4.1 Physico-chemical characterisation of sawdust and inoculum

The VS content of sawdust and calorific value were found to be 77.1 % and 3206.7 kCal/kg, respectively indicating the utility of sawdust can be used as a substrate for biogas production. The moisture content was estimated as 3.7 %. The lack of moisture content can be adjusted by the addition of water to provide the adequate amount of moisture for anaerobic digestion. The C, H, and N content was 50.6, 6.1 and 0.6 %, respectively. The cell wall components were estimated as cellulose 49.6 %, hemicellulose 13.1 % and lignin 29 % as shown in Table 4.1.

Table 4.1 Characterisation of native sawdust and inoculum

Parameter	Native sawdust	Inoculum
Proximate analysis (wt %)		
Moisture	3.7	90.0
Total solid	96.3	10.0
Volatile solid	77.1	4.7
Fixed carbon	17.8	1
Ash content	1.4	4.3
Ultimate analysis (wt %)		
Carbon	50.6	38
Hydrogen	6.1	-
Nitrogen	0.6	13
Compositional analysis (wt %)		
Cellulose	49.6	-
Hemicellulose	13.1	-
Lignin	29	-
Gross calorific value (kCal/kg)	3206.7	-
pH	-	7.01
sCOD (mg/L)	-	2102.5

Presence of lignin in abundant amount reduces the accessibility of microbe towards substrate and also results in less productivity. Therefore, lignin reduction is a primary goal to achieve the better product yield.

4.2 Effect of pretreatment on sawdust solubilisation

4.2.1 Effect of biological treatment on sawdust

The main aim of pretreatment was to reduce lignin so that microbial species can penetrate into the substrate and utilise it more efficiently. Therefore, white rot fungus P. *chrysosporuim* (PCT) and *P. ostreatus* (POT) were employed to degrade the lignin. The cellulose, hemicellulose and lignin content were slightly decreased after the both treatments. However the lignin reduction was higher in *P. chrysosporium* treated sample, i.e 20 % in 30 days which was 27.5 % more as compared to *P. ostreatus* treated (POT) sample.

Table 4.2 Effect of biological treatment on compositional components of sawdust

Fungal strain	Time (d)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Lignin degradation (%)
P. chrysosporium	10	49	12.4	28.0	3.5
	20	46.3	11.6	26.6	8.3
	30	43.7	10.2	23.2	20.0
P. ostreatus	10	48.5	12.6	28.3	2.4
	20	45.4	11.4	27.1	6.6
	30	42.5	10.5	24.8	14.5

The cellulose was decreased from 49-43.7 % and 48.5-42.5 %, respectively after PCT and POT treatments. The hemicellulose content was also decreased 12.4-10.2 % and 12.5-10.6 %, respectively after PCT and POT as represented in Table 4.2.

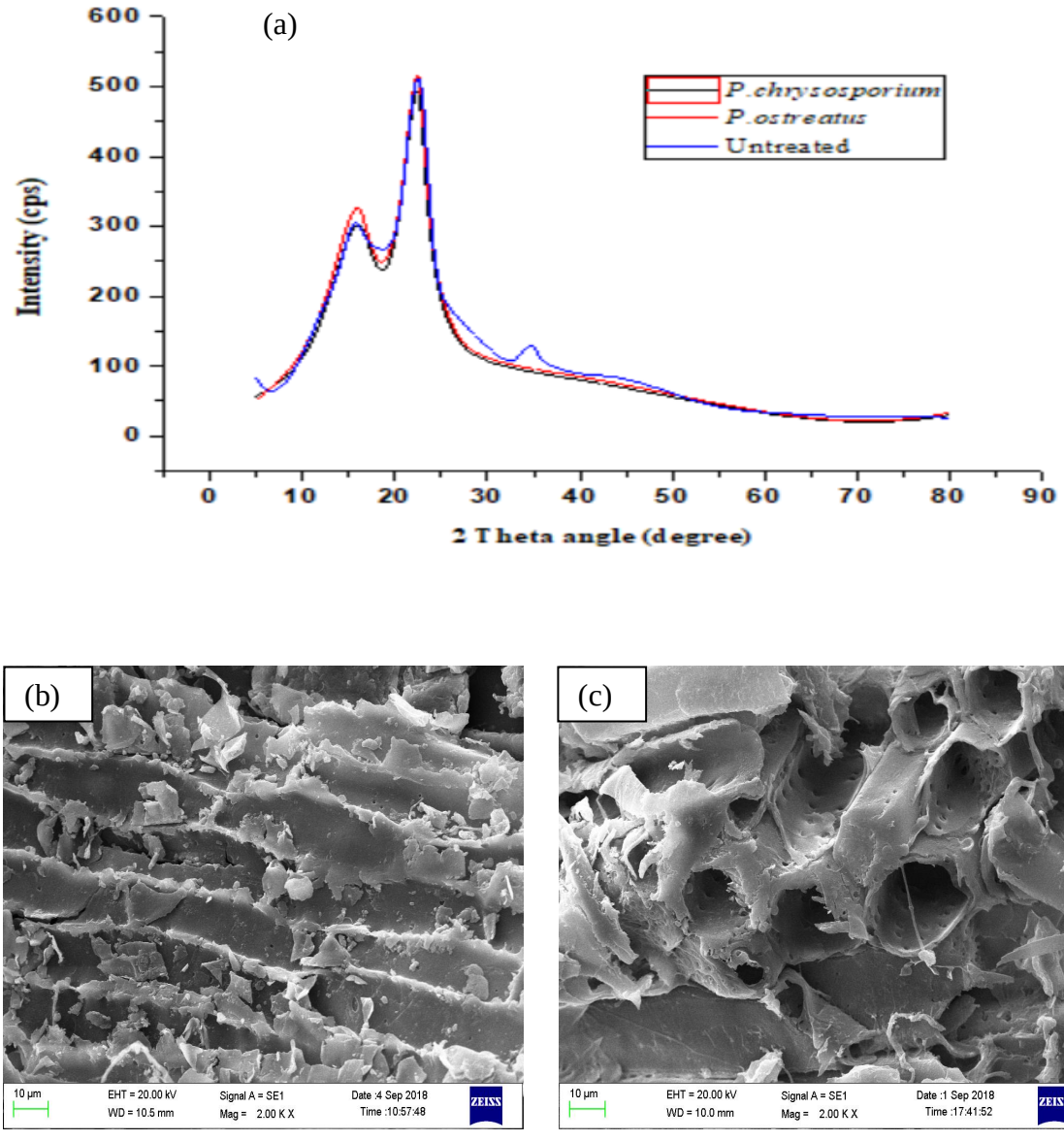


Figure 4.1 Effect of biological treatment on sawdust (a) XRD profile (b) SEM micrograph of *P. chrysosporium* and (c) *P. ostreatus* treated sample

XRD and SEM analysis was also done to study the changes in cell wall structure after treatment. XRD and SEM results indicated that there are no significant variations occurred after both of the treatments. PCT resulted in slightly reduced intensity of amorphous region near 2 theta angle of 18 °. The intensity of highest peak was found to be same in untreated and microbial treated samples. The results of XRD and SEM are represented in Figure 4.1. The cell wall surface was found slightly rough in PCT samples as compared to POT. The *P. chrysosporium* efficiently degraded lignin than *P. ostreatus* and resulted into break down of complex cell wall. White rot fungi degrades lignin by production of lignin peroxidase, manganese proxidase and laccase (Chio et al., 2019). Lignin peroxidase and manganese peroxidase require H_2O_2 for the degradation of lignin (Have and Teunissen, 2001). Lignin degradation occurs due to interaction of these three enzymes.

4.2.2 Selection of potential chemical reagent

Chemical treatment is known to be an effective treatment method as it reduces lignin efficiently from lignocellulosic biomass. Therefore, sawdust was treated with different chemical reagents (HCl, H_2SO_4, ethanol, NaOH, $Ca(OH)_2$, and aqueous ammonia) for determining the best among them in order to degrade lignin. The sCOD and phenolic content were estimated as an indirect measure of lignin reduction because lignin break down results in production of phenolic compounds and also the formation of simpler sugar from complex matter cause the elevation to the sCOD yield (Pellera and Gidarakos, 2018). sCOD value and phenolic content were more in alkali and ethanol treated samples as compared to acid treated samples. The ethanol treated sample contained 9850 mg/L sCOD and 364 mg/L phenolic content. The highest yield of sCOD and VFA was found in NaOH treated sample (Table 4.3). Therefore, NaOH was selected for further pretreatment approaches.

4.2.3 Effect of NaOH and combined treatment on sCOD and VFA

The effect of NaOH was estimated alone and in combination with thermal treatment by autoclave or microwave. The increment in COD, VFA and phenolic compounds yield was taken place by increasing the concentration of NaOH after every treatment as shown in Figure 4.2. The sCOD was increased with respect to 8 % NaOH concentration in NaOH treatment whereas; it was increased with increasing NaOH concentration to 10 % for NaOH-autoclave and NaOH-microwave treatment. NaOH-autoclave treatment turned out to be best among three in increasing the yield of solubilisation parameters. The variation associated with the cell wall structure might have resulted into release of simpler sugar into hydrolysate and caused the rise in sCOD value (Pellera et al., 2016; Wang et al., 2015, Michalska et al., 2015). VFAs are the small carbon chain (C_2-C_6) organic acids formed by available sugars. Pretreatment increased the destruction of cell wall that enhanced the sugar production. Therefore, VFA formation increased after each treatment.

4.2.4 Effect of pretreatment on phenolic content and glucose

The degradation of lignin causes production of phenolic by-products. The phenolic content was increased with increasing concentration of NaOH. The combination of NaOH and temperature caused the more destruction to the lignin molecule and hence resulted into increment in phenolic content. The hydrolysate of NaOH-autoclave treatment showed the highest yield of phenolic content. The break-down of cell wall led to the release of monomeric sugars. Therefore the glucose concentration was higher in treated samples as compared to untreated sample. Regarding the effect of NaOH concentration, the glucose yield was increased upto 2 % NaOH concentration in all treated samples and then significantly diminished at higher NaOH dose. The reduction could be associated with dehydration of glucose moieties in alkaline environment (Yang

and Montgomery, 1996). Also, temperature synergistically reduced the glucose yield with NaOH concentration. Therefore, glucose yield was more in NaOH treated sample and was reduced when combined with temperature by either autoclave or microwave treatment.

Table 4.3 Effect of chemical reagents on solubilisation of sawdust

Chemical (2 %)	sCOD (mg/L)	Phenol (mg/L)
HCl	2090	330
H_2SO_4	4340	293
Ethanol	9850	364
NaOH	10560	1137
$Ca(OH)_2$	5080	450
NH_3	9398	724

