Chemical Pretreatment of Rice and Wheat Straws to Reduce the Recalcitrant Structure: Comparative and Kinetic Studies with Different Chemicals for Biogas Production

Nikita Kanaujia,* Shalu Rawat* and Jiwan Singh*† (iD)

*Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, India
†Corresponding author: Jiwan Singh; jiwansingh95@gmail.com

ABSTRACT

In this study, a comprehensive comparison of two different chemical pretreatments of wheat straw (WS) and rice straw (RS) was made. The pretreatment was performed using piranha solution and *p*-toluenesulfonic acid (PTSA) to dissolve the biomass's complex lignin and hemicellulose matrix to enhance its methane production. Energy dispersive spectroscopy (EDS), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopes (SEM), and X-ray diffraction (XRD) analysis, were used to analyze the characteristics of untreated and pretreated feedstock. WS and RS treated with piranha solution showed maximum methane yield (1234 mL and 1196 mL, respectively). The piranha pretreatment increased the methane yield of wheat straw by 2.37 folds and rice straws by 2.31 folds. The maximum VFA concentration was observed in WS on the 14th day in the piranha-treated sample, 1553.33 ± 2.8 mg.L^{-1}, while in RS on the 21st day in the untreated sample, 676 ± 5.77 mg.L^{-1}. SEM analysis of piranha-treated WS indicated a reduction in recalcitrant structure. Deformation of C-O, C=C, C-C-O, and C-H bonds in cellulose, hemicellulosic, and lignin as a result of chemical pretreatment in WS and RS was also indicated by FTIR analysis. The modified Gompertz model (MGM) and logistic function model (LFM) appropriately defined the degradation process and explained cumulative biogas' kinetic. Pretreatment with piranha solution reduces the complexity of WS and RS, thus increasing methane production by reducing the retention time.

INTRODUCTION

In a time of rapid fossil fuel depletion, air pollution, and increased waste generation, it is vital to encourage the production of a sustainable, reliable, and affordable energy source. Production of clean energy has now become necessary since the power demand is ever-increasing (Owusu et al. 2016, Karuppiah & Azariah 2019, Korys et al. 2019). The fuel shortage could adversely affect economic activity; therefore, the dependency on fossil fuels must be reduced by opting for renewable energy. The utilization of agricultural residue in the energy sector is growing continuously as it is generated in huge quantities and is inexhaustible. The biomass is being replenished continuously with an increase in agricultural activities. Unscientific burning of agricultural waste produces a significant amount of hazardous gases, most notably nitrogen dioxide, sulfur dioxide, nitrous oxide, carbon monoxide, methane, polycyclic aromatic hydrocarbons,

particulate matter, and volatile organic compounds (Dar et al. 2016, Singh et al. 2020). Sustainable agricultural waste management through anaerobic digestion can efficiently resolve some energy crises. According to a report from the Food and Agriculture Organization (FAO RMM 2018), rice comes in the third position after maize and wheat among the most cultivated crops, producing a significant amount of lignocellulosic trash (Singh & Kumar 2019, Kainthola et al. 2019a). The three primary components of any lignocellulosic biomass are lignin, hemicellulose, and cellulose, along with some portion of soluble and insoluble materials like pectin, protein, and minerals (Maldonado-Bustamante et al. 2022). Different biomass has different ratios of these constituents according to crop variety, environmental condition, and soil quality (Kumar et al. 2021). Both amorphous and crystalline forms of cellulose are present in rice straw and wheat straw. Amorphous cellulose degrades effortlessly, while crystalline cellulose is hard to degrade due to microfibril binding. Hemicellulose is linear and highly branched heteropolymer with C-5 and C-6 sugars as its main components. It is

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ORCID details of the authors:

Jiwan Singh: https://orcid.org/0000-0003-3851-7331

susceptible to hydrolysis during anaerobic digestion owing to low molecular weight and low degree of polymerization (Ahmad et al. 2018). Lignin is a hard, hydrophobic, and complicated biopolymer that provides structural strength by producing a barrier that prevents the digestion of WS and RS (Kumar et al. 2021). Cellulose is a linear polymer of D-glucose units linked together by -1,4 bonds and closely maintained by inter and intra-chain hydrogen bonding to form microfiber, which protects the plant cell wall and provides mechanical strength to them (Ma et al. 2019). According to Halac & Ragauskas (2011), RS has more polymerization degree than WS. Hemicellulose is interconnected to lignin by covalent bonds, while hemicellulose and cellulose are linked by hydrogen bonding. Lignin comprises propyl phenol units, namely coniferyl alcohol, sinapyl alcohol, and some amount of p-coumaryl alcohol. These are connected by various ether and carbon-carbon linkages, such as β-O-4, 4-O-5, β-β, β-1, and β-5, to produce phenylpropanoid units like guaiacyl (G), ayringyl (S), and p-hydroxyphenyls (H). The β-O4-linkage is the leading ether bond (60%-40) in the lignin of RS (Sheng et al. 2021). Lignin is difficult to remove because of its attachment with carbohydrates through benzyl esters, benzyl ethers, and phenyl glycosides which provide a robust structure to RS (Satlewal et al. 2018). Pretreatment of WS and RS is necessary as various interlinked bonds mentioned above are hard to digest (Zoghlami & Paes 2019). High silica content in RS and WS also inhibits enzymatic hydrolysis; hence the removal of silica content is required to improve enzymatic accessibility (Athira et al. 2019). Anaerobic digestion of lignocellulosic biomass without pretreatment is un-remunerative. There are numerous pretreatment methods to reduce the recalcitrant nature, such as physical, chemical, and biological among them, chemical pretreatment has become the most approachable and promising method. Chemical pretreatment effectively increases cellulose biodegradability by reducing the complexity of lignin and hemicelluloses (Mancini et al., 2018). Chemical pretreatment techniques include oxidative, ozonolysis, organosolv, acidic, alkaline, and ionic liquid pretreatment. According to the current studies reported in the literature, acidic pretreatment is perhaps the best among these chemical pretreatments if lignocellulose is pretreated with a diluted acid at a high temperature (Mahmood et al. 2019). Oxidative pretreatment of lignocellulose by oxidant is referred to as an effective process. However, it has been studied rarely (Chen et al. 2017). Chemicals varying from oxidizing agents, alkali, acids, and salts are capable of degrading lignin, cellulose, and hemicellulose from lignocellulosic biomass; moreover, organic (formic, acetic, and propionic) inorganic (Sulfuric, nitric, hydrochloric and phosphoric) acids are also used to break the internal bond of lignin and hemicellulose

