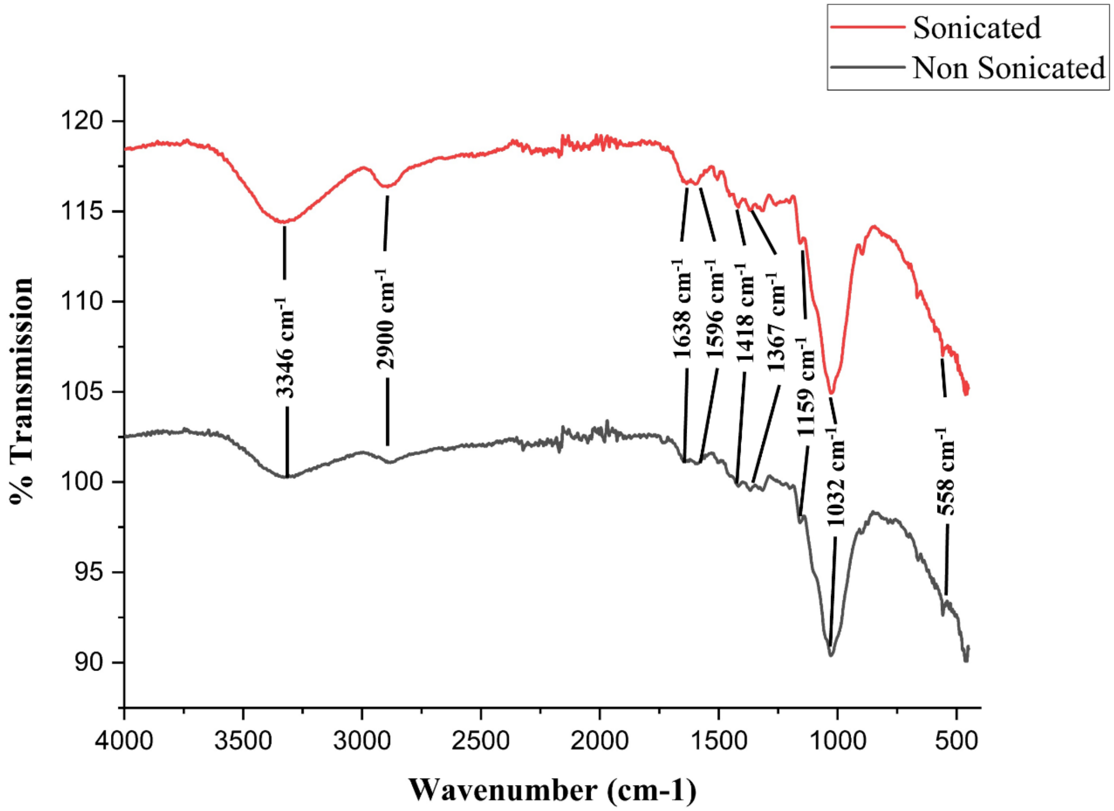


Fig. 6: IR spectrum of Rice husk derived cellulose

Hemicellulose:

The FTIR spectrum of hemicellulose after ethanolic precipitation of the pretreatment filtrate followed by drying has been performed. After plotting % transmission with respect to wavenumber, it has been seen that the native hemicellulose functional groups are present in the spectra. A very broad band has been observed at 3320 cm^{-1} indicating the presence of hydroxyl group (O-H stretching), while the peak at 2870 cm^{-1} suggested the C-H stretching in the CH_2 and CH_3 groups. Besides that, the presence of ester bond (C=O ester) has been found at 1750 cm^{-1} . The strong band at wavenumber 1045 cm^{-1} has been associated with the C-O stretching, C-OH bending and C-O-C stretching (Fig. 7).

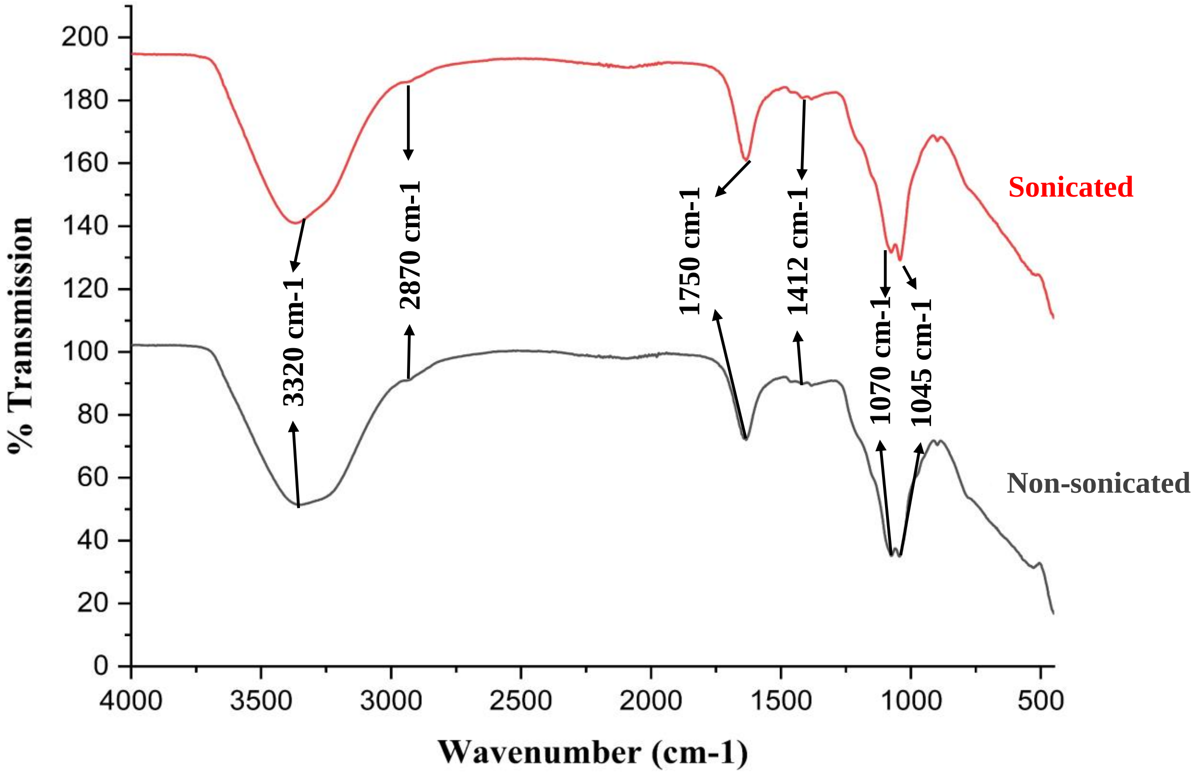


Fig. 7: IR spectrum of Hemicellulose derived from Rice Husk

Lignin:

After pretreatment of rice husk with ultrasonication assisted acid-alkali pretreatment, lignin has been extracted from the filtrate after hemicellulose extraction and precipitated fusing HCl and freeze dried to get powder lignin. In order to identify the functional groups present in the extracted lignin, FTIR analysis was performed. The spectra depicted the presence of O-H group stretching at 3342 cm^{-1} along with the C-H stretching at peak 2982 cm^{-1}. On the other hand , the aromatic ring skeleton consisting carbonyl stretching has been observed at 1645 cm^{-1}. From 1650 cm^{-1} to 1000 cm^{-1} , due to the presence of guaiacyl and syringyl aromatic units, the aromaticity of lignin structure has been observed in the spectra. At wavenumber 1430 cm^{-1} ,the peak of C-H plane has been observed couples with aromatic skeletal vibration, while at 1370 cm^{-1} and 1090 cm^{-1} presence of Phenolic OH and C-O (ester) deformation of secondary alcohols have been identified respectively (Fig. 8).

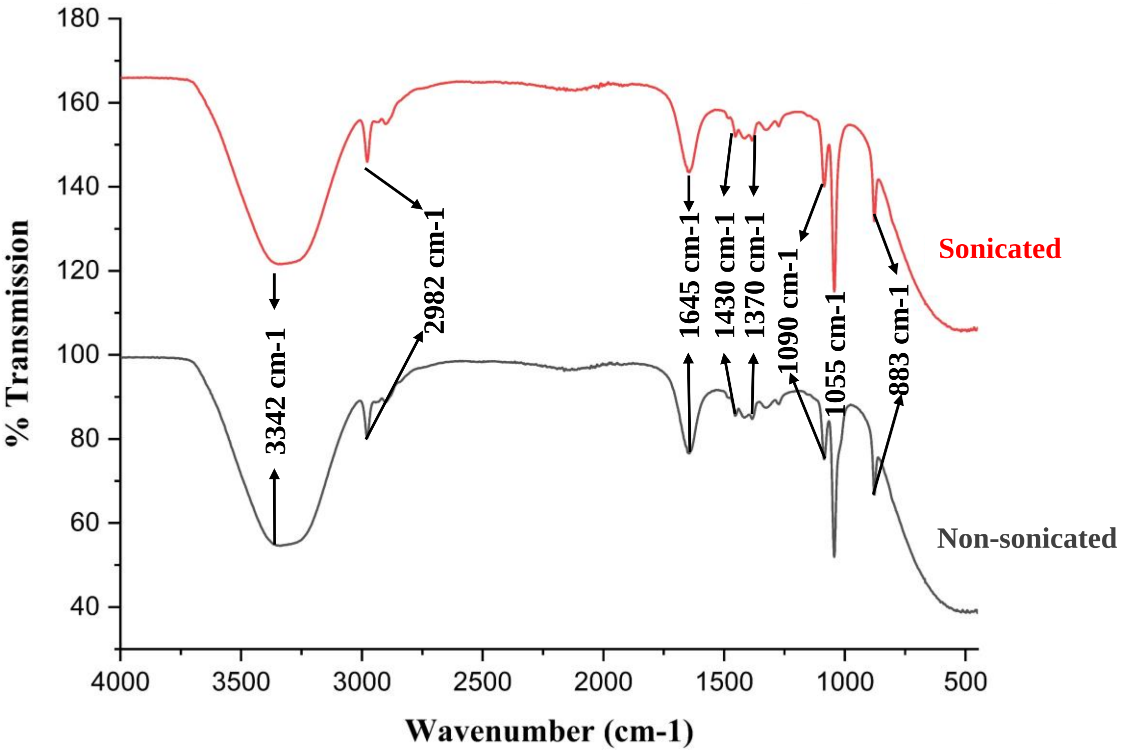


Fig. 8: IR spectrum of Lignin derived from Rice Husk

Microcrystalline Cellulose:

The FTIR spectrum of rice husk derived microcrystalline cellulose has clearly depicted the presence of O-H stretching at the peak of 3323cm^{-1} which stabilises the molecular structure. The absence of any significant bond vibrations in the range of 1750- 2700cm^{-1} has been previously reported by Morrison and Stewart (1992). Along with that, at 1180cm^{-1} wavenumber, the presence of asymmetrical C-O-C linkage in ether and esters (Bhat et al 2009). Besides that, C-H bending has been obtained at peak 2884 cm^{-1}. Along with that a strong C-O bond is found at the wavenumber 1026 cm^{-1} (Gelbrich et al 2013) (Fig. 9).