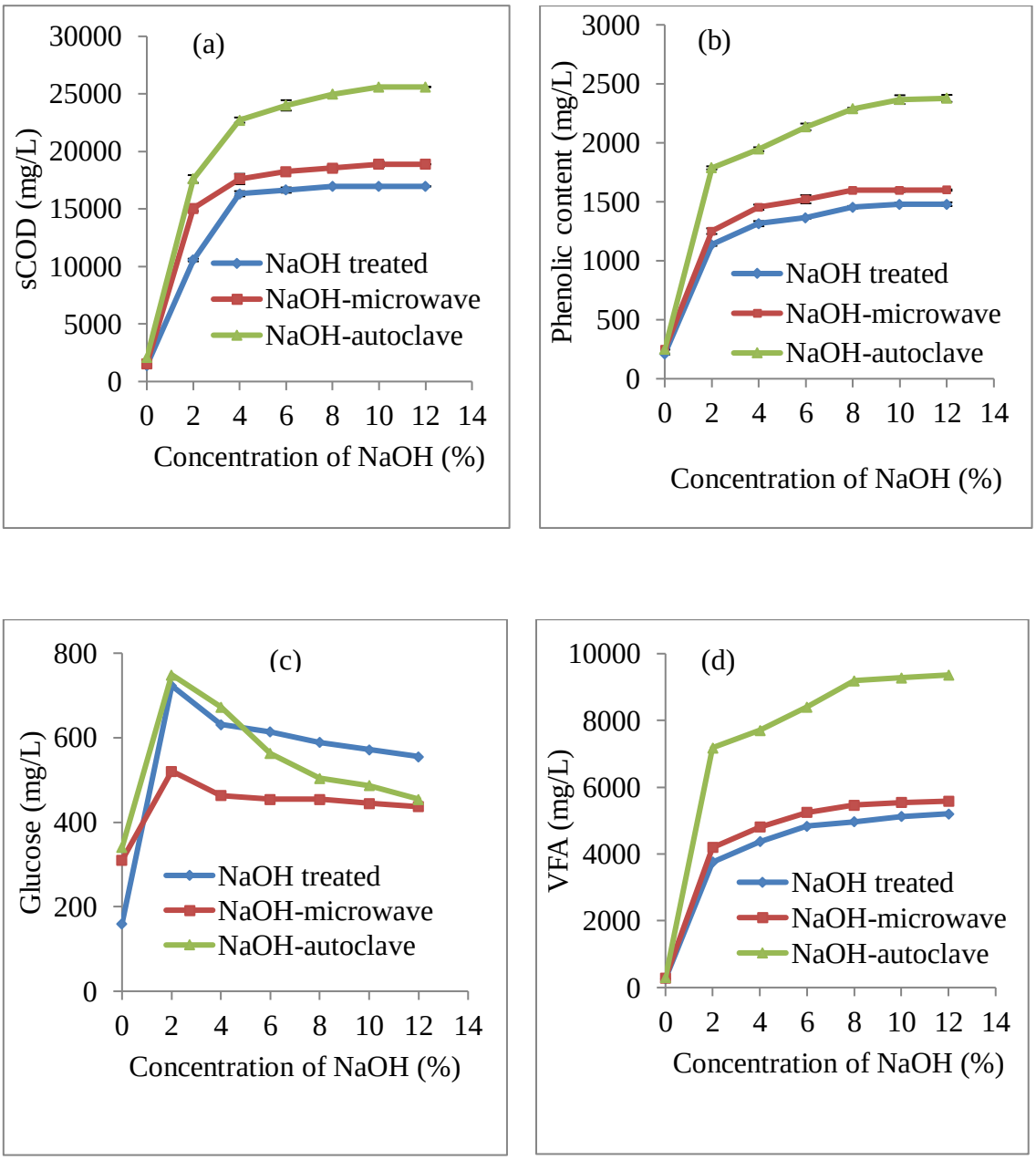


Figure 4.2 Effect of NaOH pretreatment of sawdust on (a) sCOD, (b) phenolic content, (c) glucose and (d) VFA

4.2.5 Effect of pretreatment on sawdust composition

The amount of cellulose was increased after pretreatment whereas hemicellulose and lignin content were decreased after pretreatment. The cellulose was increased in the range of 49.6-52, 49.9-54.5 and 49.6-60 % in NaOH, NaOH-microwave and NaOH-autoclave treated samples, respectively as represented in Table 4.4. The lignin concentration was decreased as 29-27, 29.2-25.4 and 29-21.7 % with increasing the NaOH concentration in NaOH, NaOH-microwave and NaOH-autoclave, respectively. NaOH causes break down of lignin main bonds and also peeling reactions that degrade carbohydrate. There are three main types of reaction responsible in lignin breakdown during alkali treatment. It includes (a) cleavage of phenol-type α-aryl ethers or α-alkyl ethers (b) cleavage of phenol-type β-aryl ethers and (c) cleavage of the non-phenol-type β-aryl ethers (Xu at al., 2016). It also causes break down of ester bonds between lignin and xylan by saponification and salvation reactions. From the results, it was found that NaOH-autoclave treatment was more effective in lignin solubilisation as compared to other pretreatments.

4.2.6 FTIR study of treated and native sawdust

The FTIR analysis was carried out to study the chemical composition variation in terms of functional group of sawdust before and after pretreatment (Table 4.5). A range of wavenumber 4000-400 cm^{-1} was used to record the FTIR absorption for qualitative analysis of the chemical structure of native and treated sawdust (Figure 4.3). Peaks near band positions 3409, 3379, 3433, 3441, 3339, 3408, 3310, 3424 cm^{-1} were considered to be associated with intermolecular hydrogen bonded O-H stretching that indicated the presence of cellulose (Taherdanak and Zilouei, 2014; Fougere et al., 2016; Lei et al., 2013). Peaks position in between 2869-2924 cm^{-1} was attributed to C-H stretch that indicated the presence of cellulose (Jiangtao et al., 2012; Ang et al., 2012). A new peak

was noted at 2691 cm^{-1} band position after NaOH (10 %)-autoclave treatment that assigned to CHO functional group and signified the presence of hemicellulose. The peak at band position 1739 cm^{-1} was attributed to C=O stretching of acetyl or carboxylic acid that represented the presence of hemicellulose and lignin (Fougere et al., 2016). A prominent peak attributed to non-conjugated C=C stretching and C=O stretching vibrations of the aromatic ring was generated at band position 1628 cm^{-1}. It signified the presence of lignin (Taherdanak and Zilouei, 2014; Lie et al., 2013; Sua et al., 2015). The intensity of this absorption peak was found to be decreased in all treated samples; it might be due to deformation and solubilisation of lignin. An absorption peak for CH_2 bending was obtained at 1439 cm^{-1} that illustrated the presence of cellulose (Lie et al., 2013). A new peak was appeared near 1353 cm^{-1} after 8 % NaOH and NaOH (8 %)-microwave pretreatment. It was attributed to C-H deformation in cellulose and hemicellulose (Pandey and Pitman, 2003). The peak near band position 1249 cm^{-1} was associated with the syringyl ring and C-O stretch in lignin and xylan (Pandey and Pitman, 2003). Syringyl type of lignin is the characteristic of hardwood which explains the nature of *Tectona grandis* sawdust. Its intensity was also decreased after pretreatment denoting the change in sawdust structure. The peaks near 1054-1032 cm^{-1} were aassigned to C-O vibrations of cellulose/hemicellulose and lignin that described the lignocellulosic nature of sawdust (Ang et al., 2012; Jiangtao et al., 2012; Pandey and Pitman, 2003).

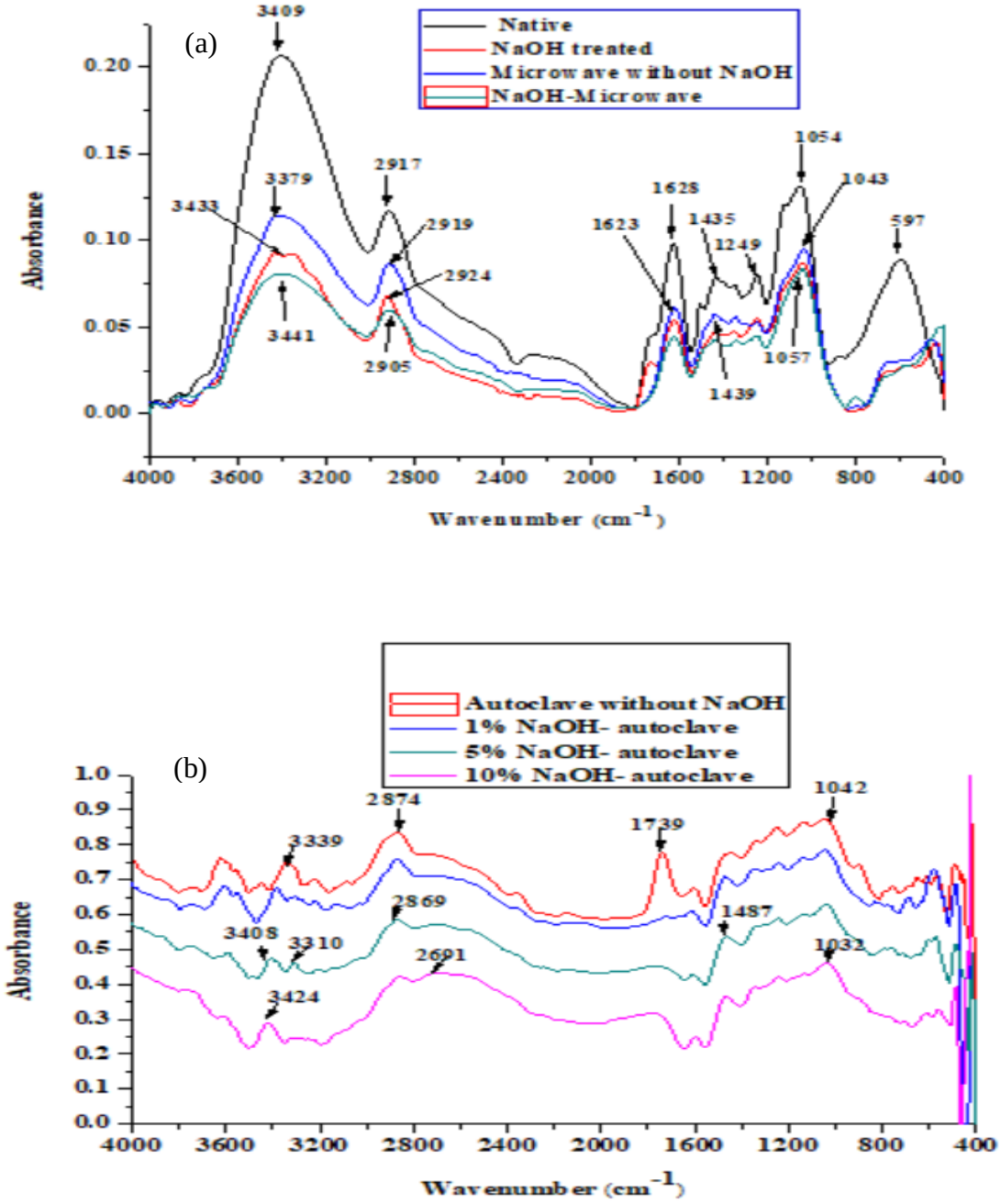


Figure 4.3 FTIR spectra: (a) NaOH and NaOH-microwave pretreatment (b) NaOH-autoclave pretreatment of sawdust

Table 4.4 Compositional characterisation of sawdust before and after NaOH pretreatment

Concentration of NaOH (%)	Pretreatment condition								
	NaOH			NaOH-microwave			NaOH-autoclave		
	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
0				49.9	13.1	29.2	49.6	13.0	29.0
2	49.9	13	28.9	50.8	12.8	28.3	52.9	11.8	25.1
4	50.2	12.9	28.5	52.3	12.4	27.1	54.2	11.2	24.3
6	51	12.3	28	53.6	11.8	25.8	56.7	10.3	24.0
8	51.5	12	27.2	54.4	10.6	24.7	58.7	9.5	22.9
10	51.8	11.8	27	54.5	10.4	24.7	60.1	9.0	21.7
12	52	11.5	27	54.5	10.5	24.5	60.0	8.8	21.7

Cellulose, hemicellulose and lignin are in wt %

Table 4.5 FTIR spectroscopic characterisation of native and NaOH treated sawdust

Control	Microwave without NaOH	10 % NaOH treated	10 %NaOH-microwave	Autoclave without NaOH	5 % NaOH-autoclave	10% NaOH-autoclave	Assigned functional group	Significance
3409	3379	3433	3441	3339	3408, 3310	3424	O-H stretching	Cellulose
2917	2919	2924	2905	2874	2869	-	C-H Stretching	Cellulose
-	-	-	-		-	2691	CHO	Hemicellulose
-	-	-	-	1739	-	-	C=O stretching of acetyl or carboxylic acid	Hemicellulose and lignin
1628	1623	1623	1623	-	1487	-	C=C aromatic bending	Lignin
1435	1439	-	-	-	-	-	CH_2 bending	Cellulose
-	-	1353	1345	-	-	-	C-H deformation	Cellulose and hemicellulose
1249	1250	1249	-	-	-	-	C-O stretch	Lignin and xylan
1054	1043	1043	1057	1042	-	1032	C-O stretching vibration	Cellulose/ hemicellulose and lignin

4.2.7 Effect of pretreatment on sawdust crystallinity

In lignocellulosic biomass, cellulose is presents in crystalline as well as in amorphous form whereas, hemicellulose and lignin are considered as amorphous material (Zhang et al., 2017). XRD measurements were performed for crystallinity analysis of sawdust samples in terms of CI. XRD pattern is depicted in Figure 4.4 and CI values are given in Table 4.6. The Peak of the crystalline plane was observed at $2\theta \approx 22^{\circ}$ and lowest value of amorphous region was found to be at $2\theta \approx 18^{\circ}$. The intensity of crystalline cellulose was increased after pretreatment as shown in Figure 4.4. Crystallinity pattern of this study was similar to that of the XRD spectra of NaOH treated *Spartina alterniflora* (Chen et al., 2014).