(Behera et al. 2014). Chiranjeevi et al. (2018) reported that a novel assisted dilute acid pretreatment with boric acid (1% w/v) + H_2SO_4 (0.75%v/v) + glycerol (0.5% v/v) at 150 C for 20 min significantly enhanced the delignification of RS and consequently glucan-to-glucose conversion. An extensive study was performed by Pellera et al. (2018) in which they assessed how chemical pretreatment affected the solubilization of agro-industrial waste. The study selected different wastes such as wineries, cotton gins, olive pomace, and the juice industry. For the pretreatment, eight reagents were used: NaOH, NaHCO$_3$, NaCl, H$_3$Cit, AcOH, H$_2$O$_2$, Me$_2$CO, and EtOH. Among all the reagents, H3Cit, H$_2$O$_2$, and EtOH were effective as they altered the substrate's structure and broke the bond between cellulose hemicellulose and lignin. Wet oxidation comprises oxidizing agents such as oxygen, ozone, and hydrogen peroxide. The wet oxidation process affects all three components of lignocellulosic biomass. Hemicellulose is enormously broken into monomeric sugars and reduced into organic acids, the crystallinity of cellulose reduces, and the lignin breaks and oxidizes (Den et al. 2018). Wet oxidation is quite effective on maize straw (Chen et al. 2017). It is analyzed that p-toluene sulfonic acid has tremendous potential for the delignification of biomass (Wang et al. 2020). Therefore, the current study compares the piranha solution with p-toluene sulfonic acid pretreatment on WS and RS. As piranha solutions are strong oxidizing agents, p-toluene sulfonic acid is strong. At the same time, no study has been conducted on the pretreatment of WS and RS with piranha solution. In this study, a comprehensive comparison of novel chemical pretreatment on RS and WS was performed also tries to understand the significance of silica reduction as much literature is present on the reduction of lignin from agricultural residue. However, the reduction of silica was neglected.

MATERIALS AND METHODS

Chemicals and Reagents

All experiments were conducted with analytical-grade chemicals bought from Fisher Scientific India. To prepare concentrated piranha solution, 3 parts of concentrated sulfuric acids and 1 part of 30 % hydrogen peroxide solution were mixed, as Shrivash et al. (2017) reported. In addition, Para toluene sulfonic acid ($CH_3C_6H_4SO_3$), phosphate buffer, iron chloride ($FeCl_3.7H_2O$), magnesium sulfate ($MgSO_4.7H_2O$), calcium chloride ($CaCl_2.2H_2O$), cobalt nitrate ($Co(NO_3)_2.6H_2O$), nickel chloride ($NiCl_2.2H_2O$), sodium hydroxide (NaOH), ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$), potassium dichromate ($K_2Cr_2O_7$), silver sulfate (Ag_2SO_4) and ferroin indicator were also used in this study. All the reagents used in this study were prepared using double distilled water.

Collection and Analysis of WS, RS and Inoculum

WS and RS were collected from Mohanlalganj (Lucknow, Uttar Pradesh, India). Both the biomasses were kept in an oven for one week separately at 45°C to dry them, afterward crushed using a grinder and kept in air-tight plastic bags at room temperature, cow dung (CD) was collected from a nearby dairy in Rajnikhand (Lucknow, Uttar Pradesh, India). The physicochemical characteristics of WS, RS, and CD were analyzed and individually shown in Table 1. Moisture content (MC), volatile solids (VS), and soluble chemical oxygen demand (sCOD) were determined with the help of methods mentioned in the American public health association (APHA). The direct titration method measured volatile fatty acids (VFA) (DiLallo & Albertson, 1961), and pH values were measured by water analyzer 371 (Systronics, India)

Pretreatment of WS and RS

For pretreatment, 50 g of WS was taken in two 250 mL conical flasks (a and b), then in the flask (a), 150 mL of 10% piranha solution, and in flask (b), 150 mL of PTSA solution was added separately. Then these flasks were kept for shaking for 24 h at a magnetic stirrer. A similar procedure was also done for RS for pretreatment. Afterward, the pretreated biomass was filtered and dried for further use.

Each experiment was performed in triplicates in this work. The untreated WS and RS were denoted as WSU and RSU, respectively, and WS and RS treated with piranha solution were denoted as WSP and RSP. And WS and RS treated with PTSA were denoted as WSPT and RSPT, respectively.

Batch Study for Anaerobic Digestion of WS and RS

The influence of chemical pretreatment of WS and RS on methane production was analyzed through the daily water displacement technique for 35 days. Borosil glass bottles of 1000 mL capacity were used as reactors in the batch study. For the anaerobic digestion of WS, 20.9 g of WSU, WSP, and WSPT were added to the reactor bottles separately. A 50 g of CD was mixed in each reactor, and the volume of the reaction mixture was maintained at 700 mL using double distilled water. Macronutrients (phosphate buffer of 7 pH) and micronutrients $MgSO_4$ (400 mg.L^{-1}), $CaCl_2$ (50 mg.L^{-1}), $FeCl_3$ (40 mg.L^{-1}), $CoCl_2$ (10 mg.L^{-1}), $ZnCl_2$ (0.5 mg.L^{-1}) and $NiCl_2$ (0.5 mg.L^{-1}) were added in reactor along with feedstock and inoculum. A similar setup was also maintained for the anaerobic digestion of RS using 23.6 g of RSU, RSP, and RSPT. Anaerobic digestion of WSU and RSU were set as control experiments. The amount of WS and RS (20.9 and 23.6 g, respectively) was selected per food-to-microbe ratio (F/M) 2. The selection of this F/M ratio was
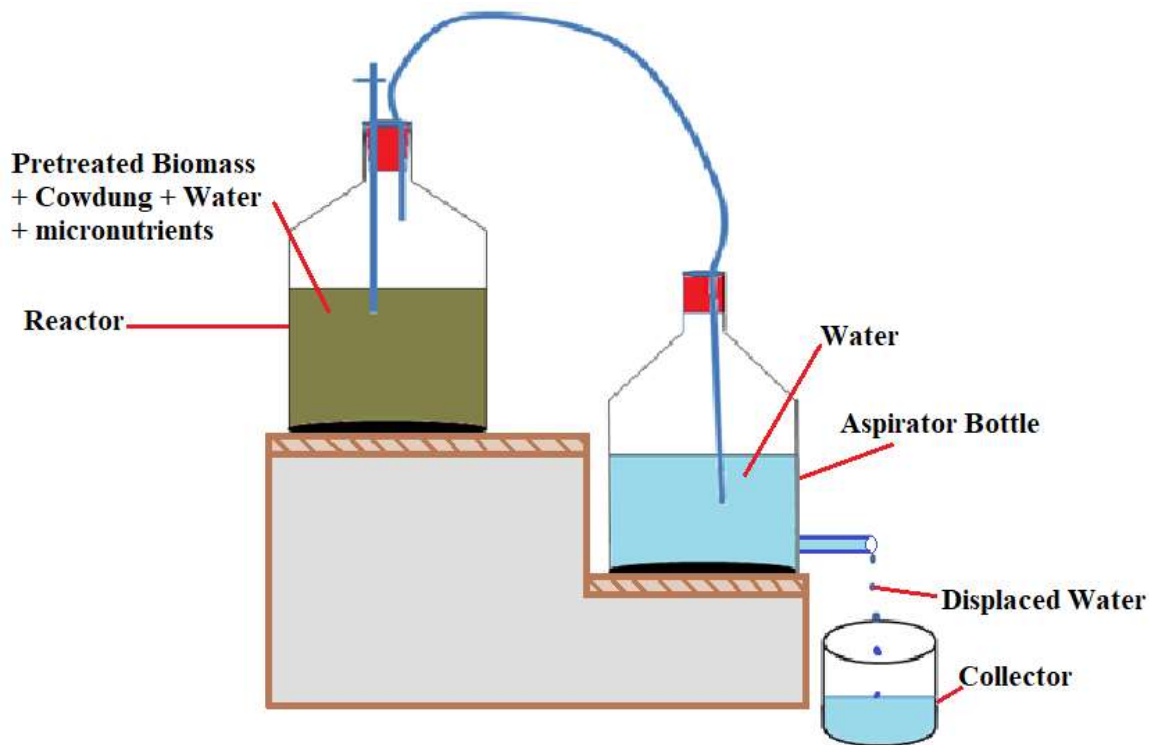

Fig. 1: Set up for anaerobic digestion study.

made based on previous literature, which reported it best for anaerobic digestion (Kainthola et al. 2019b, Saha et al. 2018, Veluchamy et al. 2017). The reactor bottles were connected with 1000 mL aspirator bottles through a pipe filled with distilled water. The water of the aspirator bottle displaced on the methane generation was collected in a collector placed at the end. A rubber cork was fitted on the reactor to sustain the anaerobic condition inside the glass bottles (Fig. 1). All the experiments were performed in triplicate.