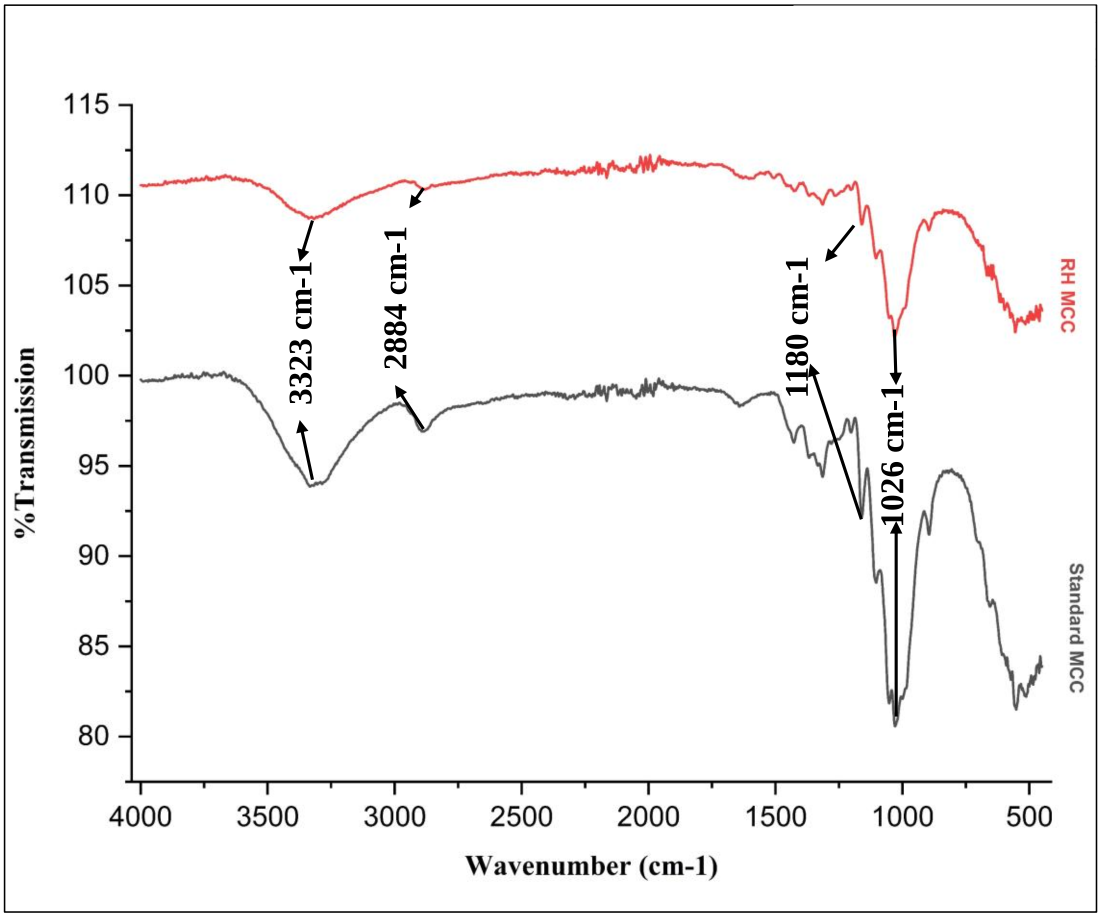


Fig. 9: IR spectrum of MCC derived from Rice Husk

X-Ray diffraction study:

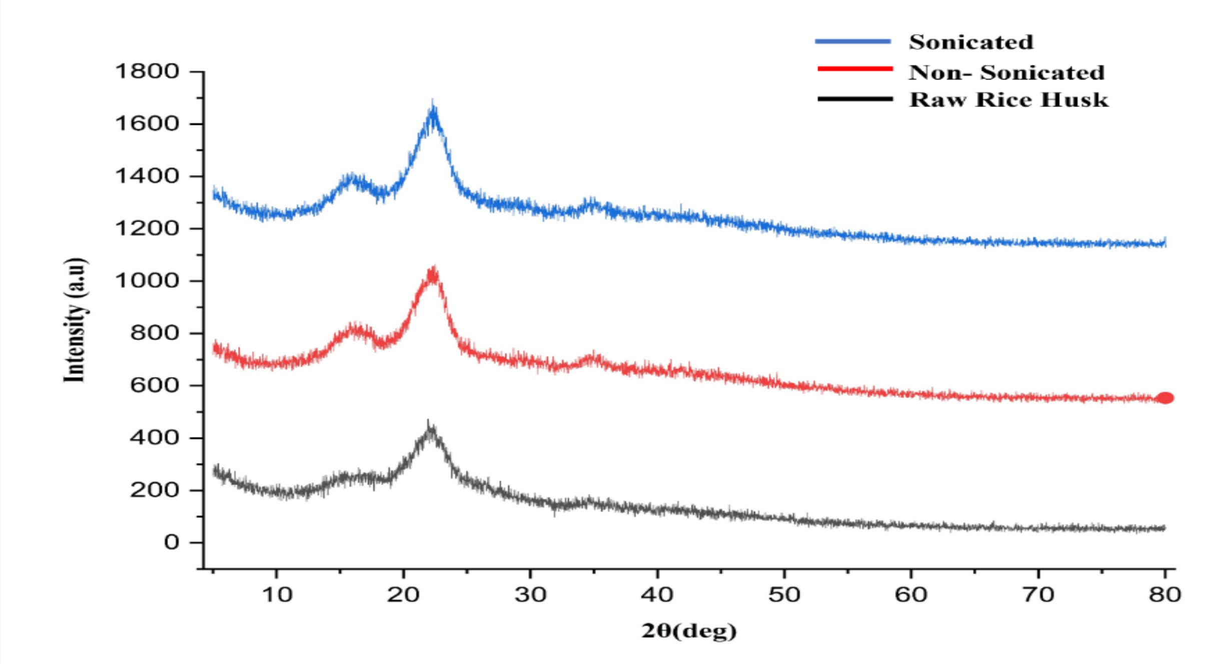


Fig. 10: Crystallographic structure analysis of Cellulose derived from Rice Husk

From Fig. 10 it was observed that a prominent peak is present at 2*Θ*= 22° representing the crystallographic plane of cellulose I present in the NaOH pretreated rice husk-derived cellulose along with two other peaks at 2*Θ*= 16° and 34° (Fig. 10). In comparison to the XRD plot of raw rice husk, the crystallinity of cellulose resulting from sonication and non-sonication coupled with NaOH treatment have found to be increased from 30.77% to 43.36 % and 41.96% respectively, signifying the removal of amorphous constituents such as lignin and hemicellulose due to the pretreatment and better exposure of cellulose crystalline structure after sonication (Fig. 10) (Jiang and Hu, 2019; Bhandari et al. 2020).

Scanning electron microscopy:

Cellulose:

Fig. 4 represents the SEM image of alkaline pre-treated rice husk with and without sonication at 600X magnification. From Fig. 11, it can be easily understood that the roughness of the surface has significantly increased due to the pretreatment of NaOH assisted with or without ultrasonication. The pretreatment method was effective in removing non-cellulosic constituents of rice husk such as lignin, hemicellulose and pectin. It has been observed that sonication can decrease the width of the fibre after alkaline treatment resulting in increased surface area for interaction...

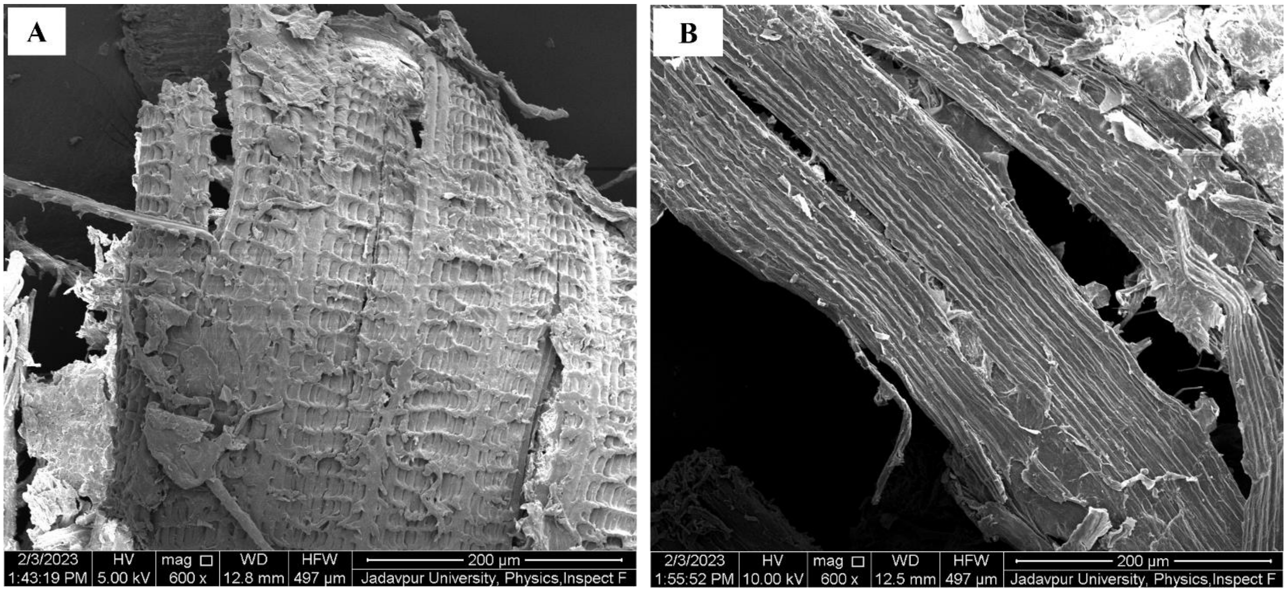


Fig. 11: Micrographic Structure of Cellulose derived from Rice Husk

Lignin:

The surface morphology of lignin extracted from rice husk has been characterized using SEM analysis. The micrographs of lignin have suggested the irregular structure of lignin with compact interior and uneven step like surface (Fig. 12).