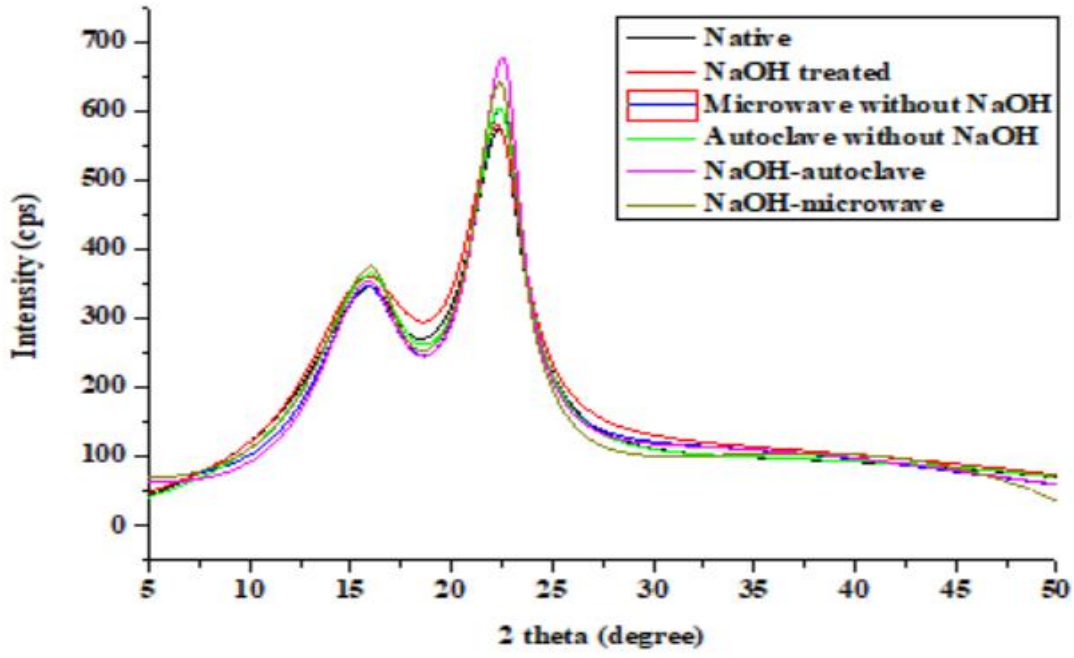


Figure 4.4 XRD pattern of native and treated sawdust

The CI values were found to increase from 64 (native without NaOH addition) to 70.2 % (with NaOH), 65.2 (microwave without NaOH) to 71.8 % (NaOH-microwave) and 66.37 (autoclave without NaOH) to 74.13 % (NaOH-autoclave). It might be due to peeling reaction of NaOH affected amorphous region more than crystalline region. Previous studies also reported that pretreatment of biomass resulted in the breakage of lignin-carbohydrate complex, recrystallisation of cellulose, removal of lignin and hemicellulose which in turn caused high CI values (Barman et al., 2014; Mancini et al., 2018; Kristiania et al., 2015). So accordingly, increased CI values could be associated with increase in crystallinity and elimination of amorphous material from sawdust. Figure 4.5 signified a positive correlation of CI and cellulose content.

Table 4.6 Crystallinity index obtained from XRD data of sawdust

Concentration of NaOH (%)	Crystallinity index (%)		
	NaOH	NaOH-microwave	NaOH-autoclave
0	64.00	65.20	66.37
2	65.44	67.35	69.71
4	66.70	68.70	70.88
6	68.25	69.83	71.34
8	69.60	71.12	73.16
10	70.20	71.80	74.13

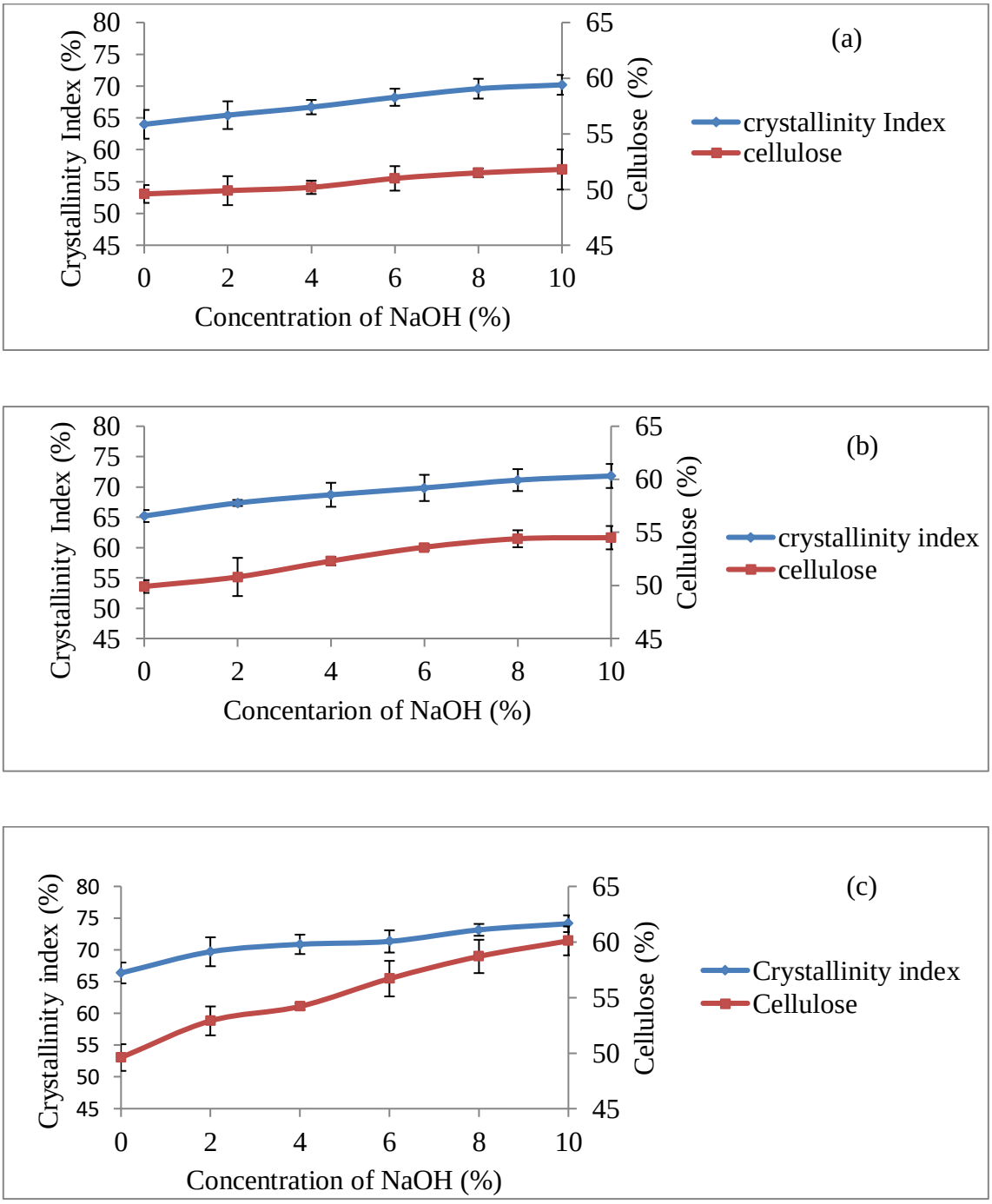


Figure 4.5 Crystallinity and cellulose profile: (a) NaOH, (b) NaOH-microwave and (c) NaOH-autoclave sawdust after pretreatment

4.2.8 Effect on surface morphological structure of treated and native sawdust

The SEM images of native and treated sawdust samples were captured in the range of 500-5000 X magnification to study the morphological change before and after treatment. The SEM micrographs clearly illustrated the cell wall structural differences of native and treated sawdust as represented in Figure 4.6. The surface structure of native sawdust was observed as rigid and compact. It seemed highly ordered, rigid with smooth surface because of lignin coating over cellulose and hemicellulose fibers (Mohtar et al., 2017). Cell bundles were found to be flaky, brittle and disaggregated in treated sawdust in comparison to native sawdust. Thermal treatment without NaOH, mainly autoclave treatment had a little effect on cell surface (Figure 4.6(e)). Whereas, combination of NaOH and thermal treatment caused significant destruction to cell wall that signified the importance of NaOH in substrate solubilisation (Figure 4.6 (d) and (f)). As described earlier, NaOH caused distortion of cross-linkages between lignin molecules like main ether bonds and ester bonds between carbohydrate and lignin that degraded a part of lignin and hemicelluloses and in turn caused the formation of cracks and holes in cell structure and exposed the cellulose. Also, its solvating nature was responsible for swelling of biomass that turned the cell wall structure disorganised and visually fragile as seen in Figure 4.6 (d) (Nargotra et al., 2018). Therefore, disruption of cell surface could be associated with breakdown and solubilisation of lignin and hemicelluloses as compositional analysis of native and treated sawdust also confirmed that lignin and hemicellulose content was decreased with increasing concentration of NaOH. The NaOH and thermal treatment ruptured the rigidity of sawdust by reducing lignin content, making sawdust fragile that can vulnerable to microbial enzymes. Increased fragile nature can facilitate the accessibility of microorganisms and increase the biogas production (Hesami et al., 2015).

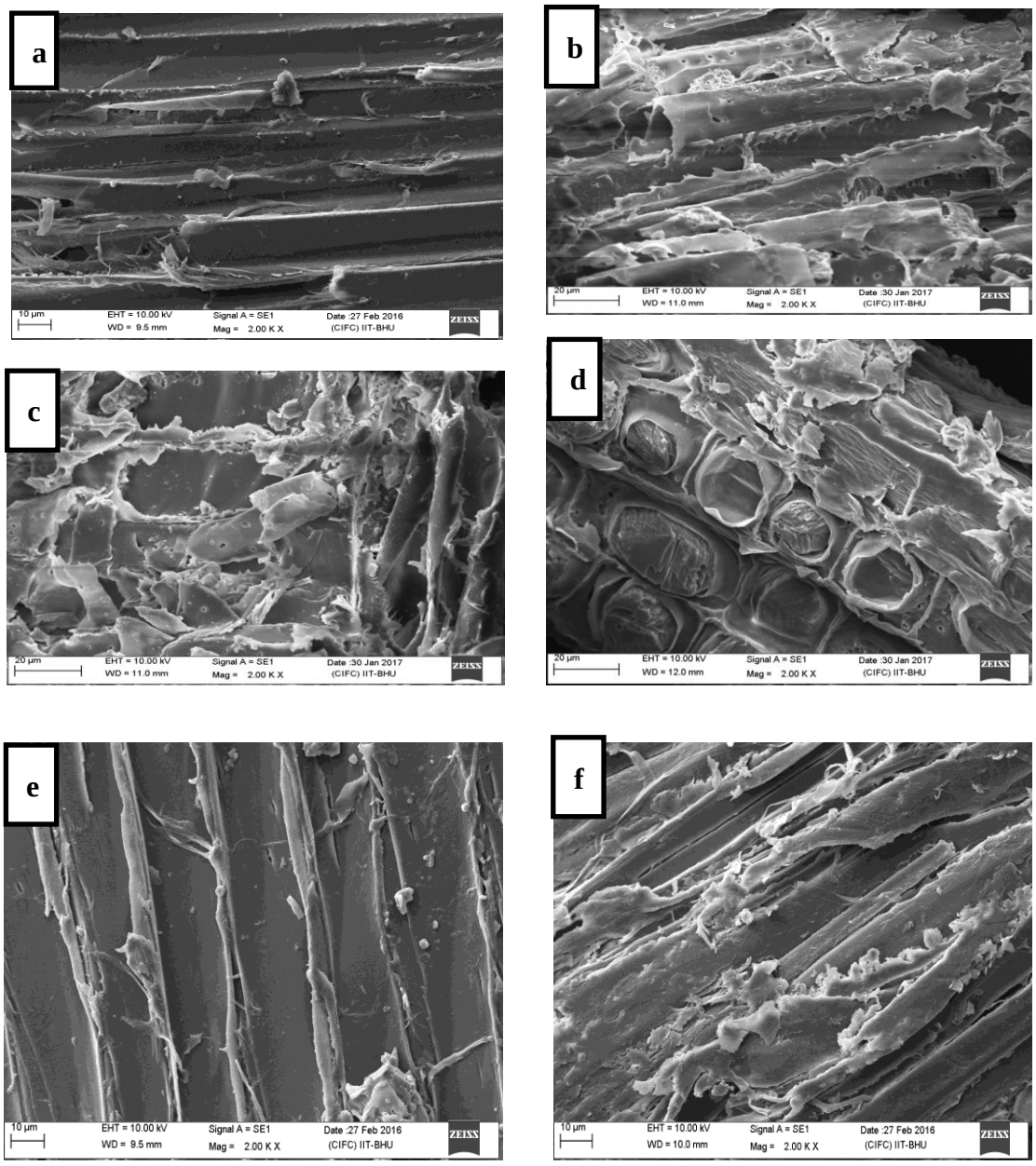


Figure 4.6 SEM images of sawdust samples: (a) native, (b) microwave without NaOH, (c) NaOH , (d) NaOH-microwave , (e) autoclave without NaOH and (f) NaOH-autoclave