Instrumental Analysis

SEM and EDS analysis for the morphological and elemental analysis of WSP, WSPT, WSM, WSU and RSP, RSPT, RSM, and RSU were done by JSM 4490 (JEOL, Japan). Surface functional group analysis was done using an FTIR model NICOLET6700 (Thermo Fisher Scientific, U.S.A). XRD was used to determine the degree of crystallinity in untreated and pretreated feedstock using D8 Advance Eco (Bruker, Germany); moreover, the crystallinity index (CI) was determined as the following equation (Kumar et al. 2016).

$$Crystallinity\ index(\%) =$$
$$\frac{\text{Area of all the crystalline peaks}}{\text{Area of all the crystalline and amorphous peaks}} \times 100$$
$$\dots(1)$$

Analysis of Data through Kinetic Models

MGM and LFM were used to model the kinetics of methane generation. To calculate the methane production and the kinetic parameters for the current investigation, IBM SPSS statistics were employed. The following equations and nonlinear regression analysis used the experimental cumulative methane yield data to determine the bio-kinetic parameters.

$$y(t) = P * Exp\left(-Exp\left(\frac{R*Exp}{P}\right)(L-1) + 1\right)$$
$$\dots(2)$$

$$y(t) = \frac{P}{1+\exp\{\frac{4R(L-1)}{P}+2\}} \qquad \dots(3)$$

Where y (t) is cumulative methane potential (mL), P represents the maximum yield of biogas (mL), R denotes the highest production rate of biogas (mL), L is the lag phase constant (day), and the value of the constant e is 2.7183. The parameters P, R, and L were calculated by curve fitting IBM SPSS 20. Regression analysis was done to estimate the coefficient of correlation R^2.

RESULTS AND DISCUSSION

Effect of Pretreatment on the Degree of Solubilization

Piranha solution and PTSA can break down the interunit of lignocellulosic biomass. The high oxidative properties and generation of singlet oxygen radicals are responsible for the degradation of feedstock. The chemical pretreatment effect on the solubilization of WS and RS was measured by the change in VFA, sCOD, VS, and pH alterations of the digestate as shown in Fig. 2 and Fig. 3. The large molecule partially degraded while pretreatment and consecutively converted into VFA during the digestion process.

At the initial stage of anaerobic digestion, the concentration of VFA varied from 233.33 ± 28.86 mg.L^{-1} to 986.66 ± 2.88 mg.L^{-1}. Minimum VFA was observed in RSU, while maximum VFA was detected in WSP. The VFA concentration rapidly increased with digestion time in all the reactors (Fig. 2c and 2d). As the VFA increased, the pH decreased (Fig. 2a and 2b), indicating the formation of acidic conditions in the reactors. VFA is produced in the acidogenesis phase and contains acetic acid, propionic acid, butyric acid, and valeric acid, which causes a drop in pH. At last, they get converted into methane. VFA is utilized as a substrate in anaerobic digestion for methane production as it preserves all chemical energy of the substrate (Valentino et

Table 1: Initial physicochemical characteristics of WS, RS and CD.

Parameters	WS	RS	CD
pH	7.48 ± 0.5	7.79 ± 0.5	7.31
Moisture content [%]	8.1 ± 0.33	5.94 ± 1.5	81.235 ± 0.6
Volatile solids [% Total solid]	92.53 ± 0.613	80 ± 0.7	82.39 ± 0.09
Ash content [% Total solid]	14.47 ± 0.99	20 ± 0.56	17.61 ± 1.25
TS [%]	90 ± 0.25	94.09 ± 0.76	18.27 ± 0.07
Lignin [%]	18.52 ± 0.55	14.76 ± 0.87	NA
Hemicellulose [%]	25 ± 1.99	29.09 ± 0.45	NA
Cellulose [%]	39 ± 1.25	37.98 ± 0.56	NA

*NA - Not Analyzed

al. 2021). The maximum concentration of VFA was achieved on the 14th day, and WSP showed a maximum concentration of VFA (1553.33 ± 2.8 mg.L^{-1}) followed by WSPT (1414.66 ± 25 mg/L), RSP (1333.33 ± 28.86 mg.L^{-1}), RSPT (1269.33 ± 19 mg.L^{-1}) while in WSU and RSU maximum VFA found on 21st day that is 752.33 ± 4.04 mg.L^{-1} and 676 ± 5.77 mg.L^{-1} respectively. Subsequently, an abrupt decrease was observed in all the reactors due to the commencing of the methanogenic phase. WSP has 1.09 fold higher VFA than WSPT, and RSP has 1.05 fold higher VFA than RSPT. A higher concentration of VFA in piranha-treated biomasses may be due to fast hydrolysis caused by piranha solution, which potentially breaks the lignocellulosic matrix because of its strong oxidative nature. Compared to the inhibitory concentration, i.e., 8000 mg.L^{-1}, the VFA concentration was lower during the digesting time in all eight reactors, demonstrating favorable conditions for the growth of acid-producing bacteria.

sCOD indicates the degree of substrate solubilization attained by microbes to generate methane (Kavitha et al.

2014). As WS and RS are anaerobically digested, the change in an sCOD is depicted in Fig. 3a and 3d, respectively. The initial sCOD for WS ranged from 6612 ± 97 mg.L^{-1} to 6166.6 ± 57 mg.L^{-1}, and that of RS ranged from 5157.3 ± 136 mg.L^{-1} to 7844 ± 135 mg.L^{-1}. In all the reactors, sCOD increased with digestion time and achieved the maximum value on the 14th day in all chemically pretreated substrates, while the maximum sCOD in WSU and RSU was found on the 21st day. Afterward, sCOD gradually decreased in all the reactors. Cellulose is hydrolyzed to produce fermentable reducing sugars, which help to raise sCOD levels (Panigrahi et al. 2020). Among all the pretreated substrates highest sCOD was found in WSP, i.e., 16333.33 ± 144.33 mg.L^{-1}. The sCOD of piranha-treated WS and RS was 1.05 times and 1.04 times higher than PTSA treatment, respectively. The lowest sCOD was found in WSU and RSU due to their recalcitrant nature, which made them difficult to solubilize.