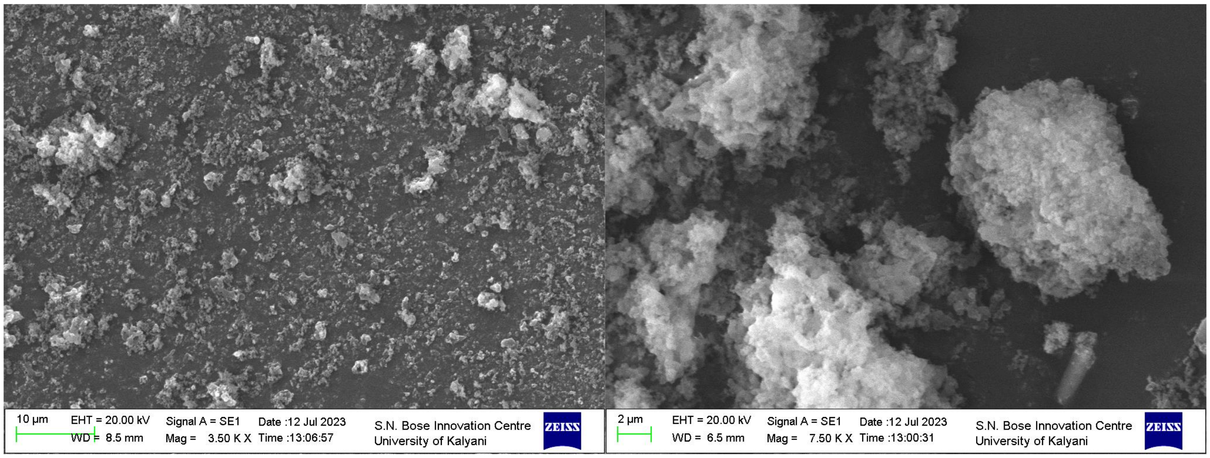


Fig. 12: Micrographic Structure of Lignin derived from Rice Husk

Hemicellulose:

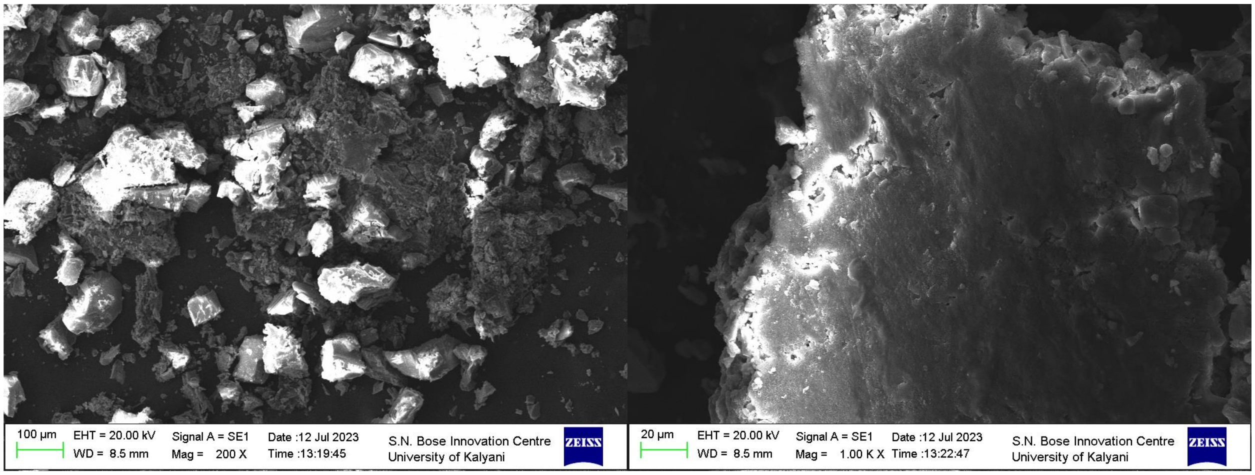
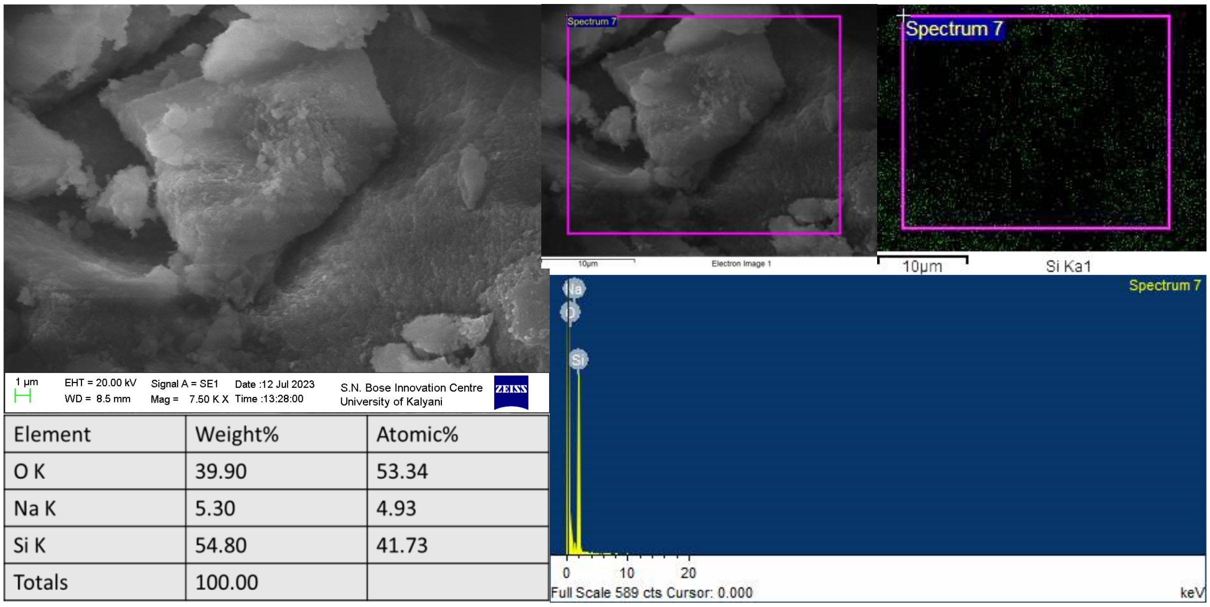


Fig. 13: Micrographic Structure of Hemicellulose derived from Rice Husk

The scanning electron microscopy (SEM) analysis has been done to study the morphology of hemicellulose extracted from rice husk. From the analysis it was observed that the hemicellulose component consists of small crystal-like structure with rough surface. Kaur et al., 2020 reported that the surface morphology of hemicellulose can change with the temperature and the smoothness of surface increases with the increase of temperature. Small pore like structures and cracks were also observed on the surface that confirms the higher internal surface area of that compound.

Silica

The SEM micrograph of Silica particles synthesised from rice husk has been observed to be agglomerated form of orientation with a magnification of 7500x . While performing the EDAX mapping and analysis, it was found that the presence of silica in the spectrum is 54.8 weight % while abundance of oxygen and sodium is 39.9 % and 5.3% respectively (Fig.14).

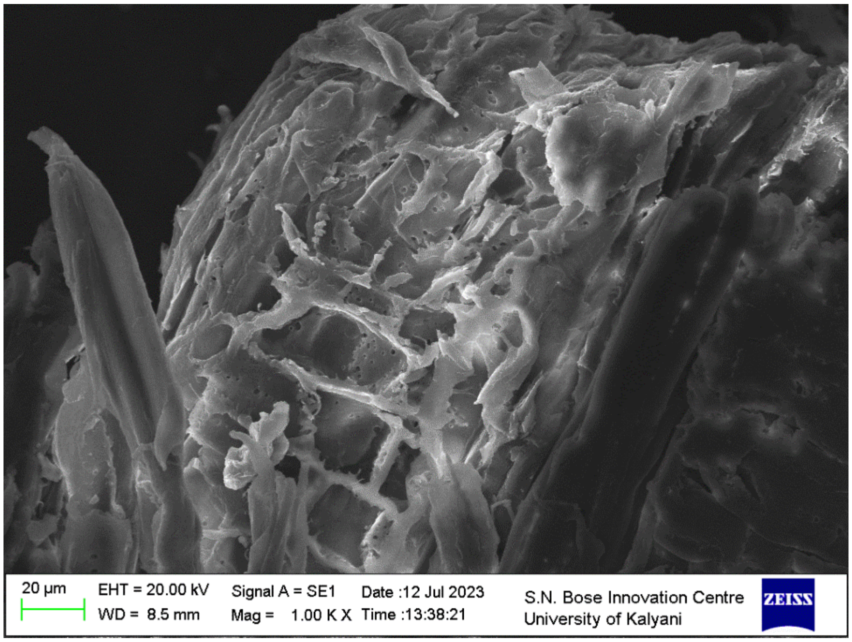


Element	Weight%	Atomic%
O K	39.90	53.34
Na K	5.30	4.93
Si K	54.80	41.73
Totals	100.00	

Fig. 14: Micrographic Structure and EDAX mapping of Silica particle derived from Rice Husk

Microcrystalline Cellulose:

Micrographic image of Microcrystalline Cellulose produced from rice husk has depicted a porous structure on the fiber. The unequal distribution of microfibers in cellulose suggested the treatment has caused fibrillation of cellulose. Due to the high porosity of cellulose fiber, the surface area has increased significantly. Along with that, the EDAX analysis has revealed the abundance of higher carbon percentage in the structure (Fig. 15).