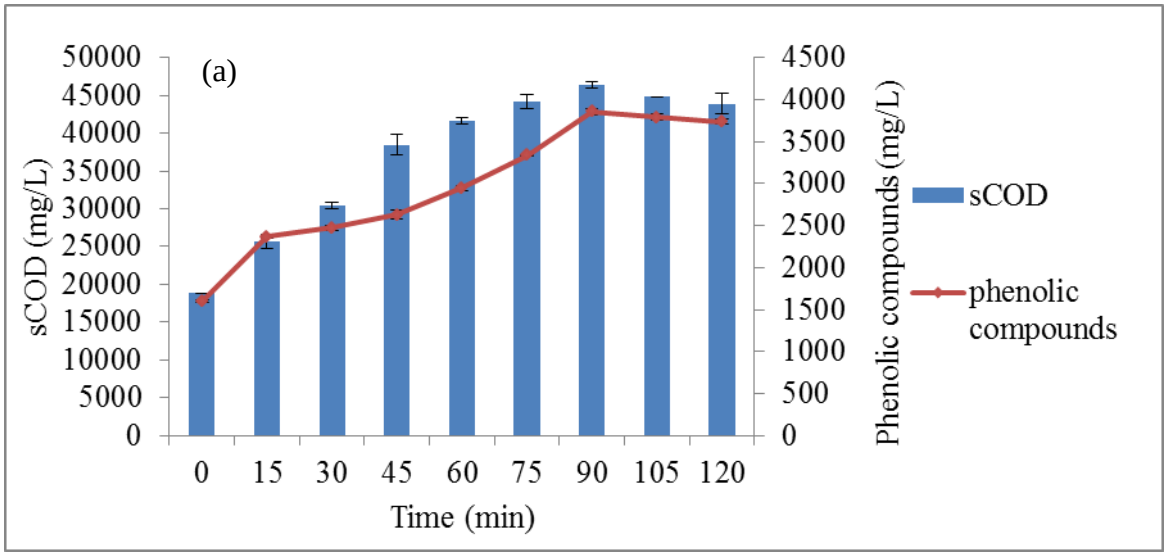


Figure 4.7 Influence of autoclaving time for NaOH-autoclave pretreatment (a) sCOD and phenolic compound variation and (b) compositional changes and lignin solubilisation

4.2.9 Influence of time for NaOH-autoclave pretreatment

Results illustrated that NaOH-autoclave pretreatment has the potential to maximize the lignin solubilization and it is more effective in sawdust hydrolysis in comparison to other pretreatment methods used in the experimentation. Therefore, effect of

autoclaving time for NaOH-autoclave pretreatment was studied for improving hydrolysis effect on sawdust. The 10 % NaOH was selected as ideal concentration since it turned out to be the best NaOH dose for lignin solubilization. The sCOD, phenolic content and lignin solubilization were increased with respect to autoclaving time as illustrated in Figure 4.7 (a). The 10 % NaOH-autoclave pretreatment with 90 min of autoclaving time was found to be the best pretreatment method which solubilized 58.6 % lignin as compared to native sawdust as found from Figure 4.7 (b).

4.3 Anaerobic digestion of sawdust

4.3.1Biogas production from sawdust

The effect of best pretreatment condition was examined on biodegradability and biogas production from treated sawdust. The cumulative biogas yield is represented in Figure 4.8. Reactor was used to agitate daily for 1 min manually before recording the biogas volume in water displacement column. There was no production of biogas observed in first 5 days of anaerobic digestion of native sawdust. Afterwards, it was increased slowly and attained a maximum rate of 6.8 $NmLg^{-1}VSd^{-1}$ biogas production in 27 days whereas; biogas production from treated (10 % NaOH and 90 min autoclaving) sawdust attained a maximum rate of 13.06 $NmLg^{-1}VS\ d^{-1}$ in 23 days with a lag phase of 2 days. The cumulative production of biogas from treated sawdust was 289 $NmLg^{-1}VS\ d^{-1}$ which was 103.5 % more than native sawdust in 40 days of digestion period. Pretreatment solubilised a part of lignin by dissolving the carbohydrate-lignin linkages present in sawdust. Lignin solubilisation made cellulose accessible to microorganisms and in turn increased the production. Previous researchers have also reported that pretreatment conditions increased the surface area of substrate and made it more vulnerable to microbial enzymes and also enhanced the biodegradability and biogas production (Bjornsson et al., 2000). pH observations were also noted during anaerobic

digestion with the help of inserted pH probe attached with pH meter. The initial pH of digester was 7.1. The pH was decreased to 5.8 upto the 15th day of digestion. Accumulation of organic acid might have reduced the pH. It is previously reported that organic acid formation took place in earlier days of anaerobic digestion that was responsible for pH drop in digester (Monte et al., 2018). Consumption of these organic acids by methanogens in order to produce biogas raised the pH of digester slowly afterwards. pH was found to be 7.5 after 40 days of digestion. Improvement in methane concentration enhances the ignitable properties of biogas and increases its heating value (Dabir et al., 2017). Biogas production from treated sawdust attained a maximum methane concentration of 62 % in 28 days of digestion.

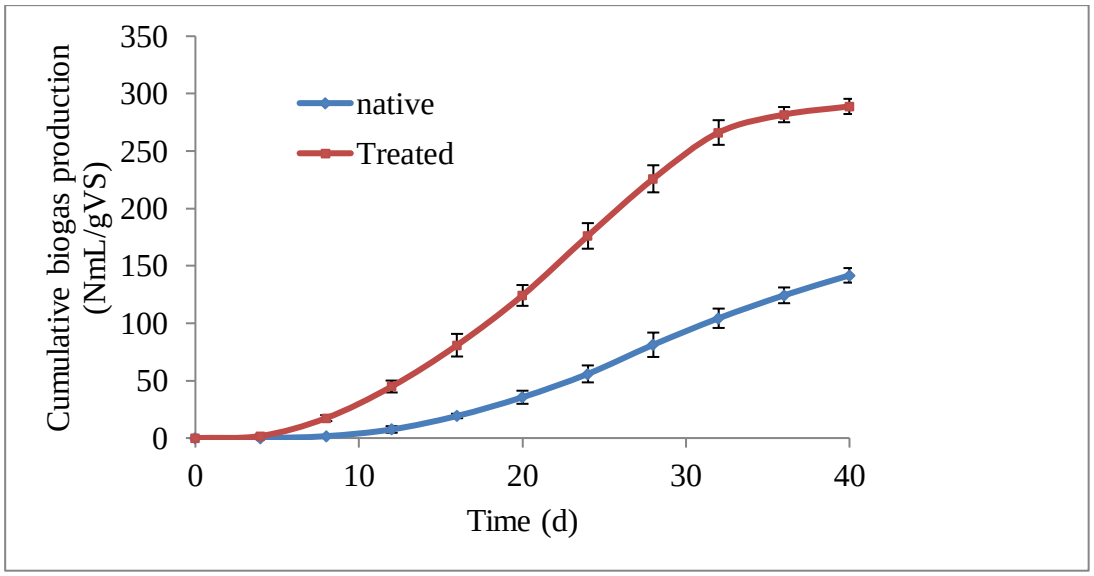
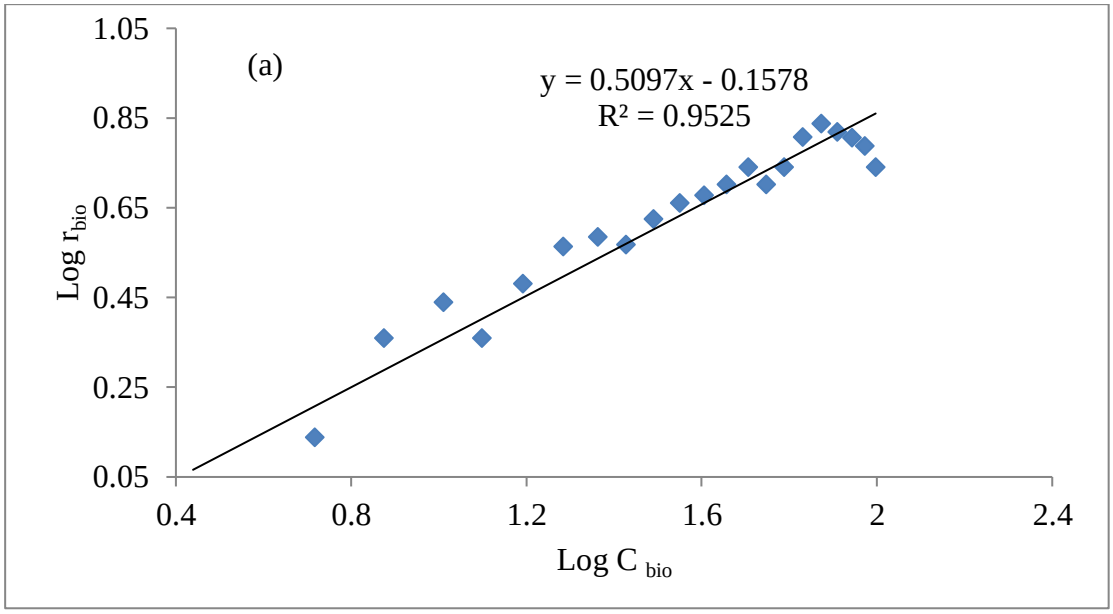


Figure 4.8 Effect of pretreatment on anaerobic digestion for biogas yield from sawdust

4.3.2 Bio-energy conversion rate

The rate constants for anaerobic digestion of native and treated sawdust were calculated by using standard rate equation (3.23). The plots of rate vs. concentration of biogas are represented in Figure 4.9. The value of rate constant was calculated as 0.697 $(NmL/gVS)^{0.491}$ d^{-1} for biogas production from native sawdust whereas the biogas production from treated sawdust it was found 1.47 $(NmL/gVS)^{0.577}$ d^{-1}. The value of order of conversion in anaerobic digestion of native and treated sawdust was 0.509 and 0.423, respectively. The increased rate constant and order signified the increase in rate of sawdust conversion into biogas. Pretreatment condition might have increased the accessibility of microorganisms to substrate by breaking the recalcitrance that in turn increased the production rate and yield of biogas.



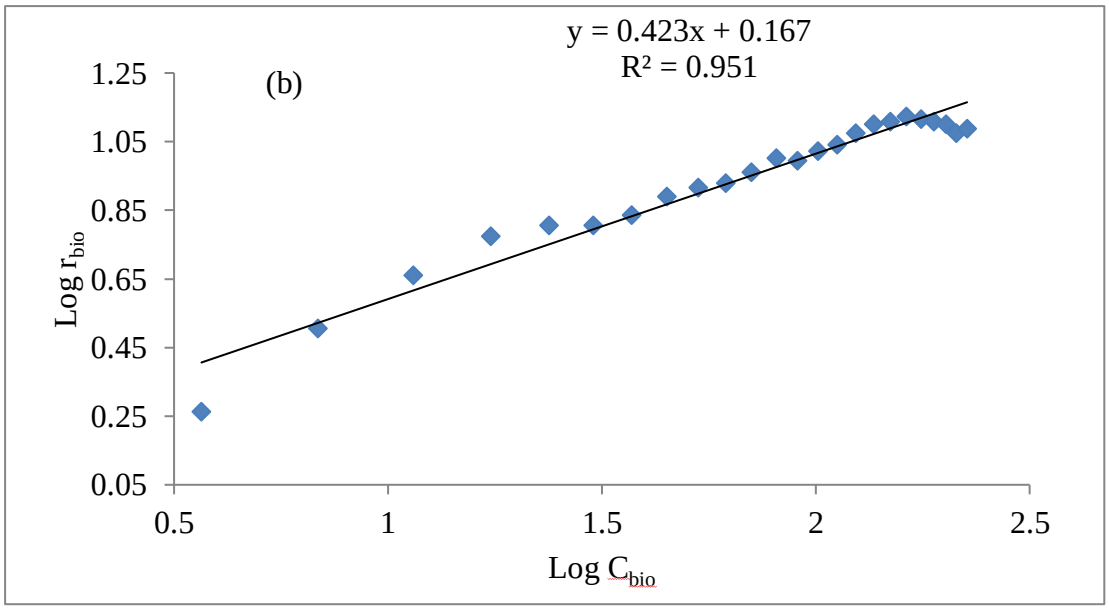


Figure 4.9 Effect of pretreatment on rate constant of bioconversion (a) native and (b) treated sawdust

4.4 Physico-chemical characterisation of OFMSW and inoculum

The OFMSW as received (raw OFMSW) was mixed and ground by a kitchen mixture grinder to form slurry. The raw OFMSW and slurry were physico-chemically characterised and values are depicted in Table 4.7. The moisture content of OFMSW was 26.85 % in raw OFMSW. It was increased to 91.3 % in slurry of liquidised OFMSW due to water addition. The total solid was obtained as 73.15 and 8.75 % in raw and liquidised slurry, respectively. The raw and liquidised slurry contained 68.34 and 7.7 % volatile solid, respectively.