The initial VS of WSP, WSPT, and WSU was 87.3 ± 2%, 89.3 ± 0.57%, and 91.6 ± 2%, respectively, and the initial

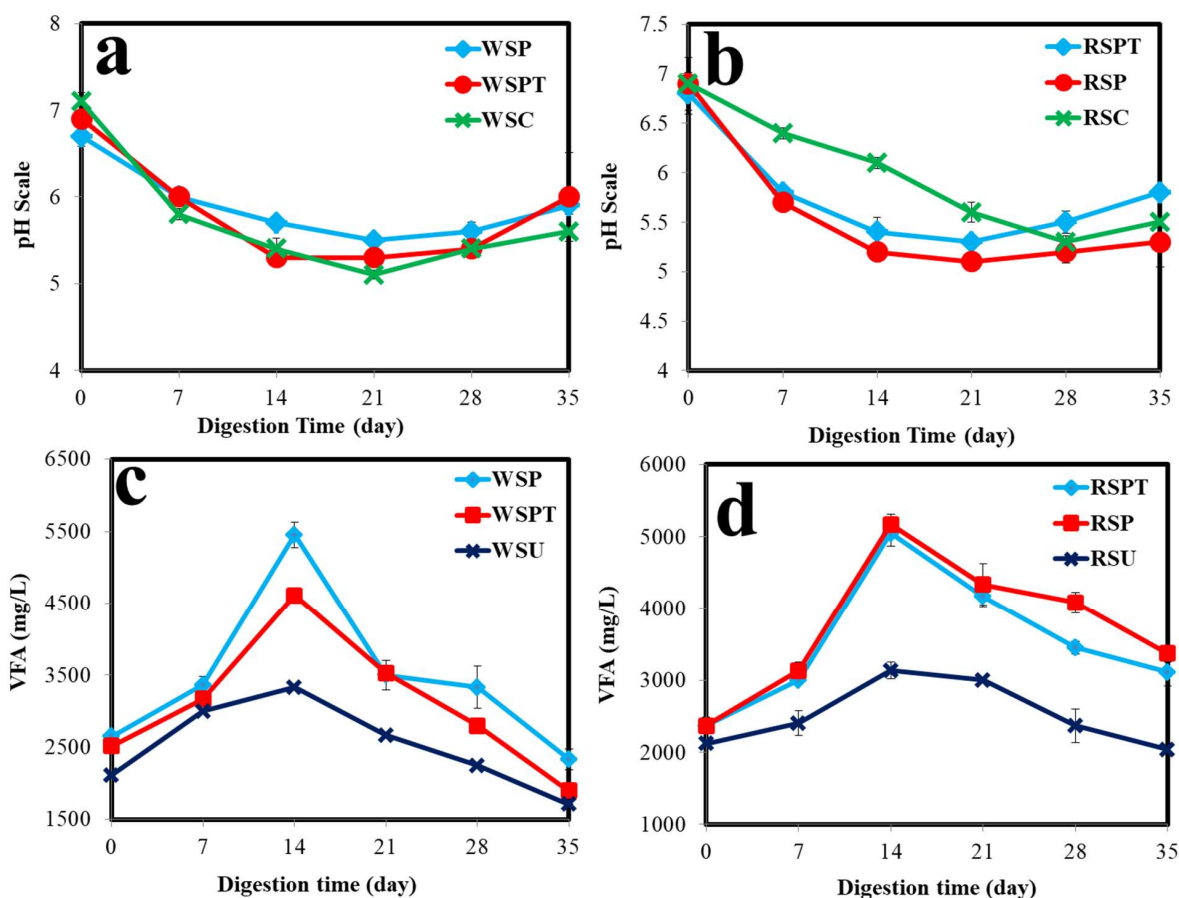
Fig. 2: Effect of Pretreatment on (a) and (b) pH change and (c) and (d) VFA of WS and RS.

VS of RSP, RSPT and RSU were 82.66 ± 1.5%, 85.33 ± 1%, and 89 ± 1%, respectively. VS content of piranha-treated biomasses was lower initially may be due to the removal of non-structural components present in WS and RS during pretreatment. The VS content in anaerobic digestion of WS decreased up to 43.6 ± 1% (WSP), 55.33 ± 0.57% (WSPT), and 61.3 ± 1.5% (WSU), and in RS, the VS was decreased up to 55.66% (RSP), 56.33% (RSPT) 65.66% (RSU). The VS content decreased by increasing digestion time (Fig. 3c and 3d). The reduction in VS is directly proportional to the conversion of organic matter and methane production (Miah et al. 2016). During the delignification process, the β-O-4 linkage is cleaved and converted into a stable C-C bond which makes the enzyme more accessible to the cellulose and consequently degrades it to produce methane (Li et al. 2020, Esposito et al. 2012).

In WSPT VS, the reduction was less (56 ± 2%), probably due to PTSA being inconsiderable to degrade lignin in WS; hence microbes were unable to degrade WSPT, while in WSP VS, the reduction is (43.6 ± 1%) because piranha solution efficiently reduces the rigid structure of WS and RS subsequently it digests effortlessly.

Effect of Pretreatment on Methane Yield

The chemical pretreatment had a consequential impact on methane yield from the initial days. In the pretreated samples, the lag phase ranges from 2 to 4 days; in untreated samples, the lag phase was between 5 to 6 days. Pretreatment improved the lag phase as organic content was solubilized during pretreatment and was easily converted into methane by microbes. The value of the highest daily methane yield in WSP (95 mL.d^{-1}) and WSPT (90 mL.d^{-1}) was observed on the 17th and 18th day, respectively, and in RSP (81 mL.d^{-1}) and RSPT (74 mL.d^{-1}) was observed on the 16th day and 13th day, respectively (Fig. 4a and 4b). Whereas, in untreated samples, the highest value of daily methane production was 23 mL.d^{-1} (WSU) and 27 mL.d^{-1} (RSU) obtained on the 35th day. Compared to the treated samples, the untreated samples produced a very low amount of methane over a comparatively longer time. The cumulative methane yield acquired in this study was 1234 mL, 1208 mL, 1120 mL, 992 mL, 520 mL, and 483 mL for WSP, WSPT, RSP, RSPT, WSU, and RSU, respectively. As shown in Fig. 4c and 4d, the maximum cumulative methane yield for WS was obtained in WSP. Compared with WSU, the cumulative
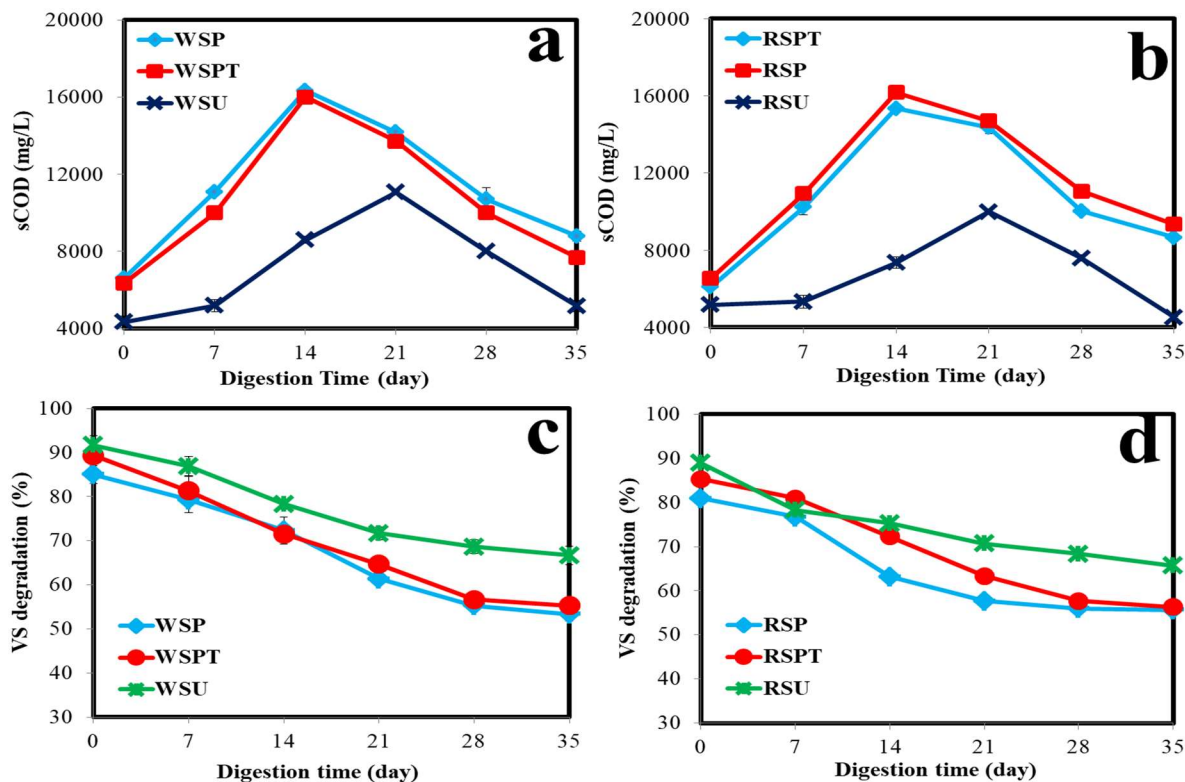