Element	Weight%	Atomic%
C K	56.59	63.45
O K	43.42	36.55
Totals	100.00	

Fig. 15: SEM and EDAX analysis of Microcrystalline Cellulose derived from Rice Husk

Particle Size Analysis of Silica particles:

The activity and effectiveness of a particle is directly related to the size of the particle. In order to measure the size of the silica particle synthesized from rice husk, dynamic light scattering has been used in Zetasizer Nano ZS Size Analyzer from Malvern Panalytical. In case of RH derived Silica, the average size of the silica particle has been found to in the range of 32.67 nm. The polydispersity index of 1.00 signifies the homogeneous distribution of particle in the solution (Fig.16).

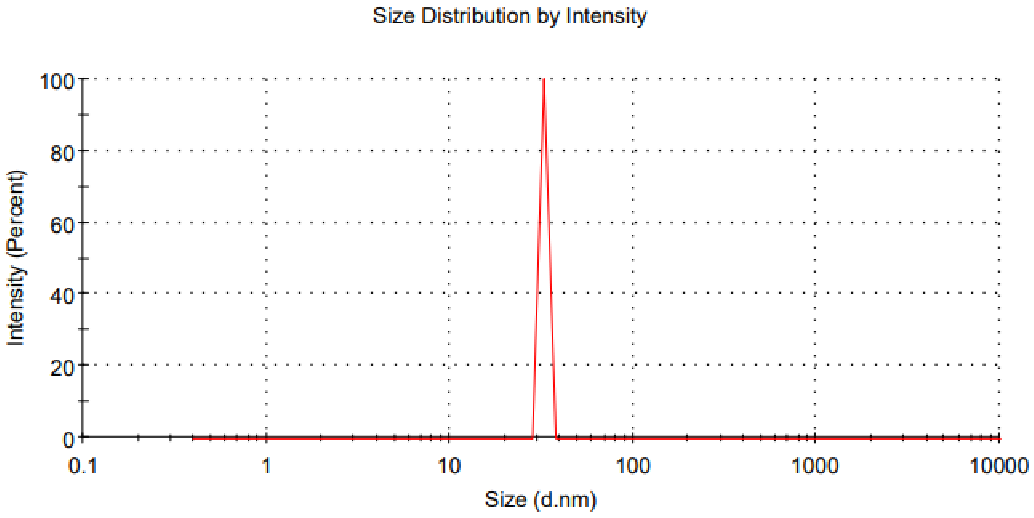


Fig. 16: Particle Size Analysis of Silica NP derived from Rice Husk

Quantification of Furfural using HPLC:

To measure the amount of furfural synthesized from rice husk derived cellulose, High Performance Liquid Chromatography analysis was performed. In order to make the standard curve, solution of three different concentrations of furfural were made (2.5mg/mL, 5mg/mL and 10mg/mL). The chromatogram was detected on a UV/Visible detector with 280nM wavelength. The retention time was 10 min. After plotting the chromatogram with respect to retention time, the area of the peak situated at 3.876 minutes were determined as the peak for furfural.

In case of furfural synthesis from cellulose derived from rice husk, it has been found that 1gm of cellulose residue is able to synthesize 26 to 37 mg of furfural (Fig.17).

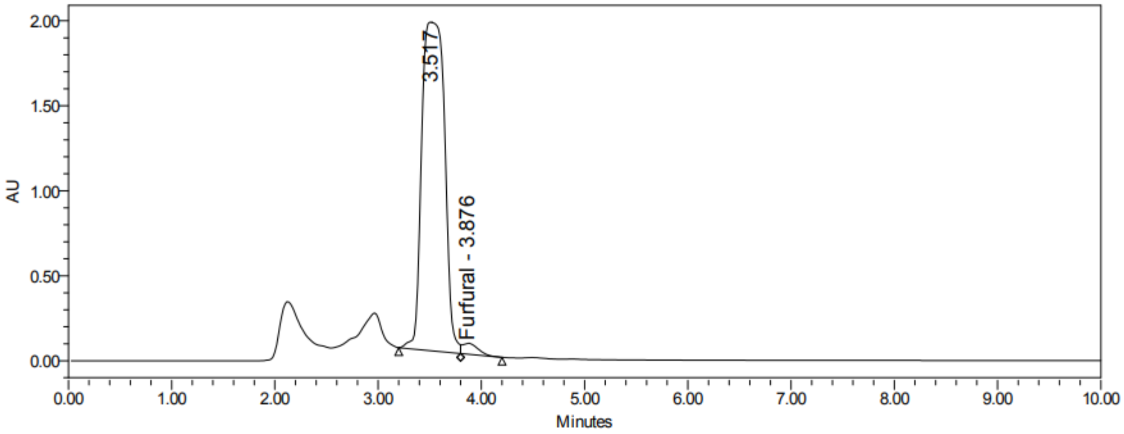


Fig. 17: Chromatogram of Furfural Synthesized from Rice Husk derived cellulose

Estimation of Total Reducing Sugar after Saccharification:

The amount of TRS produced by hydrolysis process on the cellulose extracted from rice husk pretreatment by *Aspergillus sp.* is shown in the table below.

Pretreatment of Rice Husk	**TRS mg per gm of Cellulose residue**
2% H_2SO_4 Non-sonicated	11.59791309
4% H_2SO_4 Non-sonicated	8.276353738
6% H_2SO_4 Non-sonicated	12.58627953
2% H_2SO_4 Sonicated	12.47286043
4% H_2SO_4 Sonicated	9.507761107
6% H_2SO_4 Sonicated	17.81976085
2% NaOH Non-sonicated	31.43005282
4% NaOH Non-sonicated	26.61784244
6% NaOH Non-sonicated	5.943160828
2% NaOH Sonicated	33.30956933
4% NaOH Sonicated	7.061149098
6% NaOH Sonicated	8.730030137

Table 2: Estimation of Total Reducing Sugar

It has been found that the highest amount of cellulose was synthesized from cellulose after pretreatment with Ultrasonication after 2% NaOH pretreatment (33.31mg/g of cellulose residue) , followed by 2% NaOH Non-sonicated (31.43mg/g of cellulose residue) and 4% NaOH Non-sonicated (26.62 mg/g of cellulose residue). Among the acidic pretreatment, 6% H2SO4 Sonicated has found to have the highest yield (17.82mg/g of cellulose residue) (Table 2).

Estimation of Bioethanol:

After fermentation, the amount of ethanol has been estimated using Dichromate method. The highest amount of ethanol fermented by *Saccharomyces cerevisiae* has been 2.90 g/g (2% NaOH Sonicated) followed by 2.86 g/g (4% NaOH Non-sonicated) and 2.42 g/g (2% NaOH Non-sonicated). Among H2SO4 pretreated samples, the maximum amount of ethanol has been generated in 2% H2SO4 sonicated (1.47g/g) followed by 2% H2SO4 non-sonicated (1.45 g/g) and 6% H2SO4 Non-sonicated (1.41g/g) (Table 3).

Pretreatment of Rice Husk	**g of ethanol /g of rice husk**
2% H_2SO_4 Non-sonicated	1.45
4% H_2SO_4 Non-sonicated	0.97
6% H_2SO_4 Non-sonicated	1.41
2% H_2SO_4 Sonicated	1.47
4% H_2SO_4 Sonicated	1.16
6% H_2SO_4 Sonicated	1.74
2% NaOH Non-sonicated	2.42
4% NaOH Non-sonicated	2.86
6% NaOH Non-sonicated	0.72
2% NaOH Sonicated	2.90
4% NaOH Sonicated	0.98
6% NaOH Sonicated	1.09

Table 3: Estimation of Bioethanol produced from rice husk cellulose

1.6.Conclusion:

Lignocellulosic biomass is the most abundant biopolymer containing cellulose, lignin and hemicellulose as the building blocks. Rice husk, the most important lignocellulosic biomass in agriculture-based countries like India, comprises cellulose (25-35 %), hemicelluloses (18-21 %), and lignin (26-31 %). Alongside that, silica (2-5%) and other high-value materials such as phenolic acids and flavonoids can be extracted from rice husk (Ludueña et al. 2011).

Various pre-treatment methods have been utilised to break down the structural integrity of lignocellulose in order to isolate various precursor and value-added products. Non-biological pretreatments such as physical and chemical treatment have been used in industries to extract cellulose, and biogas such as methane, butanol etc. from rice husk. On the other hand biological pre-treatments using microbes (fungi) have been employed to degrade the structure of lignocellulose and produce reducing sugars(Guerriero et al. 2016) (Fig. 2).

Rice husk is a lignocellulose that can be utilised as a great source of cellulose, lignin, hemicellulose and other high-value material in intend to produce various value-added-derived products. Cellulose extracted from rice husk can be used as a precursor in fermentation for the synthesis of bioethanol and other pharmaceutical substances having high value and demand (Sun et al. 2002). Lignin, one of the most prevalent polymers of nature can be extracted from rice husk and used to produce artificial flavouring vanillin and other important consumables such as pharmaceuticals, herbicides, hair oils etc (Brodin et al. 2007). On the other hand, in the production of furfural, xylitol and bioethanol, rice husk-derived hemicellulose can act as a precursor (Luo et al. 2019). Besides, rice husk is a great source of silica and activated carbon. Along with that some amount of antioxidants and phytochemicals such as phenolics and flavonoids can be found in rice husk extract having significance in ROS scavenging and anti-cancer activity (Gao et al. 2018).