Table 4.7 Physico-chemical characterisation of OFMSW

Properties (wt %)	Raw OFMSW	Liquidised OFMSW	Inoculum
Proximate analysis			
Moisture content	26.85 ± 2.9	91.30 ± 0.8	91.88 ± 2.2
Total solid	73.15 ± 2.9	8.75 ± 0.8	8.13 ± 2.2
Volatile solid	68.34 ± 3.8	7.7 ± 0.7	5.5 ± 0.4
Ultimate analysis (dry basis)			
Carbon	48.78 ± 2.2	45.87 ± 2.3	37.7 ± 1.8
Hydrogen	7.05 ± 2.0	6.86 ± 1.4	6.1 ± 1
Nitrogen	2.46 ± 0.6	1.65 ± 0.3	2./1 ± 0.3
Oxygen	42.56 ± 1.2	46.77 ± 3.4	53.48 ± 2.8
Chemical properties			
sCOD (mg /L)	-	15608 ± 62	2000 ± 47
VFA (mg CH_3COOH /L)	-	1313 ± 41	-
pH	7.21 ± 0.2	6.99 ± 0.3	7.1 ± 0.1

The C/N ratio of raw and OFMSW slurry was 19.83 and 27.8, respectively. The C/N ratio of OFMSW indicated the applicability of anaerobic digestion. The optimum C/N ratio lies in range of 15-25 for anaerobic digestion. The moisture content of 91.88 % was found in inoculum (slurry from biogas plant running on cow dung). The total solid and volatile solid found as 8.13 and 5.5, respectively. The sCOD of OFMSW slurry and inoculum was 15608 and 2000 mg/L, respectively. The pH of raw OFMSW was 7.21. It was reduced to 6.99 in OFMSW slurry and the pH of inoculum was 7.1.

4.5 Effect of thermal, chemical and thermo-chemical treatment on OFMSW solubilisation

The thermal, chemical and thermo-chemical treatments were employed on OFMSW. The achieved solubilisation was studied through physicochemical characterisation, XRD, FTIR and SEM analysis.

4.5.1 Effect of pretreatment on physico-chemical characteristics of OFMSW

The moisture content in samples was found in the range of 166.5 to 250.5 g kg^{-1} which showed the applicability of the OFMSW sample to be treated by anaerobic digestion. The volatile solid content was reduced by 1.99, 1.21 and 2.36 % for thermally, chemically and thermo-chemically treated samples, respectively. It might be due to the hydrolysis of particulate organic matter into organic acids. The COD of samples after pretreatment was increased due to the fact that pretreatment caused breakage of complex molecules like carbohydrates, proteins and fats into a mixture of free amino acids and small peptides (Yunqin et al., 2009). The carbon and nitrogen content in the samples were found in the range of 50.33-52.25 % and 2.40-3.16 %, respectively. The maximum increase in COD obtained was 11.60 % in sample after thermo-chemical pretreatment whereas an increase of 6.87 and 1.61 % was found in thermally and

chemically treated samples, respectively. The Characterisation of OFMSW samples before and after pretreatment is shown in Table 4.8.

Table 4.8 Characterisation of OFMSW samples before and after pretreatment

S.No.	Properties	Control	Thermal	Chemical	Thermo-chemical
1	Moisture content (g kg^{-1})	247.8	250.5	166.5	222
2	Total solids (g kg^{-1})	752.2	749.5	833.5	778
3	Volatile solids (g kg^{-1} of total solid)	944.1	925.3	932.7	921.8
4	Ash content (g kg^{-1})	55.9	74.7	67.3	78.2
5	pH	7.42	7.28	7.3	7.1
6	Carbon (%)	50.33	50.75	52.25	51.95
7	Hydrogen (%)	5.65	5.99	6.08	6.11
8	Nitrogen (%)	3.16	2.62	2.56	2.40
9	COD (mg/L)	9760	10480	9920	11040
10	VFA (mg CH_3COOH/L)	332.5	586.25	1137.5	1356.25

4.5.2 Crystallinity study of OFMSW by XRD

The XRD was carried out to identify the effect of treatments on the crystallinity of the OFMSW sample. Figure 4.10 (a)-(d) showed the X-ray diffraction spectrum of OFMSW samples before and after treatments. There was slight increase in the width of broad peak which indicated the reduction in the crystallinity of substrate. The broad scattering peak appeared at 17.4-25.3° in the spectrum for untreated OFMSW indicated the presence of abundant compounds with amorphous C-H structures whereas this broad

scattering peak appeared at 17.1-25.6°, 17.4-25.5° and 17.1-25.7° for thermally, chemically and thermo-chemically treated samples respectively. The crystallinity index (*CI)* value of treated sample was reduced because treatments caused reduction in the crystallinity of substrate. *CI* of thermally treated sample is higher because of the formation of melanoidins (formed when sugar and amino acids combine at high temperature) which are considered as less biodegradable. The crystallite sizes and *CI* values of the samples before and after treatment are represented in Table 4.9.

Table 4.9 Crystallite size of OFMSW samples before and after treatments

S.No.	Sample	2θ (degree)	FWHM (degree)	crystal size (nm)	CI
1.	Control	22.46	1.96	4.14107	49.51
2.	Thermal	22.45	2.8	2.89749	52.18
3.	Chemical	22.42	2.11	3.84598	46.30
4.	Thermo-chemical	22.28	3.44	2.35742	48.78

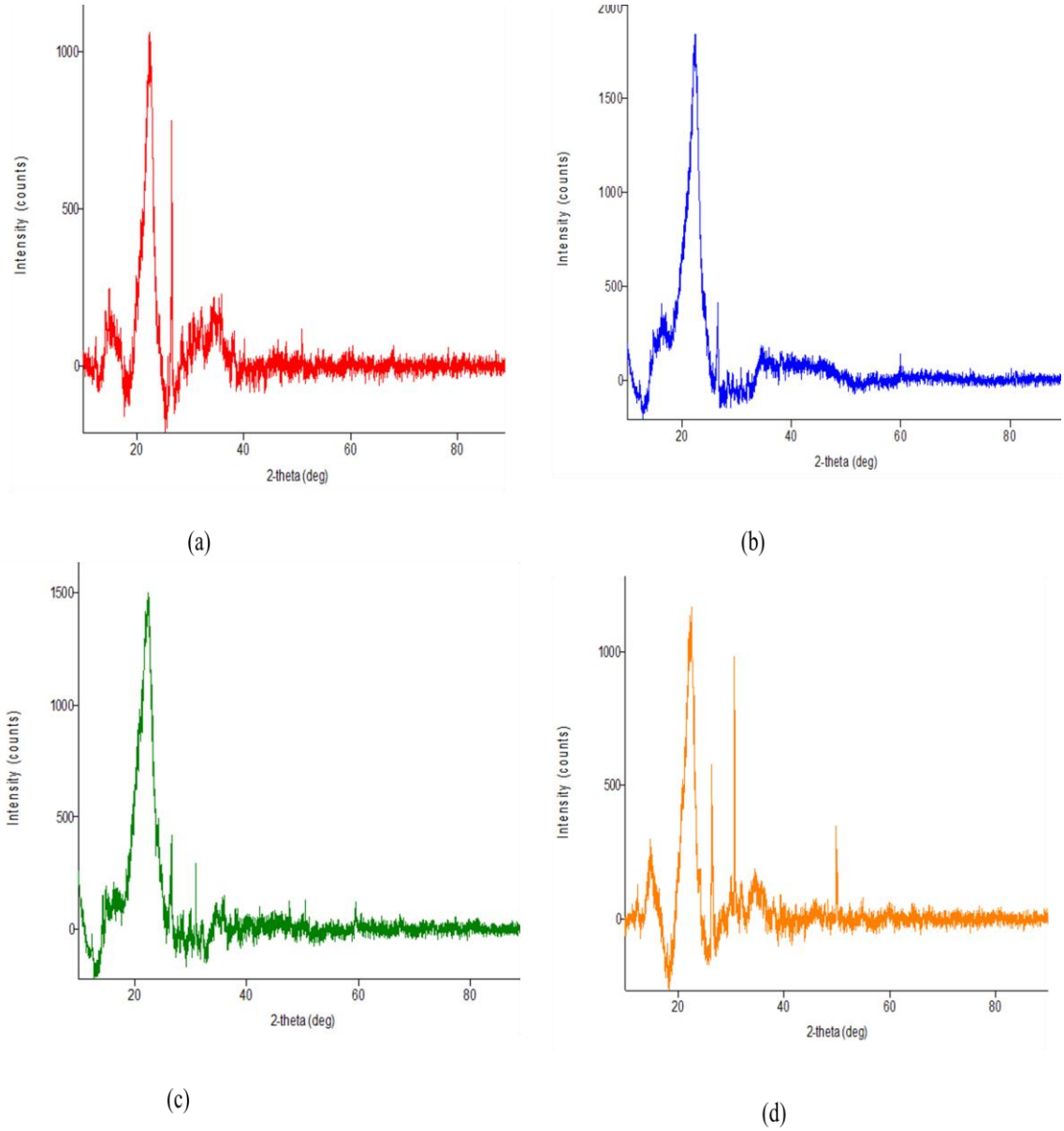


Figure 4.10 X-ray diffraction spectrum of OFMSW: (a) before pretreatment, (b) after thermal pretreatment, (c) after chemical pretreatment and (d) after thermo-chemical pretreatment

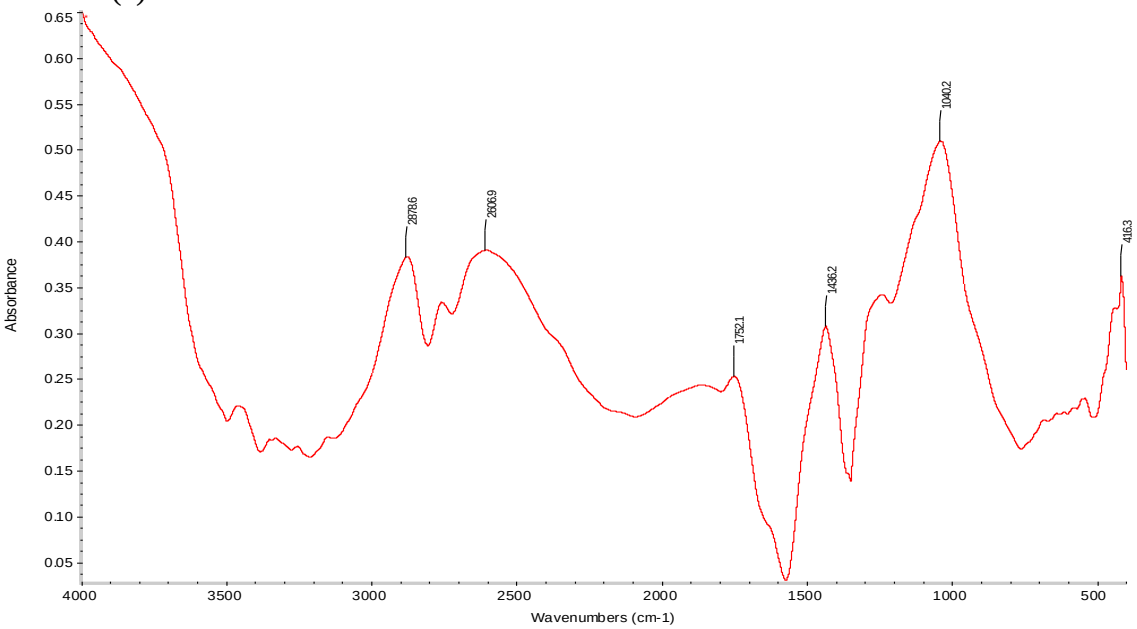
4.5.3 FTIR study of OFMSW

The FTIR absorption spectra of OFMSW samples before and after treatments are shown in Figure 4.11. The FTIR absorption spectrum was recorded in the range of 4000-400 cm^{-1} range, with the resolution of 4 cm^{-1}. It was observed that OFMSW majorly consisted of alkanes, alkenes, alcohol, carboxylic acid, ketones, aldehydes and aromatic rings. Untreated OFMSW was found to consist methylene, C-H (2878.6 cm^{-1}), carboxylic acid, O-H (2606.9 cm^{-1}), ester, C=O (1752.1 cm^{-1}), alkane, C-H (1436.2 cm^{-1}) and ether, (Chefetz et al., 1998; Wu et al., 2011; Elfels et al., 2014; Smidt et al., 2005). Peak near 1040.2 cm^{-1} was assigned to C-O stretch that demonstrated the presence of primary alcohols, polysaccharides, aromatic ethers and esters (Cano et al., 2014). Peak 3293.2 cm^{-1} was attributed to –OH of phenol (Soobhany et al., 2017). Thermal pretreatment caused slight increase in C-H and carboxylic acid, O-H peaks whereas major increase in ether, C-O was observed. Similarly, chemical and thermo-chemical treatments also caused increase in the C-H, O-H and C-O peaks which indicated the changes in the stretching and bending of these functional groups. The presence of amorphous cellulose was signified by the shift of the band from 2878.6 cm^{-1} corresponding to the C–H stretching vibration, to higher wave number values 2879.6, 2881 and 2880.2 cm^{-1} for thermal, chemical and thermo-chemical treatments, respectively as depicted in Table 4.10. The reduction in intensity or disappearance of the bands characteristics of the crystalline domains indicated the alteration in the crystalline structure.