Fig. 3: Effect of pretreatment on (a) and (b) sCOD and (c) and (d) VS degradation of WS and RS.

methane yield was 2.37 folds higher in WSP and 2.32-fold higher in WSPT.

Similarly, the maximum cumulative methane was observed in RSP at 2.31 times higher for RS, followed by RSPT with 2.05 times higher methane production than RSU. As compared to WSPT, WSP showed 1.02-fold increased cumulative methane. Piranha-treated biomasses showed the best yield, probably because the piranha solution effectively breaks the ether linkage. WSP showed the best result compared to RSP may be due to cellular degradation, and microfibrils are disrupted during pretreatment. Moreover, it successfully reduced the recalcitrant nature of RS. Cellulose is the significant structural constituent of cell walls in plants, comprised of a linear polymer of D-glucose subunits connected by β-1,4 linkage the long chain of cellulose is connected by hydrogen bond and van der Waals bond makes cellulose packed into microfibrils (Perez et al. 2002). After the pretreatment of biomasses,

the fermentable D-glucose is produced from cellulose by breaking a β-1,4 glycosidic bond.

Analysis of Untreated and Pretreated WS and RS

SEM and EDS Analysis

Morphological alterations of biomasses were observed through SEM analysis. As the SEM analysis (Fig. 5a to 5c and 6a to 6c) shows, WSU had a smooth and closed-packed surface with fiber arranged in the bundle due to the occurrence of a strong bond between lignin, hemicellulose and cellulose which is a hard-to-digest. The surface of pretreated WS and RS was very loose, scattered, and rough due to chemical pretreatment, possibly due to the dissolution or removal of complex lignin structure from the surface. Piranha solution effectively damaged WS and RS's surface since it is a strong oxidizing agent. PTSA in RS caused minimal alteration on the surface structure, as shown in Fig. 6c. Untreated RS had a very ordered structure that got disordered after
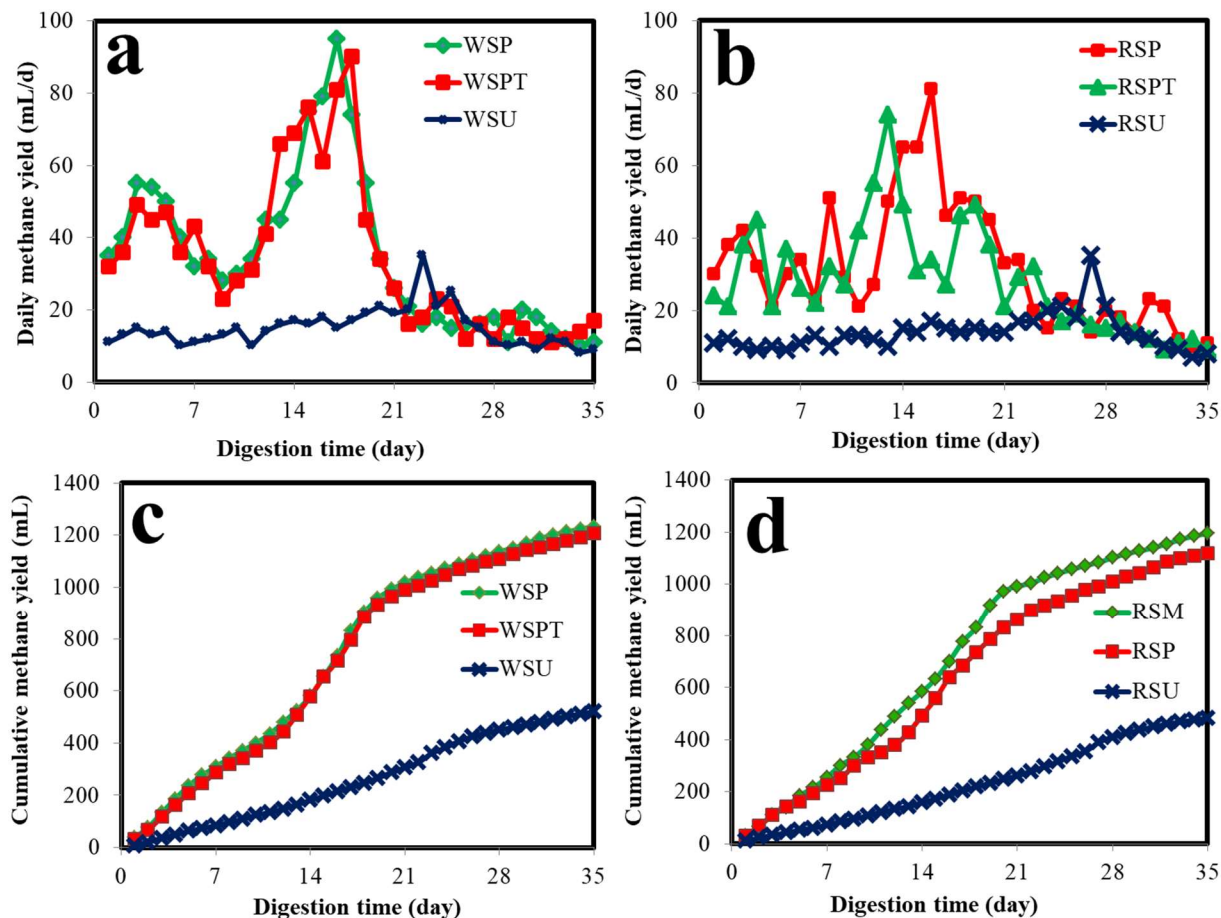