Although studies relating rice husk as an important lignocellulosic biomass have been done for decades the diversity in implications is limited. In a recent study, modified rice husk has been seen to be capable of removal of harmful dyes (such as malachite green) and metals (Cu), but the spectrum of studies is limited (Chowdhury and Saha, 2012; Jaman et al. 2009). Apart from this, the involvement of different rice-derived products in stress tolerance and prevention of biomagnification of harmful products in our food materials also need to be studied in future.

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Chapter 2:

Synthesis of Rice Husk-Derived Cellulose for Efficacious Removal of Malachite Green from Aqueous Solution.

2.1. Introduction:

Wastewater:

Water is regarded as one of the most valuable resources for a civilization's survival. However, as development progressed through industrialization, waste production increased. Since then, effluents from various industries have polluted the very source of civilizations' survival. Coloured wastewater is generated due to industry dyeing and finishing processes, further ejected into natural streams, having negative repercussions on environmental ecosystem and anthropogenic health. More than 10000 different synthetic dyes and colouring agents exist with an annual production of over 0.7 million tonnes (Gupta and Suhas, 2009). Interestingly, over 20% of the total dye produced enters the industrial discharge signifying its existence in natural streams and can originate serious harm to marine life by rising toxicity, chemical oxygen demand, and impeding photorespiration processes through reduced light penetration (Demirbas A. 2009;Ozdemir et al. 2009).

Malachite Green as a contaminant:

One such commonly used dye of cationic nature is Malachite Green (MG). MG, while useful in various industrial applications (dyeing of silk, leather, plastics, paper, and others), is also used in aquaculture industries as a fungicide, bactericide, and parasiticide. MG is an N-methylated diamino-triphenylmethane dye. In solutions, the dye produces two distinct ions: chromatic malachite green (cation) and carbinol base. This dye is chemically reduced to a leuco derivative (Culp and Beland, 1996). In nature, MG and leuco-malachite green are toxic and thus can cause harm to humans and animals through direct contact, inhalation, and ingestion. Human carcinogenesis, chromosomal mutation, genotoxic, respiratory toxicity, and decreased fertility have been reported in many studies (Ahmed et al. 2021).

Proper treatment and dye removal of wastewater is one of the major concerns in recent times. Due to the high stability of the synthetic dyes, environmental exposure to heat, water, light, and the microbial attack becomes effectless (Aksu et al. 2008). Although there are various traditional methods of dye removal is present but high operating costs, labour intensive and incomplete removal are some of the major disadvantages. In this dilemma, Adsorption has emerged as a cost-effective, easy-to-use and efficient alternative by which complete removal of the pollutant is possible even in diluted conditions. Natural materials along with waste materials have been used as efficient adsorbents for synthetic dye removal. Usage of agricultural waste such as rice husk, sawdust, banana peel, orange peel etc. has a significant edge due to the easy availability, cost-effectiveness, low toxicity and possibility of dye recovery (Chowdhury et al. 2011).

Lignocellulosic Biomass as an adsorbent:

Agricultural lignocellulosic wastes consist of a nonedible, sustainable supply of chemical components such as cellulose, hemicellulose and lignin in different ratios (Fu and Mazza, 2011). Rice being the staple food of most of the world's population, especially in South Asia and Africa, generates a massive amount of agricultural waste such as straw and husk. Recent developments have increased the processivity of rice straw into biofuels, fertilisers, paper and animal feed (Goodman B A , 2020). Burning of rice straw after harvesting is one of the major environmental concerns in countries like India. On the other hand, Rice husk comprises of cellulose, Lignin, hemicellulose, and silica in order to make it chemically and mechanically resistant (Chuah et al.2005; Ludueña et al. 2011). In order to break down rice husk and synthesise cellulose, the disintegration of the lignin-hemicellulose barrier and disruption of the silica shell become essential (Chundawat et al. 2011). The interior surface of the rice husk notably consists of lignin and cellulose, whereas inorganic silica is the main building block of the exterior surface (Moayedi et al. 2019). The extraction of cellulose after deranging the structural integrity of lignocellulose is a difficult process. Although due to the behaviour of as hydrogen bond acceptor, ionic fluids can disintegrate the complex polymer structure and solubilise cellulose (Zhu et al. 2006). Among chemical pretreatment of lignocellulosic biomass, alkaline pretreatment holds a certain edge due to its simple and cost-effective reaction conditions over acidic pretreatments. OH-forms of sodium, calcium, ammonium and potassium salts are the prevalent alkalis for the breakdown of lignocellulosic biomass. Among these, Sodium hydroxide was found to be the most used pretreatment method due to its ability to breakdown the ester and glycosidic side chains in order to swelling and crystallisation of cellulose (Ibrahim et al. 2011; McIntosh and Vancov, 2010).

This study aimed to understand the role of different concentrations of Sodium hydroxide (NaOH) pre-treatment coupled with the effect of sonication on rice husk in order to synthesise rice husk-derived-cellulose and its effectivity in the removal of Malachite green (MG) as a wastewater pollutant. This study also investigates the involvement of different parameters such as reaction temperature, dye-adsorbent contact time, initial pH value, dye concentration, and dosage of adsorbent on the adsorption dynamics.

Alongside that, an effort was made to investigate the thermodynamic parameters, adsorption kinetic models and isotherms models in the study.

2.2. Materials and Methods:

Preparation of Malachite Green solution:

For this study, malachite green (Loba Chemie, India) was selected as the pollutant of interest. To prepare the stock solution 100 mg of dye was dissolved in 1000 ml of deionized water. During the continuation of this experiment, the 100mg/L stock solution was diluted to achieve the required concentrations.

Batch biosorption experiment:

To determine the adsorption kinetics, isotherm and the reaction thermodynamics a batch study was conducted. The malachite green solution was taken in an Erlenmeyer flask with the adsorbent and the study was conducted in a BOD shaker incubator for 120min. To study the adsorption kinetics, samples were collected at 30 min intervals. The samples were centrifuged to separate the solid adsorbent from the dye solution and the final pollutant concentration was measured by a UV-Vis spectrophotometer (Thermo Scientific Orion Automate 8000, USA) at an absorbance maximum (λmax) of 617nm. Different parameters such as adsorbent dose, contact time, initial pH of dye solution, the concentration of dye in the media and reaction temperature were studied to understand their effect on the adsorption dynamics.

Influence of adsorbent dose:

100 ml of 10 mg/L dye solution was used for this study. Three different doses viz., 0.5 g/L, 1 g/L and 1.5 g/L of adsorbent were considered to conduct the experiment. All the experiments were conducted in a BOD shaker incubator at 303K for 120 min.

Influence of pH:

Three different values of initial pH viz., pH 4.0, pH 6.0, and pH 8.0 were considered for this experiment. A 0.1 M NaOH solution and 0.1 M HCl solution were used to adjust the pH of the solution. The concentration of the dye considered for this experiment was 10 mg/L and the adsorbent dose was 1.5 g/L. The study was conducted for 120min at 303K.

Influence of dye concentration:

The influence of dye concentration on the adsorption process was considered as a parameter for this study. To prepare three different concentrations viz., 10,30, and 50 mg/L, the 100 mg/l stock solution was diluted into deionized water. The study was conducted with 1.5g/L of adsorbent dose at 303K having initial pH 6.0 in a BOD shaker incubator.

Influence of temperature:

Temperature is also an important factor to determine not only the adsorption efficiency but also the isotherm model of the process. Three different temperature 298K, 303K, and 308K was considered and maintained in a BOD shaker incubator while other parameters were unchanged. 10 mg/L solution was taken for this experiment with a 1.5g/L adsorbent dose and pH 6.

From the absorbance value of the dye solution determined by the UV-Vis spectrophotometer, the percentage of malachite green removal was calculated using the mentioned equation:

$$\text{Removal } \% = \frac{C0-Ct}{C0} x100 \tag{1}$$

Where C_0 denotes the initial concentration (mg/L) of malachite green in an aqueous solution whereas, C_t is the concentration of the dye after the adsorption performed for time t.

Also, the adsorption capacity of the adsorbent at time t was determined via,

$$q_e = \frac{(C0-Ct)xV}{m} \tag{2}$$

where V signifies the volume (L) of the experimental solution utilised during batch study and m denotes the mass (g) of the adsorbent used for the experiment. q_e (mg/g) represents maximum adsorption capacity at equilibrium time t.

Isotherm study of the experiment:

From the result of the batch study, the adsorption isotherms of that reaction were analysed. A total of three isotherm models named, the Langmuir model, Freundlich model, and Temkin model were considered for analysis (Saha et al. 2009).

Considering the Langmuir isotherm model, which is applicable for monolayer biosorption on a homogeneous surface with a restricted number of binding sites, can be expressed as,

$$\frac{Ce}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0} \tag{3}$$

where, C_e represents the equilibrium dye concentration in solution (mg/L), whereas q_e is the amount of malachite green adsorbed at equilibrium (mg/L), b and Q_0 denote the Langmuir constant related to the energy of adsorption and adsorption capacity respectively.

For the calculation of Freundlich isotherm constants, the following equation was used,

$$ln q_e = ln K_f + (\frac{1}{n}) ln C_e \quad (4)$$

where K_f and n denote the Freundlich constants regarding adsorption capacity and adsorption intensity respectively, whereas q_e and C_e represents the amount of malachite green (mg/L) in adsorbent and in solution respectively.

The following equation was utilised for studying the Temkin isotherm model,

$$q_e = B_T \, ln \, C_e + B_T \, ln \, K_T \quad (5)$$

Where, q_e and C_e denotes the same as Langmuir and Freundlich isotherm model whereas K_T and B_T represents the Temkin equilibrium binding constants.