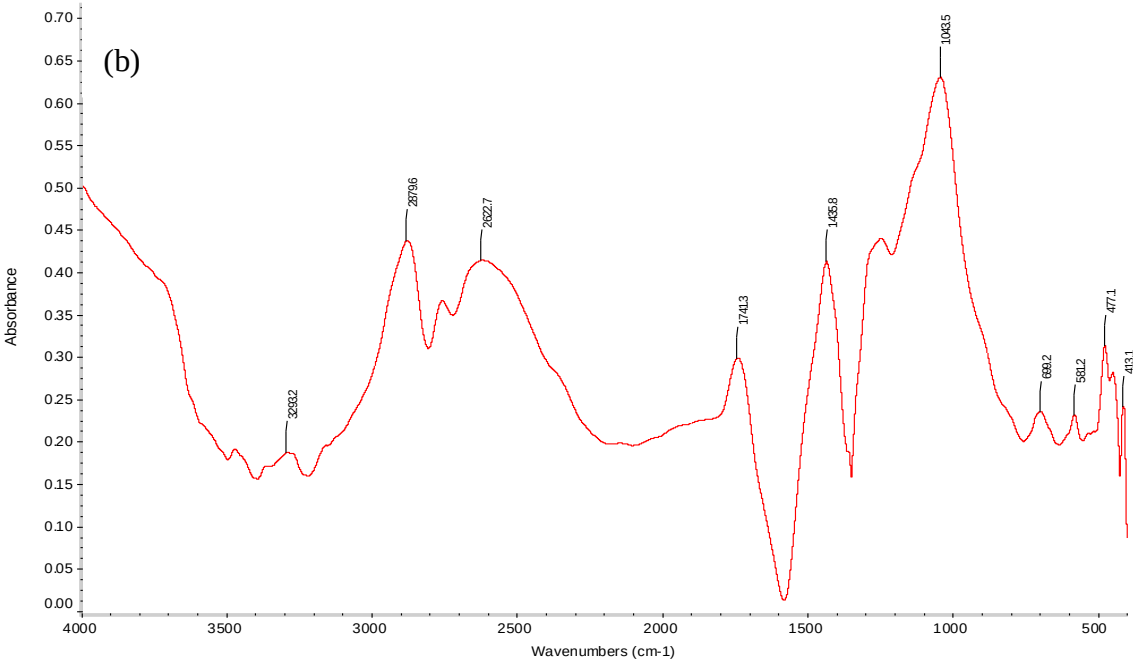
Table 4.10 FTIR analysis of OFMSW sample before and after treatments

S.No.	Characteristic absorption(s) (cm^{-1})				Functional group
	Control	Thermal	Chemical	Thermo-chemical	
1	-	3293.2	-	-	Phenol O-H stretch (small, broad)
2	2878.6	2879.6	2881	2880.2	Methylene C-H stretch (medium)
3	2606.9	2622.7	2620	2622.3	Carboxylic acid, O-H stretch (variable)
4	1752.1	1741.3	1741.7	1737.9	Ester, C=O stretch (medium)
5	1436.2	1435.8	1436.3	1436.2	Alkane, C-H bending (variable)
6	-	-	-	1246.3	Amine, C-N stretch (medium, weak)
7	1040.2	1043.5	1041.3	1042.5	Ether, C-O stretch (strong)
8	-	699.2	-	-	Aromatic C-H bending (small)

(a)


Absorbance
Wavenumbers (cm-1)
2878.6
2606.9
1752.1
1436.2
1040.2
416.3

(b)


Absorbance
Wavenumbers (cm-1)
3293.2
2879.6
2622.7
1741.3
1435.8
1043.5
699.2
581.2
477.1
413.1

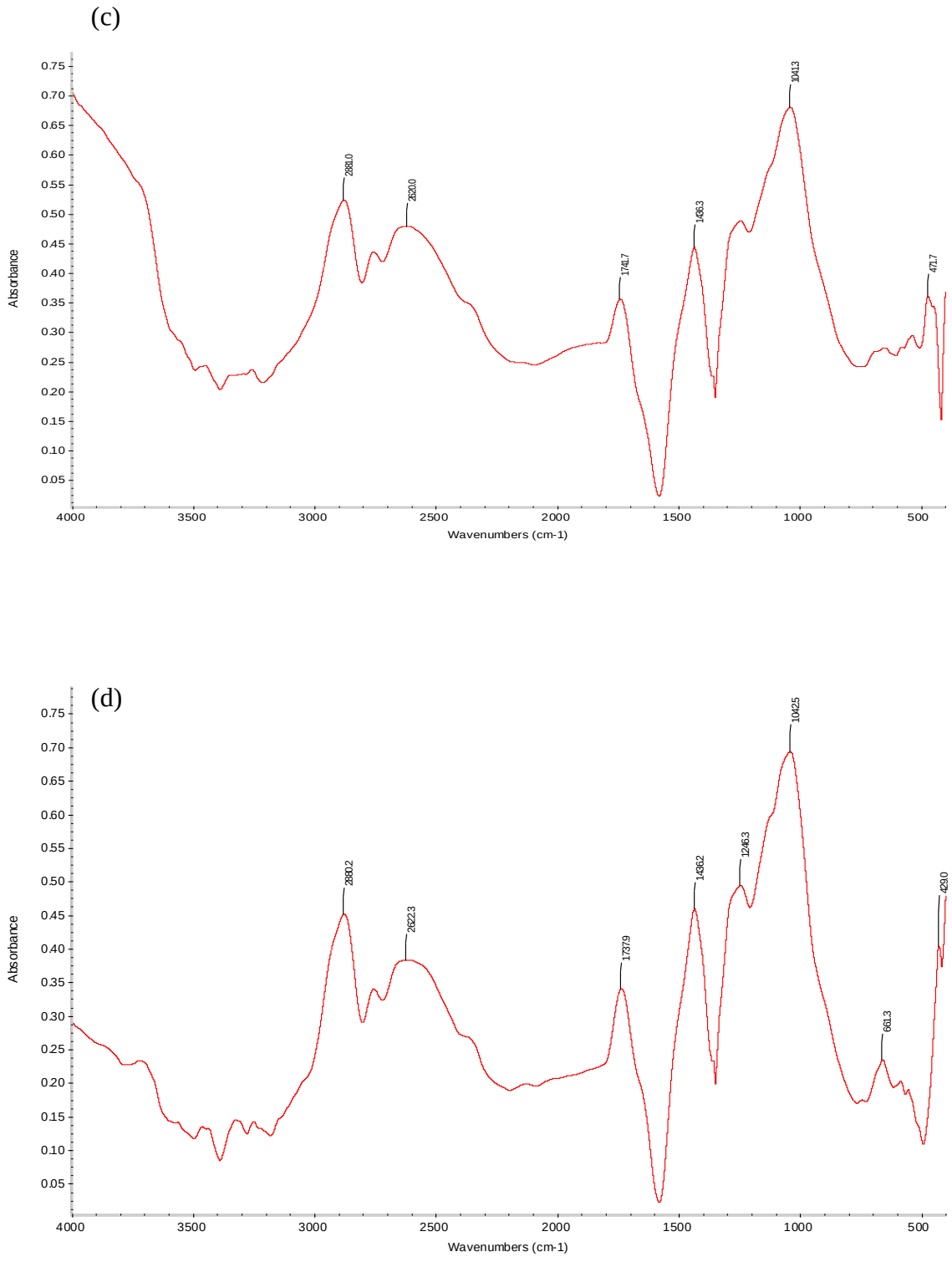


Figure 4.11 FTIR spectrum of OFMSW sample: (a) before treatment, (b) after thermal treatment, (c) after chemical treatment and (d) after thermo-chemical treatment

4.5.4 Effect of pretreatment on surface structure of OFMSW

SEM micrographs of the control and treated samples were obtained on a field emission scanning electronic microscope (FEI QUANTA 200) which showed the significant physical micro-structural difference between the four samples as shown in Figure 4.12. Image 4.12 (a) indicated the untreated OFMSW in which the fibers make up the structure of celluloses and hemicellulose whereas, the fibers structure was observed disorganized after treatment.

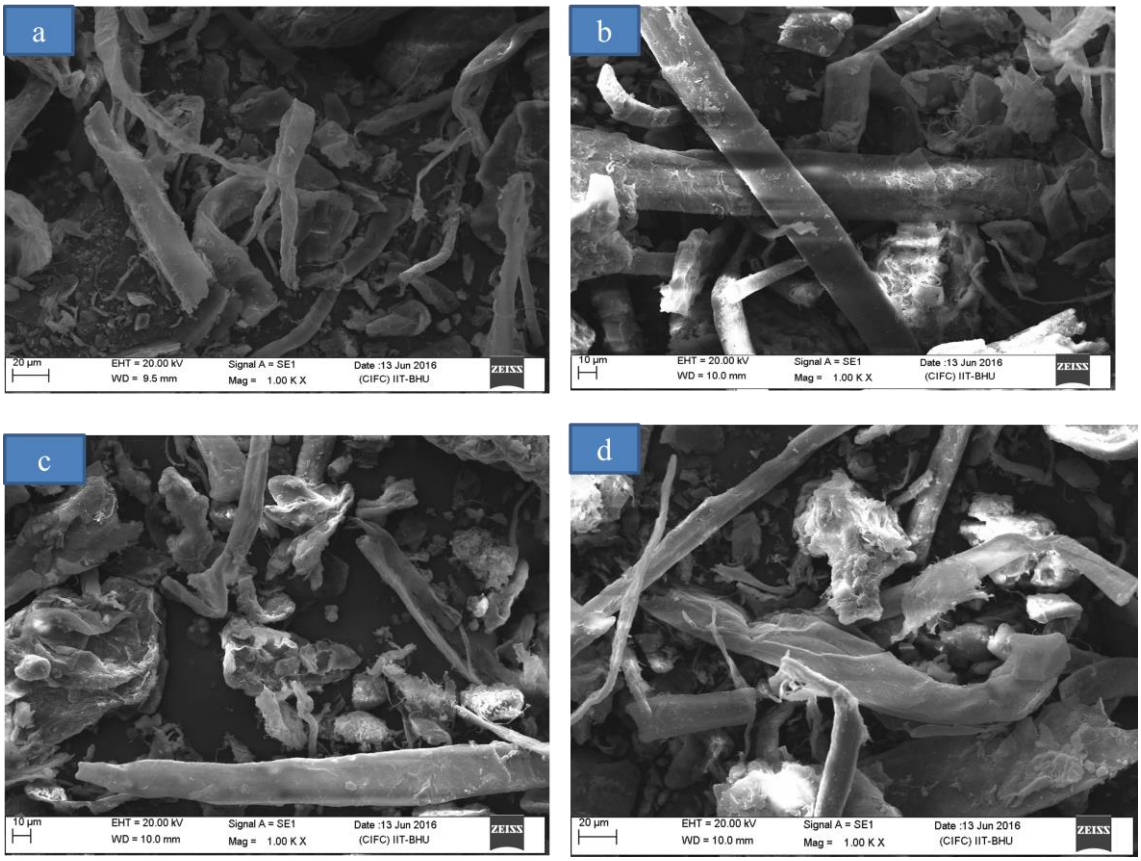


Figure 4.12 SEM micrographs of OFMSW sample: (a) before pretreatment, (b) after thermal pretreatment, (c) after chemical pretreatment and (d) after thermo-chemical pretreatment

These micrographs represented the organic fragments and debris of various shapes and sizes in which large and smooth sheet fragments were probably the vegetable cellulose, whereas the smaller adhesive porous granules were mainly mixture of starch, proteins and lipids. Treatments might have affected the biomass structure by solubilising or altering hemicelluloses, altering lignin structure and increasing the surface area and pore volume of the substrate. Chemical and thermo-chemical treatments disrupted the cellulosic structure which allowed the greater accessibility for the microbes.