Fig. 4: (a) and (b) Daily methane yield and (c) and (d) cumulative methane yield of WS and RS.
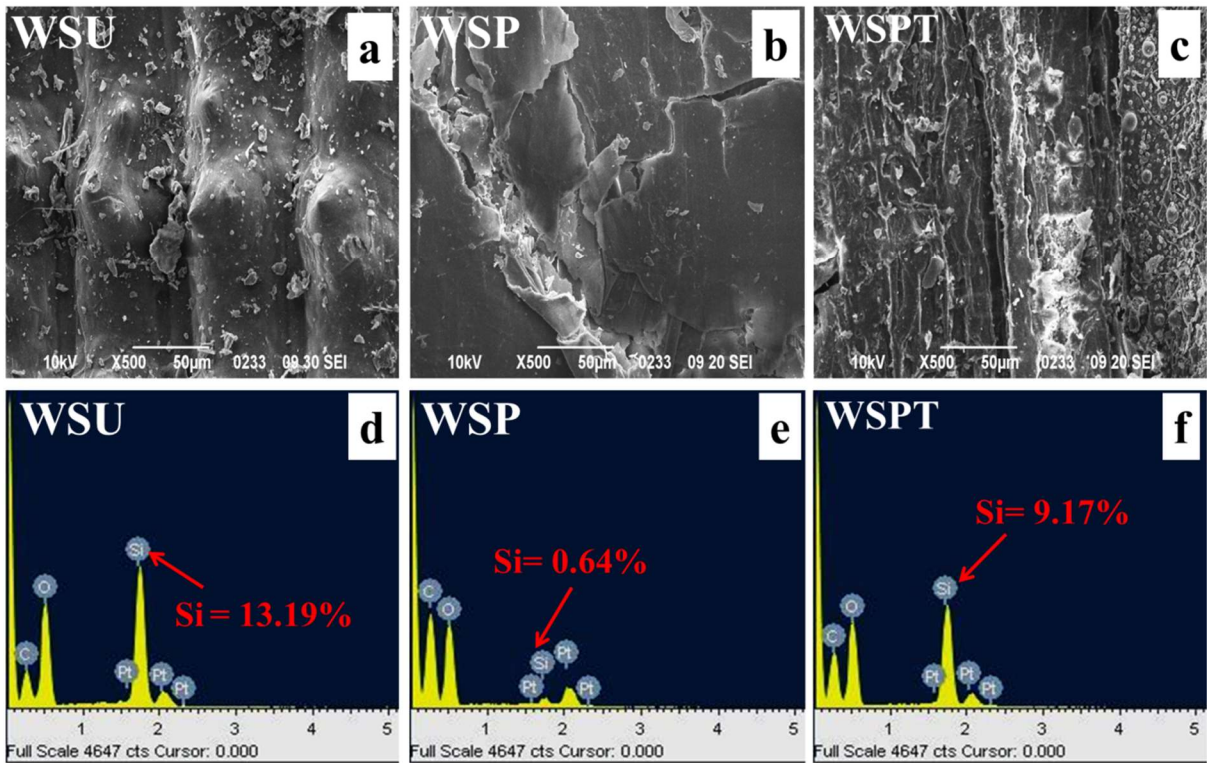
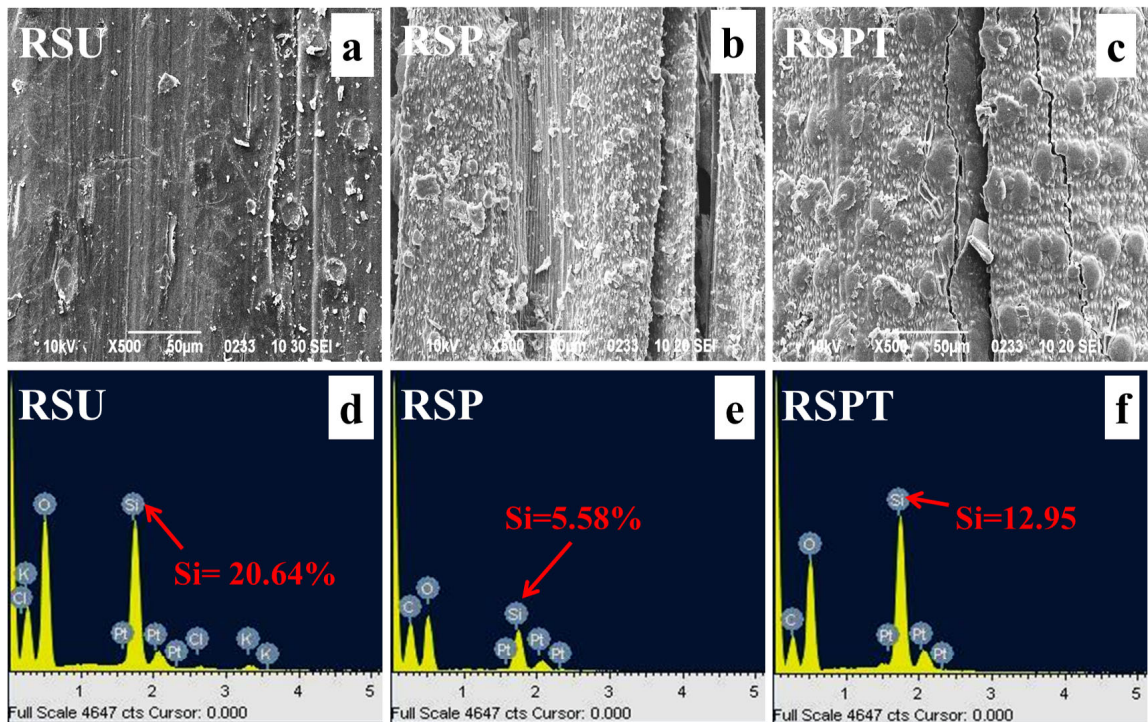Fig. 5: Morphological and elemental analysis of untreated and pretreated WS.

Fig. 6: Morphological and elemental analysis of untreated and pretreated RS.

pretreatment with piranha solution, PTSA. It is clearly shown in Fig. 6b RSP had a high degree of roughness. As shown in the EDS analysis (Fig. 5d to 5f), the peak of silica is reduced significantly in WSP by 95.07%. However, in WSPT, it was reduced to 30.47% silica. RS pretreatment also showed a reduced silica content of 72.1% in RSP and 37.25% in RSPT (Fig. 6d to 6f). The reduced peaks of silica in EDS analysis show its possible solubilization after pretreatment. Reduction in silica also enhances methane production as a high silica concentration restricts the fermentation process; therefore, it needs to reduce the concentration for effortless digestion (Satlewal et al. 2018).