Kinetic study:

The batch study was conducted at different temperatures and the data was utilized for kinetic study. For that, pseudo-first-order and pseudo-second-order models were considered for three different temperatures (298K, 303K, and 308K) (Saha et al. 2010) . The correlation coefficients (R^2) of each model were determined to analyse the appropriateness of the model and for comparative study of the experimental adsorption capacities and theoretical values.

The linearized expression of the pseudo-second-order model was as follows,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \quad (6)$$

Where q_t and q_e represent the amount of malachite green adsorbed (mg/g) at any time 't' and in equilibrium respectively. Whereas k_2 denotes the pseudo-second-order rate constant.

Thermodynamic analysis:

The thermodynamic parameters of malachite green adsorption were determined by the change in the Gibbs free energy (ΔG), changes in enthalpy (ΔH^0) and the change in standard entropy (ΔS^0) (Saeed et al. 2010). The thermodynamic factors were calculated by using the following equations:

$$\Delta G = -RT \ln K_c \qquad (7)$$

$$K_c = \frac{C_a}{C_e} \qquad (8)$$

$$\Delta G = \Delta H^0 - T\Delta S^0 \qquad (9)$$

In these equations, K_c, C_a and T represent the distribution coefficient of adsorption, the quantity of malachite green adsorbed by unit mass of adsorbent and the operational temperature in Kelvin respectively. The intercept and slope of ΔG vs T plot were used to calculate the value of ΔS^0 and ΔH^0, respectively.

2.4. Results and Discussion

Batch study:

Effect of adsorbent dose on the removal of malachite green:

In the intent to study the effect of dosage of 2%, 4% and 6% NaOH pretreated rice husk-derived cellulose with or without sonication as an adsorbent in the removal of malachite green from an aqueous solution, three different dosages (0.5 g/L, 1g/L and 1.5 g/L) of three non-sonicated (2%, 4% and 6% NaOH cellulose non-sonicated) and three sonicated (2%, 4% and 6% NaOH Sonicated) adsorbents were implemented in 100 ml of 10mg/L of Malachite green aqueous solution at 30°C for 2 hours. It has been found that with the increase in dosage, the effective removal of malachite green has increased. At 0.5g/L dose, within 30 mins of the study, 4% NaOH Cellulose sonicated has successfully removed 89.35% of the dye followed by 6% NaOH Cellulose sonicated (88.9%), 2% NaOH Cellulose sonicated (82.35%), 4% NaOH Cellulose non-sonicated (72%), 6% NaOH Cellulose non-sonicated (70.6%), and 2% NaOH Cellulose non-sonicated (69.2%). Among the sonicated adsorbents 6% NaOH Cellulose sonicated has been found to be the most efficient in the removal of malachite green (93.66%) after 60 minutes of the process whereas, 6% NaOH Cellulose non-sonicated has been observed to be the most effective in removal of the contaminant after 120 min (90.11%) among non-sonicated adsorbents (Fig. 18A).

On the other hand, at 1g/L dosage of adsorbent, 6% NaOH Cellulose non-sonicated has shown the most amount of removal after 1 hour of adsorption (96.64%) followed by 4% and 2% NaOH Cellulose non-sonicated (96.35% and 95.24% respectively) amongst non-sonicated adsorbents. Apart from the non-sonicated adsorbents, all three NaOH Cellulose sonicated adsorbents were capable of removal of >96% of malachite green in aqueous solution after 1 hour but 6% NaOH Cellulose sonicated was the most effective (96.82%). After reaching the highest amount of removal, some amount of desorption has also been observed in almost all the adsorbents (Fig. 18B).

Among all the three dosages, 1.5g/L has been proven to be the most effective dose in the removal of malachite green due to the higher availability of adsorbent surface area resulting in higher active sites for adsorption (Fig. 18C). It has been observed that, after 2 hours of study, almost all three non-sonicated adsorbents have successfully removed more than 97% of the dye whereas, the maximum amount of removal in sonicated samples has been performed by 6% NaOH Cellulose sonicated (97.28%) after 120 min. Most interestingly it has been seen that sonicated cellulose materials are associated with the rapid removal of malachite green although it reaches saturation earlier than the non-sonicated NaOH cellulose samples. Hence, increasing the dose of adsorbent might lead to the aggregation of solid particles and decrease the surface area for adsorption (Saeed et al. 2010; Banerjee et al. 2015). Therefore, for further understanding of the role of temperature, pH, and dye concentration, the adsorbent dose of 1.5g/L has been kept constant..

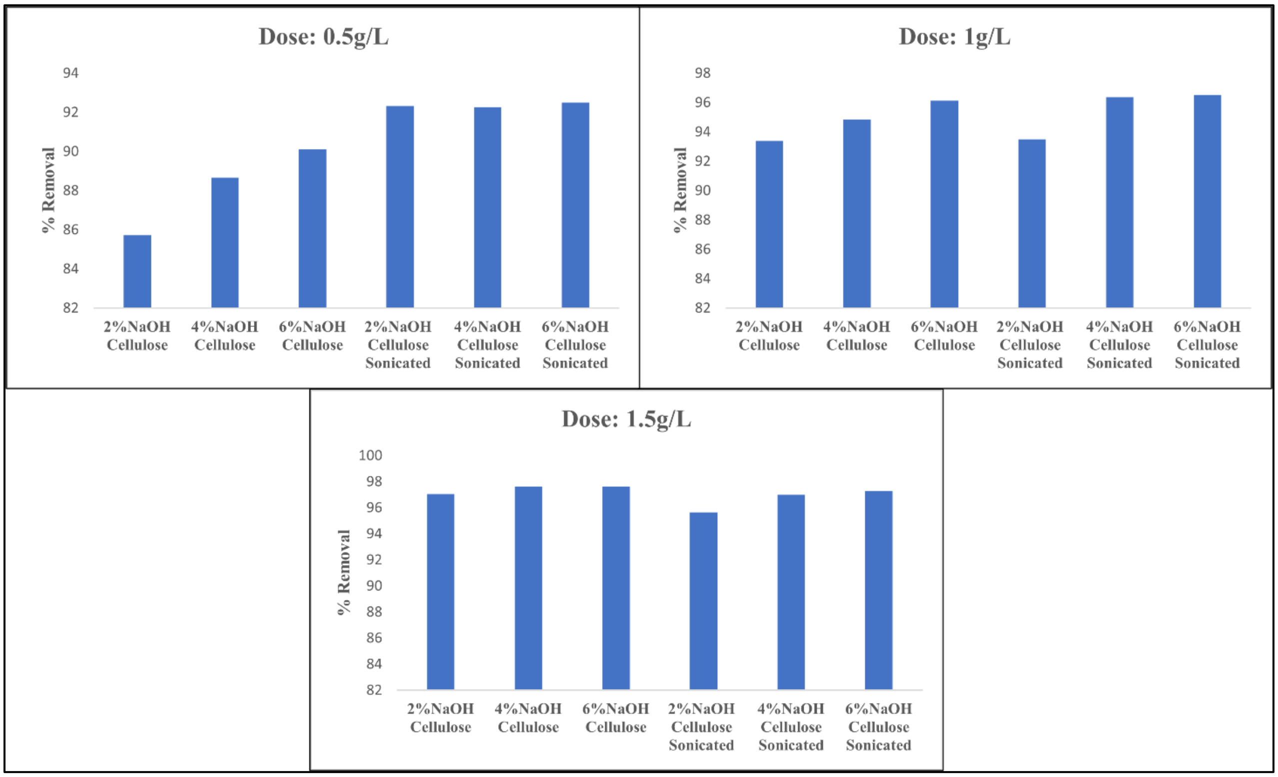

Fig. 18: Effect of adsorbent dose on the removal of malachite green

Effect of process temperature on removal of malachite green:sssss

Process temperature can play a significant role in adsorption with respect to contact time. In this study, it has been found that an increase in temperature can positively regulate the rate of adsorption of malachite green onto the cellulose adsorbents. From Fig. 6 it can be easily inferred that at 298K, the highest percentage of removal has been encountered in 6% NaOH

Cellulose sonicated (96.76%) whereas in 303 K 6% NaOH non-sonicated Cellulose has been found to be the most efficient in removal of malachite green from the solution after 90 min of the study. Temperature 308K has been identified to be the most optimal temperature for adsorption as in this temperature the highest amount of removal (97.9%) has been seen in 6% NaOH non-sonicated Cellulose and 4% NaOH non-sonicated Cellulose within 90 min and 120 min respectively (Fig. 19). This might be caused by the increase in surface area due to the increase in temperature. Along with that, due to the increase in active sites of the inner and outer surface of the adsorbent, the rate of diffusion of malachite green on cellulose adsorbent might increase (Banerjee et a. 2015).