4.5.5 Effect of biological treatment on OFMSW solubilisation

Effect of *P. chrysosporium* and *P. ostreatus* on OFMSW solubilisation was studied, and findings are represented in Figure 4.13. The concentration of sCOD and VFA were slightly increased with increasing the time of treatment. However, *P. chrysosporium* found to be more effective in improving the sCOD and VFA yield in comparison to *P. ostreatus*. Increment in sCOD and VFA value could be associated with the partial solubilisation of OFMSW sample. Hydrolysis of carbohydrates, protein and fats into sugar, amino acids and fatty acids may increase the sCOD concentration. Lignin reduction due to fungal treatment might have exposed the available cellulose and hemicellulose to microbes that in turn increased the soluble compounds and also sCOD value. The white rot fungal strains can cause degradation of lignin by secreting some lignolytic enzymes like lignin peroxidase, manganese peroxidase and laccase. The complete degradation of the lignin molecule takes place in fungal treatment due to the action of fungal enzymes without generation of the phenolic compounds. It is an advantage over other treatment methods like chemical treatment, where generation of the phenolic compound takes place with increasing degradation of lignin. The glucose and phenolic content yields were slightly decreased after treatment (Figure 4.13 (c)). The reduction in glucose yield might have associated with its consumption by

microorganisms to fulfil their energy requirement, and phenol degradation was occurred by fungal enzymes secreted by *P. chrysosporium* and *P. ostreatus*.

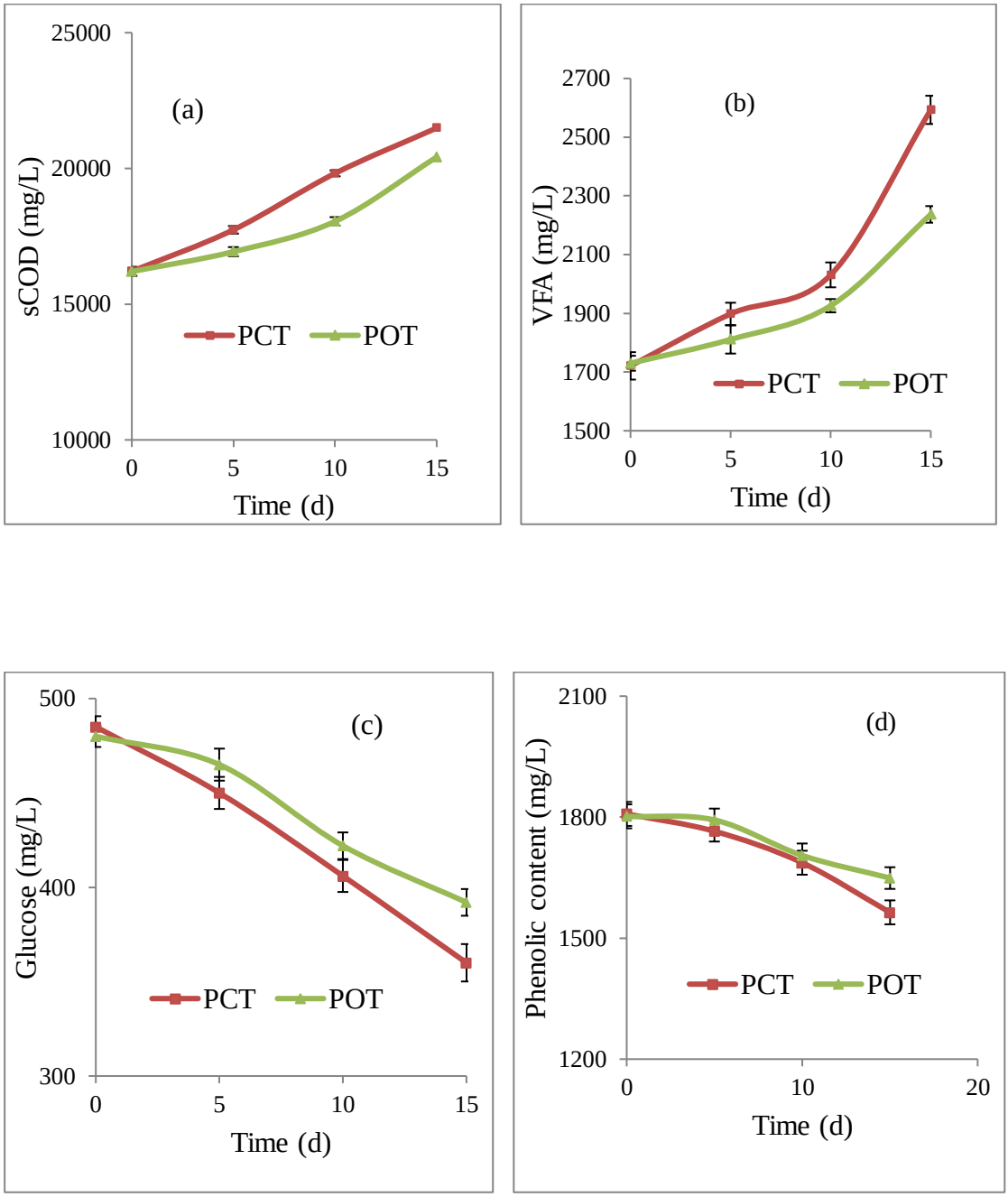


Figure 4.13 Effect of biological pretreatment by *P. chrysosporium* (PCT) and *P. ostreatus* (POT) on OFMSW solubilisation (a) sCOD (b) VFA and (c) glucose and (d) phenolic content

The *P. chrysosporium* and *P. ostreatus* treatment have been widely applied to degrade the lignocellulosic material by researchers. The effect of such type of pretreatment on OFMSW has not been reported in the literature as per our knowledge. However, fungal strain *Aspergillus awamori* was employed by Fdez.-Güelfo et al. (2011b) to solubilise the OFMSW.

4.5.6 Effect of different pretreatments on biogas production

Anaerobic digestion was done for evaluating the effect of pretreatments on OFMSW. A graphical representation of biogas yield is depicted in Fig 4.14. Cumulative biogas yield of 207 NmL/gVS was obtained in 18 days from untreated OFMSW sample. The biogas yield increased and production time decreased after treating the samples by different pretreatment methods prior to anaerobic digestion. Presence of lignin hindered the biogas production in untreated OFMSW while the treatment made it more available to microbes and resulted in elevated production. Thermal treatment turned out to be in 284 NmL/gVS cumulative biogas yield in 16 days. The Cumulative biogas yields obtained from chemically and thermo-chemically treated OFMSW were 370 NmL/gVS in 14 days and 450 NmL/gVS in 12 days, respectively. The strains of white rot fungi are capable of degrading lignin by using their ligninolytic enzymes. The *P. chrysosporium* treatment (PCT) might have degraded the lignin more efficiently than *P. ostreatus* (POT) and turned out in 13.9 % more biogas production. The biogas was produced at a slow rate in POT and PCT treatment due to less availability of dissolved organic matter. Biological treatment (PCT) slightly increased the biogas production (46.4 %) in comparison to untreated OFMSW. The POT and PCT resulted in 266 and 303 NmL/gVS biogas, respectively.

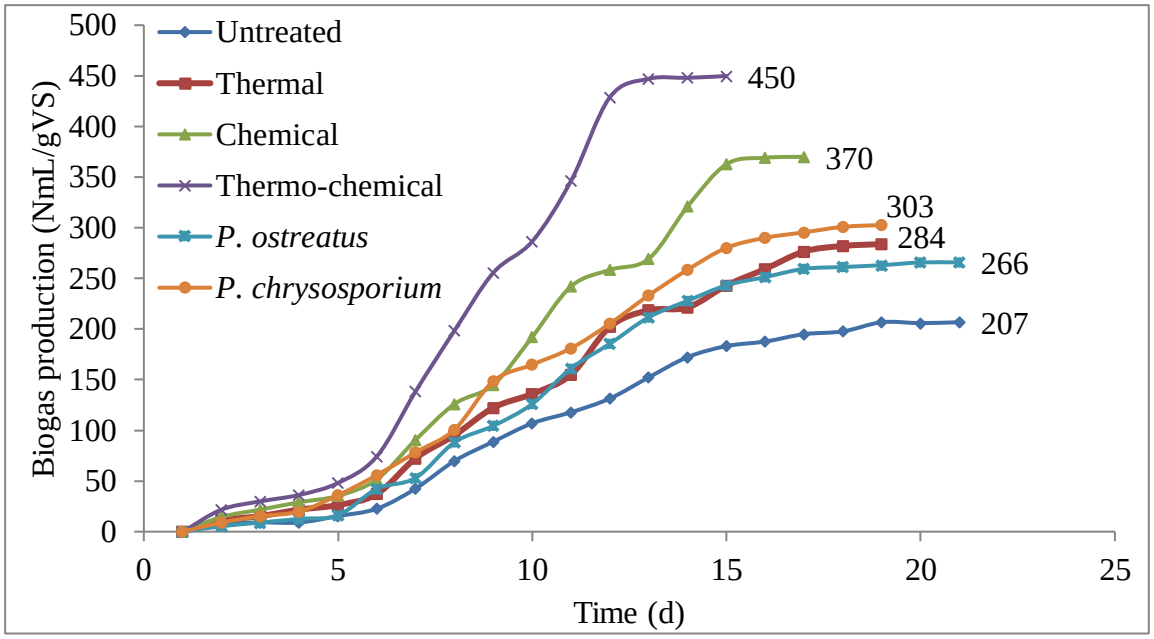


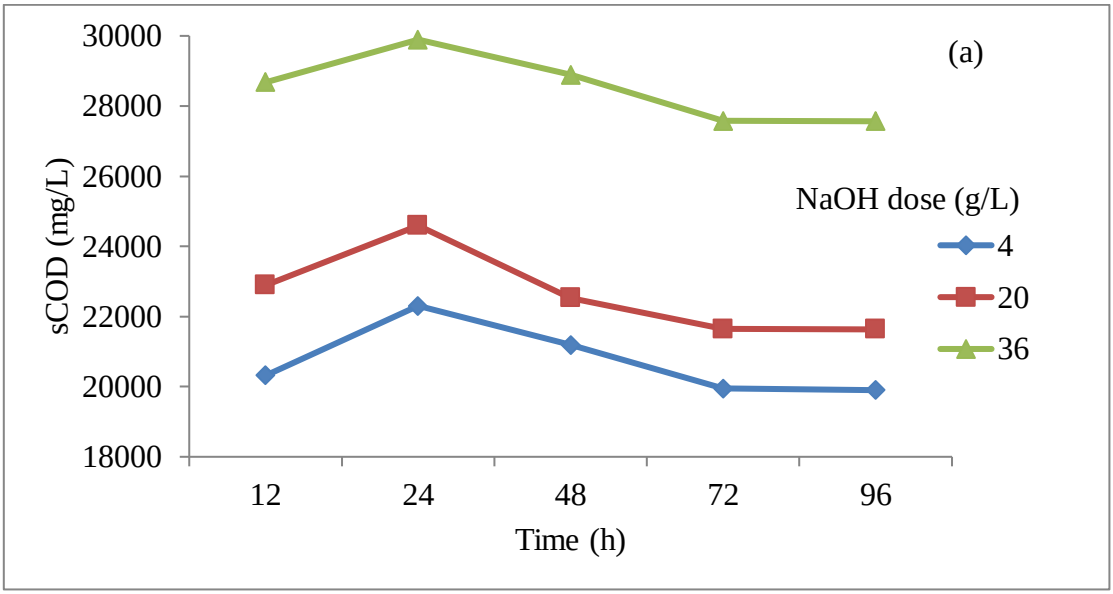
Figure 4.14 Biogas production from different pretreatments of OFMSW

4.6 Optimisation of chemical treatment of OFMSW by RSM

4.6.1 Solubilisation characteristics of NaOH treated OFMSW samples

The NaOH treated sample was analyzed for VFA and sCOD content in it. As, represented from Figure 4.15 (a), sCOD value was significantly increased upto 24 h in all cases. The sCOD value was also found to increase with increasing NaOH concentration. During hydrolysis stage, complex compounds like carbohydrate, lipid, and protein break down into monomoric sugars and other simpler compounds resulting to increase the sCOD value (Liu et al., 2018). The sCOD value started to decrease after 24 h and became almost constant after 72 h. There was negligible change in sCOD value from 72 to 96 h interval indicating the termination of hydrolysis process. Simpler compounds obtained in hydrolysis process are further converted into VFA. Therefore, the diminution of sCOD could be associated with the formation of VFA. On the other

hand, the VFA content was increased with increasing both time and concentration of NaOH as illustrated in Figure 4.15 (b).