Change in the Chemical bond of Untreated and Pretreated WS and RS

FTIR was used to examine the functional group alterations between untreated and pretreated WS and RS. The peaks of WSU, WSP, WSPT, RSU, RSP, and RSPT are shown in Fig. 7a. The peaks emerged at 3377 cm^{-1}, 2920 cm^{-1}, 1645 cm^{-1}, 1242 cm^{-1}, and 1067 cm^{-1} in FTIR spectra indicate the cellulose and hemicellulose content. The spectra of WSP, WSPT, RSP, and RSPT were different from WSU and RSU, indicating their different surface structure due to pretreatment. The absorption band at 3413 cm^{-1} was assigned to -OH stretching and 2926 cm^{-1} to C-H stretching in CH_3 and CH_2 groups showing the presence of polysaccharides in biomass (Kumar et al. 2011). Comparing the pretreated and untreated WS, in pretreated, it is observed that the intensity of the -OH group decreased, indicating the dehydration reaction takes place (Rajput et al. 2018). A decrease in peak intensity at 1242 cm^{-1} in all the pretreated WS and RS suggested breaking the -C=O hemicellulose-linked lignin stretch. The band range of 1770 -500 cm^{-1} in WS and RS is considered the lignin region. The absorbance peak at 1645 cm^{-1} assigned to aromatic C=C stretching from lignin and aliphatic C-H stretching in methyl and phenol, -OH, and syringyl units of lignin (Tian et al. 2020, Rambo et al. 2015) the decrease in peak at 1645 cm^{-1} pretreated WS and RS were due to the
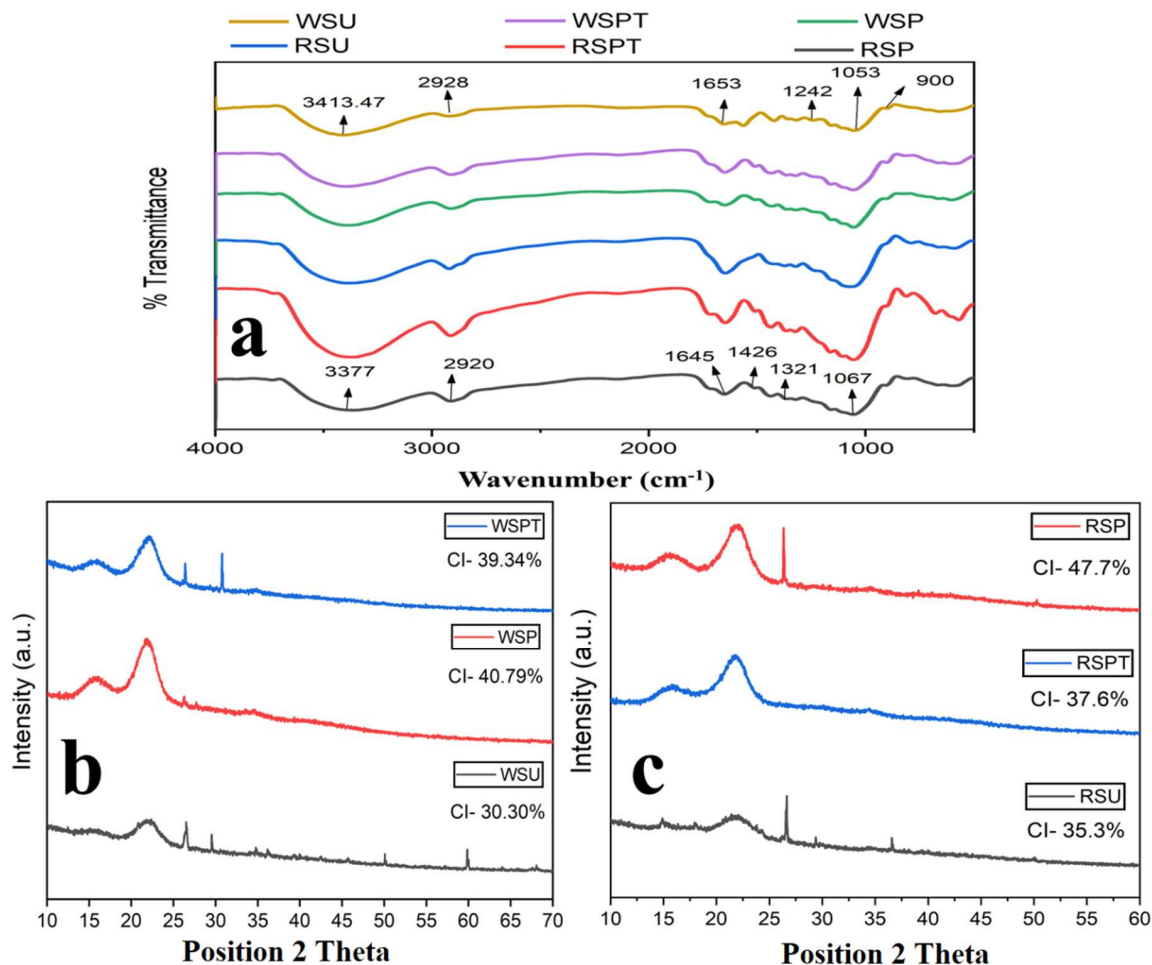

Fig. 7: (a) FTIR analysis (b) and (c) XRD analysis of WS and RS.

removal of lignin which is attributed to the polymerization of polysaccharide after chemical pretreatment. A notable drop of band intensity detected at 1067 cm^{-1} and 1321 cm^{-1} indicates the C-O, C=C, C-C-O, and C-H deformation in cellulose, hemicellulose, and lignin due to chemical pretreatment (Barua & Kalamdhad 2017).

X-ray Diffraction of Untreated and Pretreated WS and RS

The crystallinity of lignocellulosic biomass was assessed by XRD analysis. WS and RS mainly consist of cellulose, lignin, and hemicellulose, although cellulose exhibits crystalline nature, whereas lignin and hemicellulose are amorphous. The crystallinity of lignocellulosic biomass significantly affects the hydrolysis process of anaerobic digestion; therefore, it is vital to determine the degree of crystallinity. XRD analysis of untreated and pretreated WS and RS is shown in Fig. 7b and 7c, respectively. The pretreated samples show a notable change in peak intensities and broadening of the peaks. The characteristics of crystalline peaks for cellulose were identified at 2Θ =26.2°-26.5°, 13.5°, and 18.6° in untreated and pretreated samples (Sharma et al. 2020). It is evident from XRD analysis that the crystallinity of WS and RS increases after pretreatment. The crystallinity value of WS was decreasing in the order of WSP>WSPT>WSU. The CI of WSU was calculated to be 30.3%, while the CI of WSP was 40.79%, and WSPT was 39.34%. In the RS sample also, the CI of RSP (47.70%) was higher compared to RSPT (37.60%) and was lowest in RSU (35.3%). Hemicellulose, lignin, and the amorphous component of cellulose all degraded, which led to an increase in crystallinity in pretreated samples.