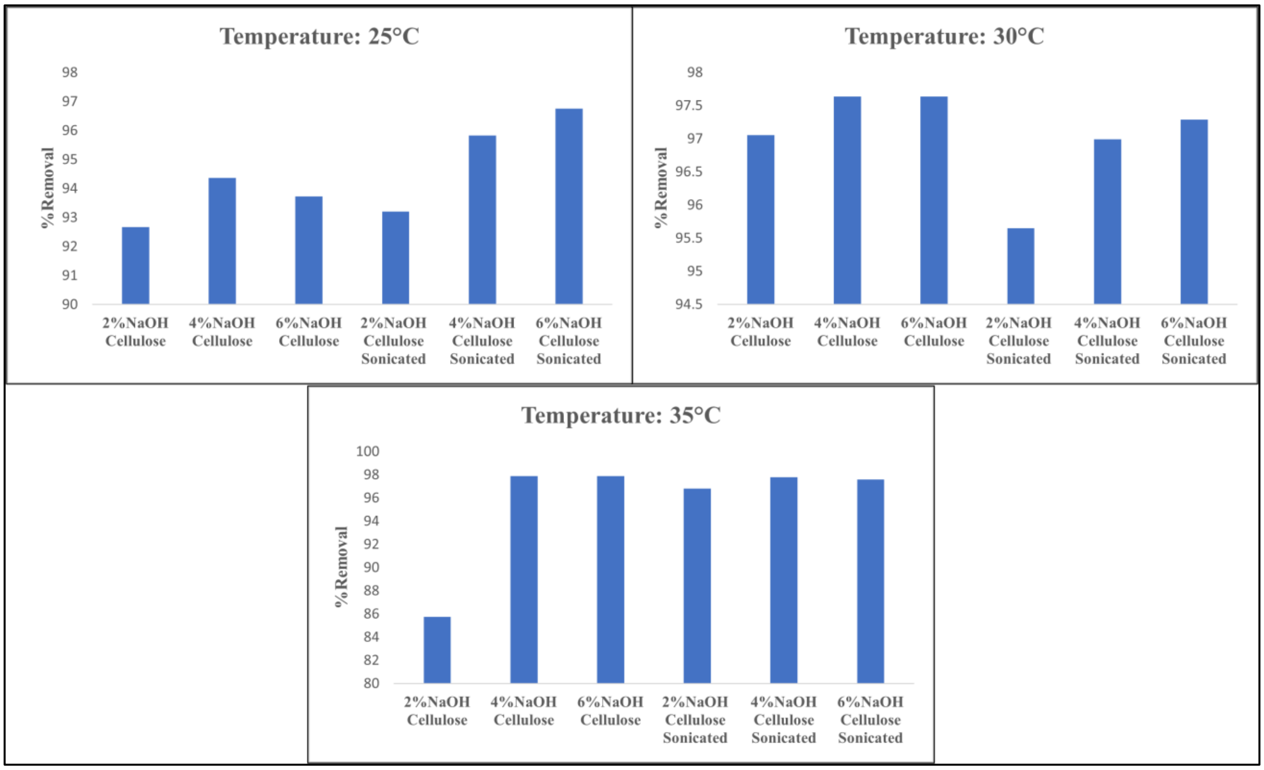

Fig. 19: Effect of process temperature on the removal of malachite green

Effect of Initial pH of the solution in adsorption of malachite green:

The involvement of pH in the adsorption of contaminants is well established due to its influence on the chemical nature of the adsorbate along with the surface characteristics of the adsorbent. In this current study of the removal of malachite green onto 6 different rice husk-derived celluloses, three different pHs (pH4, pH6 and pH8) have been accounted for understanding the role of pH on the adsorption process. From the result, it has been observed that the percentage removal has increased with the increase in pH up to pH 6 but decreases thereafter (Fig. 20). pH 6 has been identified as the most optimum pH of the solution with the highest removal of 97.63%, followed by pH 4 (96.58%) and pH 8 (96.29%) (Fig. 20). Similar to Banerjee et al. (2015), with the increase in pH, the charge of the adsorbent surface becomes more negative resulting in electrostatic attraction between the cellulose adsorbent surface and cationic dye malachite green (Banerjee et a. 2015).

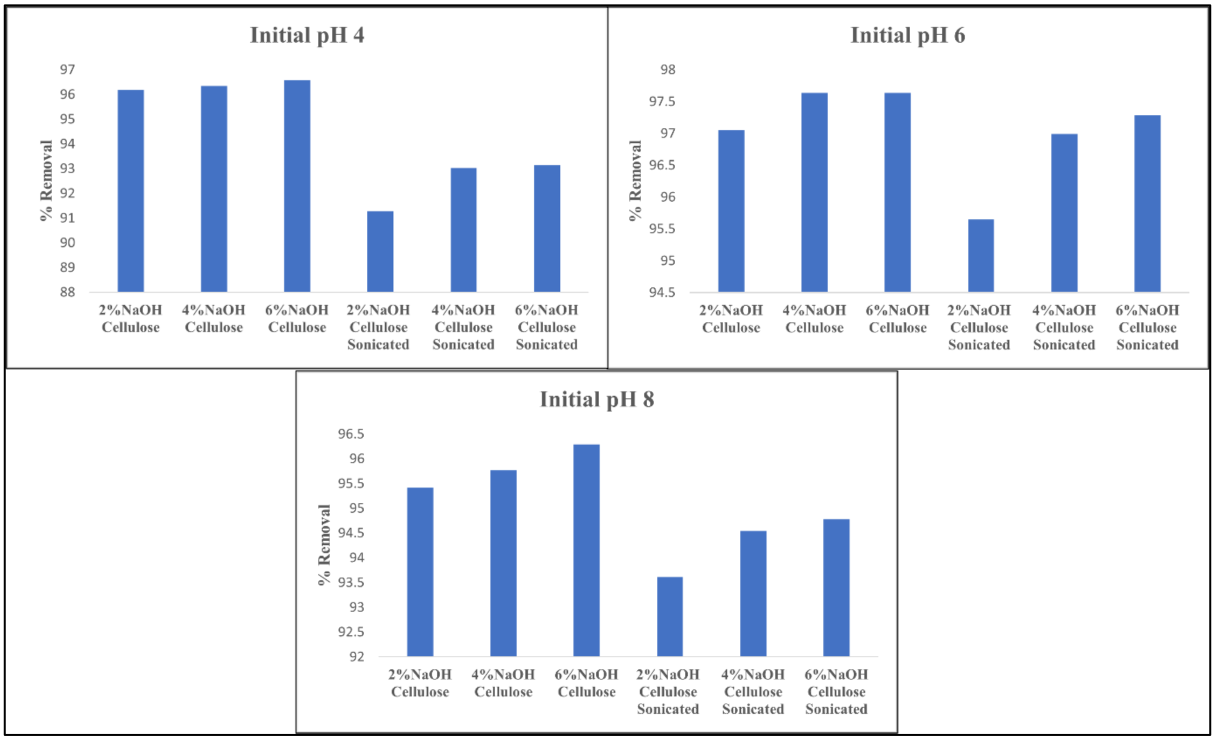

Fig. 20: Effect of initial pH on the removal of malachite green

Effect of Dye concentration in adsorption of malachite green:

In order to understand the effect of different concentrations of malachite green on the adsorption process, three solutions having MG concentrations of 10mg/L, 30mg/L and 50mg/L have been prepared and undergone adsorption with an adsorbent dose of 1.5g/L at 303K for 120 min. From Fig. 21, it has been found that the percentage removal of malachite green onto the cellulose adsorbent was slightly more at 30 mg/L (98.14%) in comparison to 10 mg/L

(97.63%) followed by 50 mg/L (97.16%) (Fig. 21). It was observed that the removal of malachite green by rice husk-derived cellulose has been maintained at over 97% in all three concentrations of dyes. This may

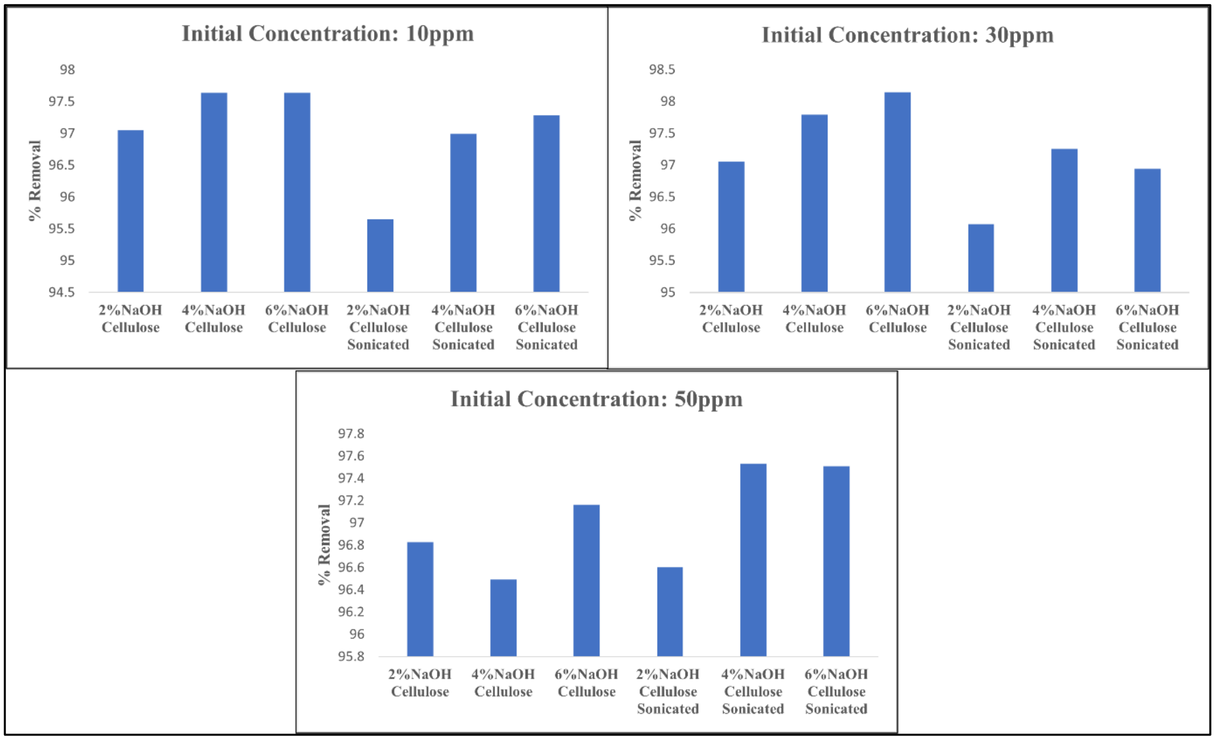

Fig. 21: Effect of Dye Concentration on the removal of malachite green

suggest that, while applying a lower concentration of dye, there is an abundance of free active sites on the adsorbent surface. It has been observed that, after 30mg/L of malachite green concentration, the percentage removal has decreased, suggesting the saturation of active sites resulted in slight descent in the percentage removal of malachite green.