Kinetic Study

In the current work, MGM and LFM were applied to estimate the methane production of rice and WS that had undergone

chemical pretreatment. The experimental cumulative methane yield was used to estimate the kinetic parameters using nonlinear regression. Table 2 outlines the obtained bio-kinetic parameters (P, R, and L) and statistical measure (R2) values for the studied kinetic model. The maximum predicted value of P (mL) was obtained in WSP (1234 mL) for MGM, which is 7.64% higher than the experimental value, while in LFM value of P is -0.12 % lower than the experimental value. The maximum percentage difference between the experimental and the predicted value was found in RSU (64.52%) for MGM, followed by WSU (28.44%) for LMF. The lag phase constant, L, describes how long bacteria can adapt to their new environment. In this study, the value of L ranges from 5.94 to 1.65 days. Lower L suggested a shorter degradation rate. It is observed from the table that pretreated WS requires the lowest time for acclimatization. Among the pretreated WS and RS, the shortest lag phase was seen in WSP, followed by RSP, WSPT, RSPT, WSU, and RSU. Maximum reduction in lag phase in piranha-treated biomasses was probably due to its higher potential for the removal and destruction of the recalcitrant structure of biomass and prior solubilization of large particles. The kinetic analysis using MGM and LFM in this investigation was able to fit the expected outcomes. The simulated kinetic values are presented in Table 2. It is evident from Fig. 8 that experimental cumulative methane yield fits with the predicted methane yield in both models. The projected values were plotted against the experimental data to assess the model's viability, and it was found that the value of R^2 varies between 0.987-0.998 of the model. For RSPT, RSU MGM fits best for WSP, WSPT, WSU, and RSP LFM fit best.

CONCLUSION

The effects of two different types of chemical pretreatments

Table 2: Generated kinetic parameters from the evaluated model.

| Sample	Model used	P[mL]	R[mL]	L[d]	Exp. data [mL]	Δ [%]	R^2	MSE
WSP	MGM	1328.38	55.72	2.22	1234	7.64	0.991	31.29
	LFM	1232.44	59.30	3.30		-0.12	0.994	26.47
WSPT	MGM	1284.39	56.39	2.78	1208	5.94	0.992	28.99
	LFM	1195.21	60.23	3.87		-1.05	0.995	23.23
WSU	MGM	714.84	19.08	4.39	520	37.46	0.997	8.36
	LFM	573.32	21.13	5.9		10.25	0.997	6.69
RSP	MGM	1226.28	48.88	3.06	1120	8.66	0.995	17.27
	LFM	1119.12	52.57	4.22		-0.07	0.997	17.27
RSPT	MGM	1095.68	44.81	2.80	992	10.45	0.998	12.65
	LFM	1011.43	47.19	3.76		1.95	0.997	12.65
RSU	MGM	807.52	17.35	5.33	483	67.08	0.997	6.59
	LFM	581.24	18.93	6.72		20.33	0.996	7.83
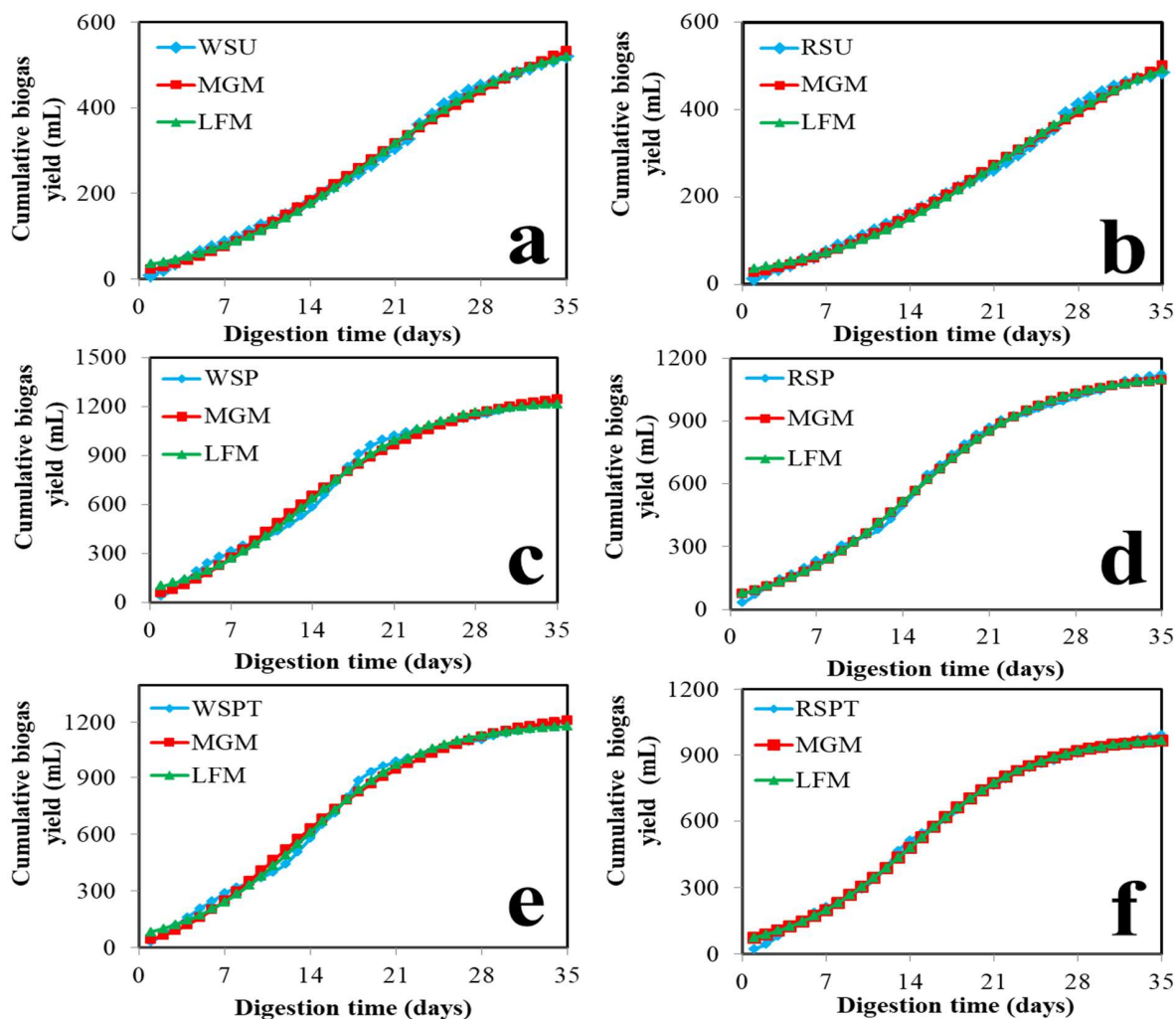
Fig. 8: MGM and LFM fitting for methane yield data of WS and RS.

(piranha and PTSA treatment) to reduce the recalcitrant nature of WS and RS for the production of enhanced methane were compared in this study. The chemical pretreatments were found successful in the enhancement of methane production of both biomasses. The present study suggests that pretreatment boosts enzymatic activity by reducing the recalcitrant structure of WS and RS. Methane production was maximum after piranha treatment 1234 mL, and 1120 mL in WSP and RSP, respectively, followed by PTSA treatment 1208 mL (WSPT) and 992 mL (RSPT), and minimum methane was produced in untreated samples 520 mL (WSU) and 483 mL (RSU). In comparison with RS, the methane production in WS was higher. Enhancement in methane production resulted from the solubilization of organic matter. Pretreatment with piranha solution was the best technique, as it effectively reduced the recalcitrant nature of RS and WS and increased methane production. SEM, EDS, FTIR, and XRD analysis also supported the positive effect of chemical pretreatment. MGM and LFM accomplished the kinetic model of methane production. The outcome of the experiments was compared with the predicted value obtained from these two models. The statistical analysis shows that LFM fitted best with the experimental results than MGM. This was verified by analyzing the root means square error and regression.

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