3.6. Biosorption Isotherm:

The concept of adsorption isotherm revolves around the equilibrium relationship between the concentration of the adsorbate in the liquid phase and its concentration on the surface of the adsorbent under specified conditions. The underlying relationship becomes pivotal in order to understand the retentivity, release or substance mobility to a solid phase from aqueous porous media. For the purpose of describing these equilibrium relationships, several isotherm models have been developed such as Langmuir, Freundlich, and Temkin models. Although, it becomes necessary to identify the most appropriate model of isotherm which represents the adsorption equilibria justifying the applicability as no single model can be universally applied due to the uncertainty of assumptions.

In the current study, three isotherm models have been utilised viz. Langmuir, Freundlich, and Temkin to identify the most suitable model of specific sorption of MG by Rice husk derived cellulose. The results, depicted in Fig. 22 clearly suggest that the representation of the experimental data is better depicted by the Langmuir isotherm model in comparison to the other three isotherm models with 100mL solution of 10mg/L malachite green dye at temperature 303K with 1.5g/L dose of 6%NaOH cellulose non-sonicated as an adsorbent. The Langmuir constant Q^0 (maximum adsorption capacity) is 5.51 mg/g and b is 45.3 L/mg with a regression coefficient of 0.9999 (Fig. 22). On the other hand, the regression coefficient in Freundlich isotherm is 0.9853 where the coefficient of adsorption capacity(K_F) is 5.62(mg mg^{-1})(L mg^{-1}) (Fig. 22). Alongside that, the regression coefficient in the Temkin isotherm model is 0.9863 (Fig. 22). According to the experimental data, the malachite green adsorption on RH-derived cellulose is monolayer adsorption.

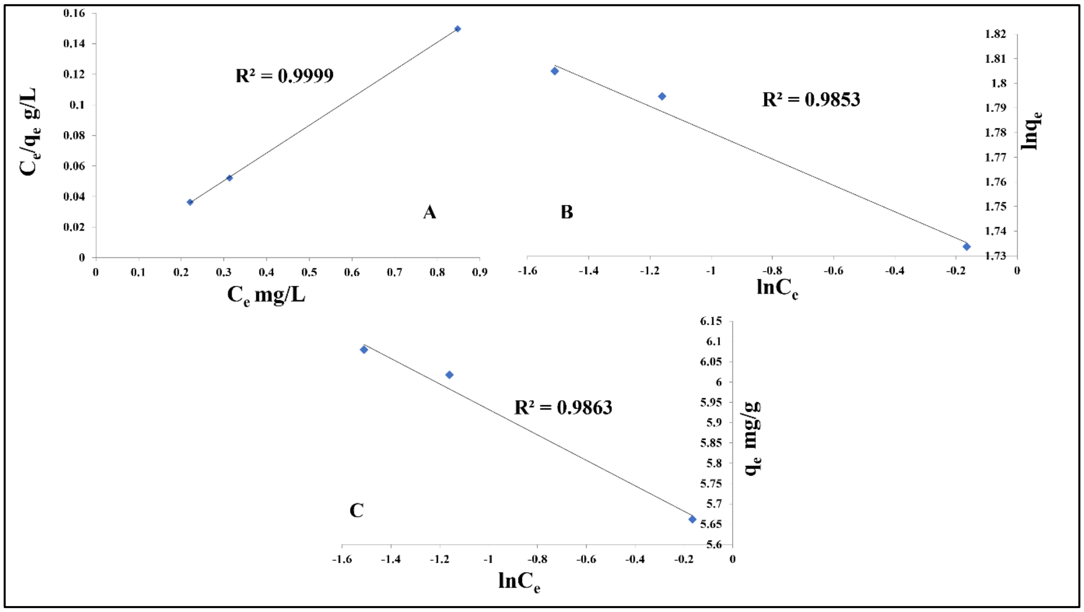

Fig. 22: Adsorption isotherm

$$R_L = \frac{1}{1+bC_0} \qquad (10)$$

In order to calculate the separation factor (R_L), the aforementioned equation can be used where C_0 denotes the initial malachite green concentration (mg/L) and b signifies the Langmuir constant. It has been found that the value of the separation factor (R_L) is 0.0024, well in the range of 0 to 1 signifying the favourability of the process (Saha et al. 2010).

3.7. Biosorption Kinetics:

In order to unravel the residual time of sorbate in a solid-solution interface encompassing the diffusion process on the adsorbent, understanding the kinetics of a biosorption process becomes necessary. With the variance of temperature from 298K to 308K, the adsorption efficiency of rice husk-derived cellulose has ranged from 4.25 to 4.55 mg/g for 50ml of 10mg/L of malachite green solution. Intending to study the kinetics of MG biosorption in contact with rice husk-derived cellulose, pseudo-first-order and pseudo-second-order models were utilised. From the experimental data, the adsorption of malachite green using rice husk-derived cellulose favours the pseudo-second-order kinetic model more than the pseudo-first-order model. The regression coefficient of the pseudo-second-order kinetic model happens to be 0.9994 in comparison to the regression coefficient of the pseudo-first-order kinetic model which is 0.8473 (Fig. 23). Along with that, the theoretical (q_e) and experimental(q_t) adsorption efficiency values derived from the pseudo-second-order model are fairly similar to each other, which confirms the adsorption process to follow the pseudo-second-order model. On the other hand, the positive rate constant (k_2) of the pseudo-second-order model inferred the endothermic nature of the adsorption process (Gupta and Bhattacharya, 2006).

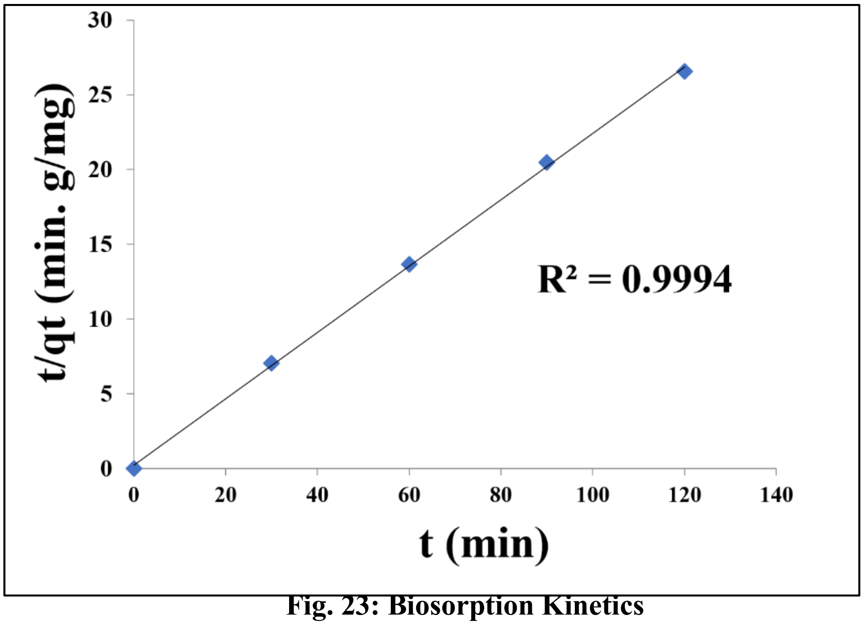

Fig. 23: Biosorption Kinetics

3.8. Thermodynamics:

Thermodynamic parameters such as changes in Gibbs free energy, enthalpy and standard entropy can be associated with the adsorption process as temperature plays a significant role. The spontaneity of the process can be determined by the classical Van't Hoff equation as the Gibbs free energy change (ΔG^0) plays a key role:

$$\Delta G^0 = -RTlnK_c \qquad (7)$$

where R denotes the universal gas constant ((8.314 J mol^{-1} K^{-1}), T signifies the absolute temperature in K and K_c is the biosorption distribution coefficient defined by the ratio of C_a (sorbate concentration at adsorbent in equilibrium, mgL^{-1}) and C_e (sorbate concentration in solution, mgL^{-1}).

According to the following reaction, the change in Gibbs free energy (ΔG^0) can be expressed as a function of change in enthalpy(ΔH^0,kJ mol^{-1}) along with the standard entropy change (ΔS^0, J mol^{-1} k^{-1})

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (9)$$

In this study, the thermodynamic parameters such as ΔG^0, ΔH^0, ΔS^0 have been calculated at different temperatures (298K, 303K and 308K). The spontaneity of the process has established the negative value of ΔG^0 in all the temperatures (-8.41, -9.01, and -9.46 kJ mol^{-1} respectively). The change in enthalpy and standard entropy was calculated from the slope and intercept of the plot lnK_c vs 1/T . The change in enthalpy has been found to be 22.849 kJ/mol, signifying the endothermic nature of the reaction. Due to the nature of energy consumption, the increase in temperature would enhance the removal of MG by cellulose. On the other hand, the change in entropy has been found to be negative (-105.00582 J mol^{-1} k^{-1}) suggesting the decrease in system randomness in the sorbate-adsorbent interface as the dye is getting removed onto the cellulose adsorbent (Chowdhury and Saha, 2011).

2.5.Conclusion:

In the current study, rice husk was utilised as a lignocellulosic source in order to produce cellulose as a result of NaOH pretreatment and further used as an effective mode for removal of wastewater pollutant malachite green from an aqueous solution. Besides that, the effect of sonication after pretreatment on the structural and functional properties of cellulose produced from rice husk and its effect on the removal of MG has also been studied. The experimental outcomes have suggested that the endothermic adsorption process can be regulated by the process conditions such as the dose of adsorbent, temperature, pH and concentration of the dye. The most efficient removal of malachite green was seen at a cellulose dose of 1.5g/L, pH 6.0, at 30°C for 120 min with a dye concentration of 10mg/L. Pretreatment of rice husk with 6% NaOH (sonicated and non-sonicated both) has been the most efficient as the sonicated cellulose can remove up to 95% of the contaminant within 30 min of the process. The adsorption process has been found to follow the Langmuir isotherm model and pseudo-second-order kinetics model with the characteristics of spontaneous thermodynamic reaction. Taking account of all the findings, it can be concluded that cellulose extracted from rice husk after NaOH pretreatment has the potential of developing a cost-effective adsorbent for efficient dye removal from wastewater and further research is needed to understand its future applications in the field of wastewater treatment.

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