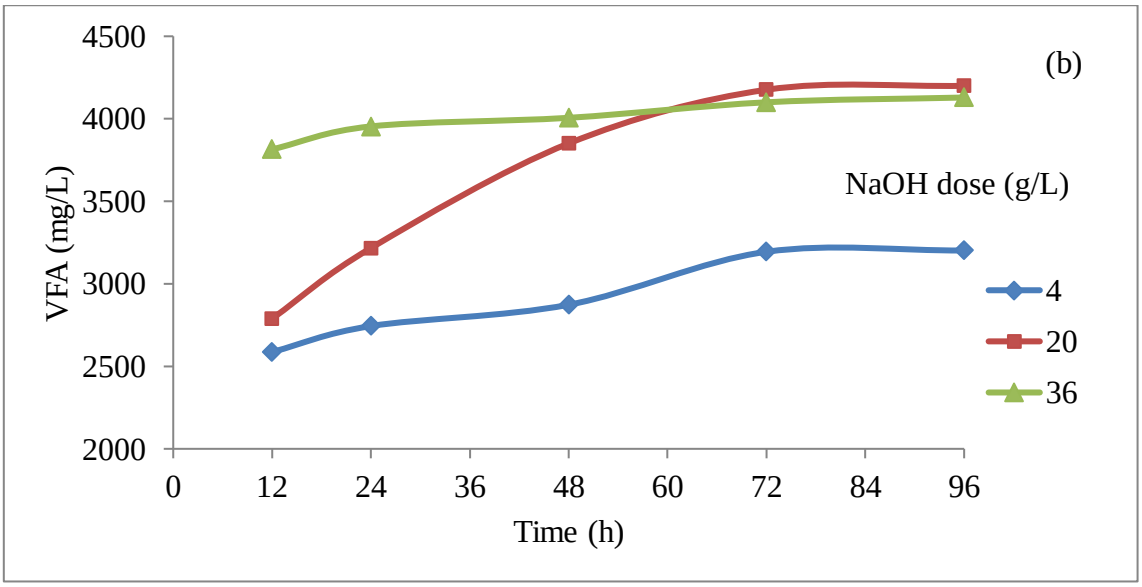


Figure 4.15 Hydrolysis of liquidised OFMSW slurry at different NaOH concentrations for (a) sCOD and (b) VFA estimation with time

4.6.2 Box-behnken design and statistical analysis of chemical treatment of OFMSW

State-Ease Design Expert 7.0 software was used for the design and statistical analysis of hydrolysis process parameters. VFAs are the substrate utilized by methanogens for biogas production. The combination of maximisation of VFA and minimisation of sCOD was considered as the optimum response of the process. Therefore, effect of NaOH dose (A), time (B) and temperature (C) was observed on sCOD value and VFA content. A BBD design suggested 17 experimental trials considering all three influencing parameters. The summary of effect of influencing factors on response is given in Table 4.11. Comparison of predicted and actual value of responses were carried out (Figure 4.16 (a) and (b)). The value of coefficient of determination (R^2) for both (VFA and sCOD) was 0.986 that indicated that regression analysis was adequate and the data fitted well with model.

4.6.3 Analysis of variance (ANOVA) for chemical treatment of OFMSW

Analysis of variance (ANOVA) was performed to evaluate the significance of the developed model (ANOVA of VFA and sCOD are represented in Table 4.12, respectively). The P value (also called Prob > F) less than 0.05 indicated that the model is statistically acceptable (Torres and Llorens, 2008). In the case of VFA, A, B, C, A^2 and C^2 were significant terms. The Predicted R-squared of 0.7915 was in reasonable agreement with Adjacent R-squared of 0.9695. In the case of sCOD, A, B C and A^2 were significant terms. The Predicted R-squared of 0.7826 was in reasonable agreement of Adjacent R-squared of 0.9689. The Adequate precision measures the signal to noise ratio. A ratio of greater than 4 is desirable. Here, the ratio was 23.898 that signified the adequate signal and suggested that model can be used to navigate the design space (Saha et al., 2018). The coefficient of variation (CV_r) is the measure of precision and

reproducibility of model. Its value was calculated as 2.34 and 2.32 for VFA and sCOD, respectively. The lower value (<10) of CV_r indicated that the model is highly reliable (Saha et al., 2018).

Table 4.11 Box-Behnken design matrix and response yield for chemical treatment

Run	NaOH concentration (g/L)	Time (h)	Temperature (℃)	VFA (mg CH_3COOH/L)	sCOD (mg/L)
1	4	48	20	2,659	22,139
2	4	24	30	2,744	22,304
3	20	24	20	3,199	23,860
4	4	72	30	3,194	19,950
5	36	48	40	4,168	29,901
6	36	72	30	4,100	27,572
7	20	72	40	4,289	21,160
8	20	72	20	3,565	19,870
9	36	24	30	3,954	29,893
10	20	48	30	3,851	22,524
11	36	48	20	3,877	27,524
12	20	48	30	3,877	22,499
13	20	24	40	3,592	25,129
14	20	48	30	3,875	22,487
15	20	48	30	3,891	22,454
16	4	48	40	3,099	22,560
17	20	48	30	3,899	22,471

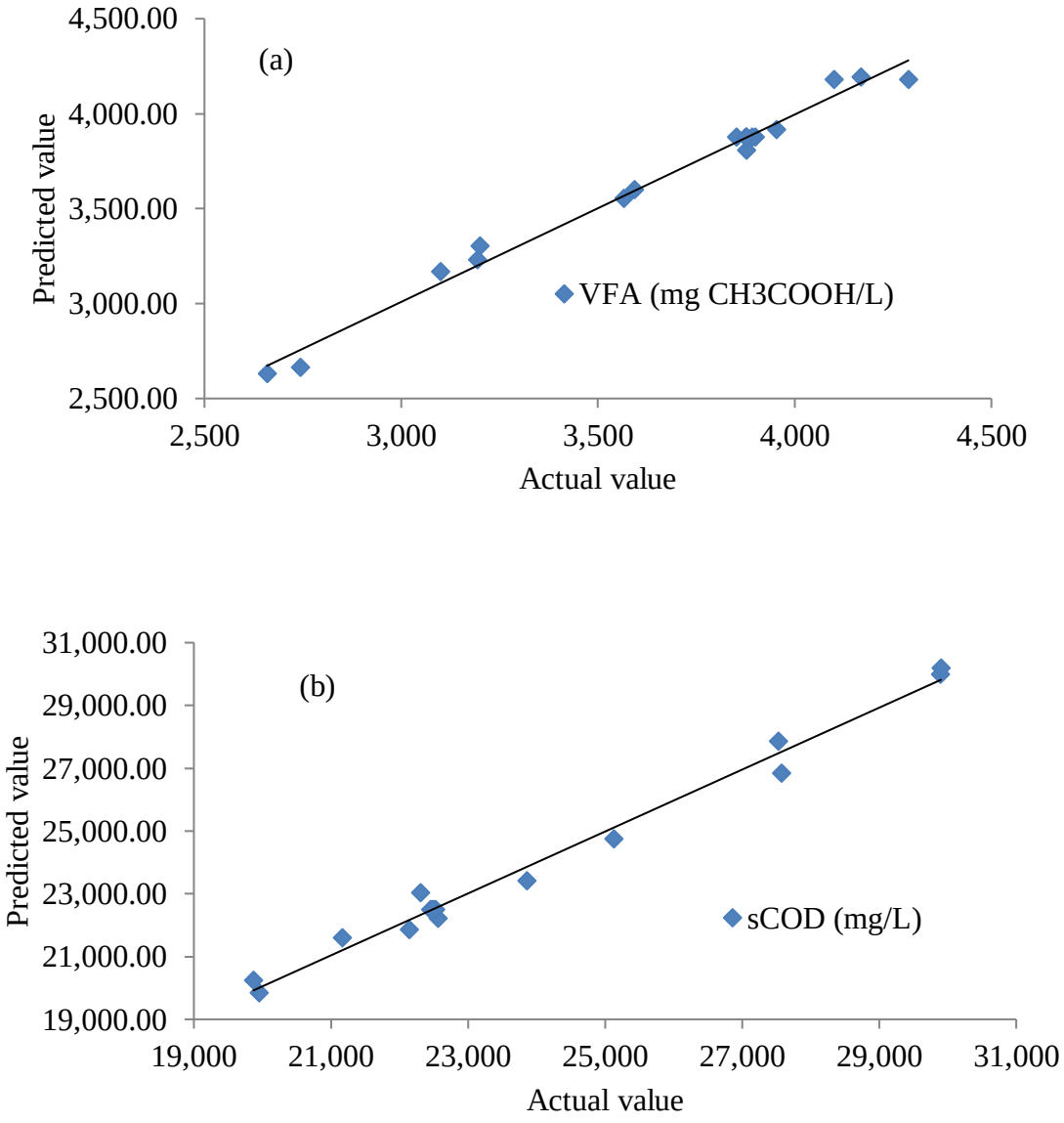


Figure 4.16 Actual vs. predicted values of response for (a) VFA and (b) sCOD chemically treated OFMSW

Table 4.12 ANOVA analysis of VFA and sCOD for chemical treatment

Source	Sum of Squares		df		Mean square		F Value		p-value	Prob > F	
Model	3.7E+06	(1.5E+09)	9	(9)	4.1E+05	(1.7E+07)	57.48	(56.40)	< 0.0001	(< 0.0001)	Significant
A-NaOH conc.	2.4E+06	(9.7E+07)	1	(1)	2.4E+06	(9.7E+07)	333.34	(320.62)	< 0.0001	(< 0.0001)	
B-Time	3.4E+05	(1.9E+07)	1	(1)	3.4E+05	(1.9E+07)	47.32	(65.57)	0.0002	(< 0.0001)	
C-Temp	4.2E+05	(3.5+06)	1	(1)	4.2E+05	(3.5E+06)	58.72	(11.79)	0.0001	(0.0109)	
AB	2.3E+04	(272.25)	1	(1)	2.3E+04	(272.25)	3.18	(0.01)	0.1178	(0.9770)	
AC	5.5E+03	(9.5E+05)	1	(1)	5.5E+03	(9.5E+05)	0.76	(3.14)	0.4112	(0.1195)	
BC	2.7E+04	(110.25)	1	(1)	2.7E+04	(110.25)	3.77	(0.01)	0.0934	(0.9853)	
A^2	3.6E+05	(3.1E+07)	1	(1)	3.6E+05	(3.1E+07)	50.59	(103.47)	0.0002	(< 0.0001)	
B^2	3.0E+04	(3.5E+05)	1	(1)	3.0E+04	(3.5E+05)	4.19	(1.18)	0.0799	(0.3138)	
C^2	7.3E+04	(4.0E+05)	1	(1)	7.3E+04	(4.0E+05)	10.14	(1.33)	0.0154	(0.2874)	
Residual	5.0E+04	(2.1E+06)	7	(7)	7.2E+03	(3.0E+05)					
Lack of Fit	4.9E+04	(2.1E+06)	3	(3)	1.6E+04	(7.0E+05)	49.03	(992.36)	0.0013	(<0.0001)	
Pure Error	1.3E+03	(2.8E+03)	4	(4)	336.8	(714.5)					
Cor Total	3.8E+06	(1.5E+09)	16	(16)							

R^2 = 0.9866 (0.9864) Adj. R^2 = 0.9695 (0.9689) Pred. R^2 = 0.7915 (0.7826) Adeq. Precision = 23.88 (24.42) CV_r = 2.34% (2.32%)

Quadratic model was suggested while cubic model was aliased by BBD design. Corresponding data correlations obtained are as follows:

$$VFA\ (\frac{mg}{L}) = 3{,}878.60 + 550.38\ A + 207.38\ B + 23{,}100\ C - 76.00\ AB - 37.25\ AC + 82.75\ BC - 295.55\ A^2 - 85.05\ B^2 - 132.30\ C^2 \quad (4.1)$$

$$sCOD\ (\frac{mg}{L}) = 22{,}487.00 + 3{,}492.13\ A - 1{,}579.25\ B + 669.63\ C + 8.25\ AB + 489.00\ AC + 5.25\ BC + 2{,}734.50\ A^2 - 291.75\ B^2 + 309.50\ C^2 \quad (4.2)$$

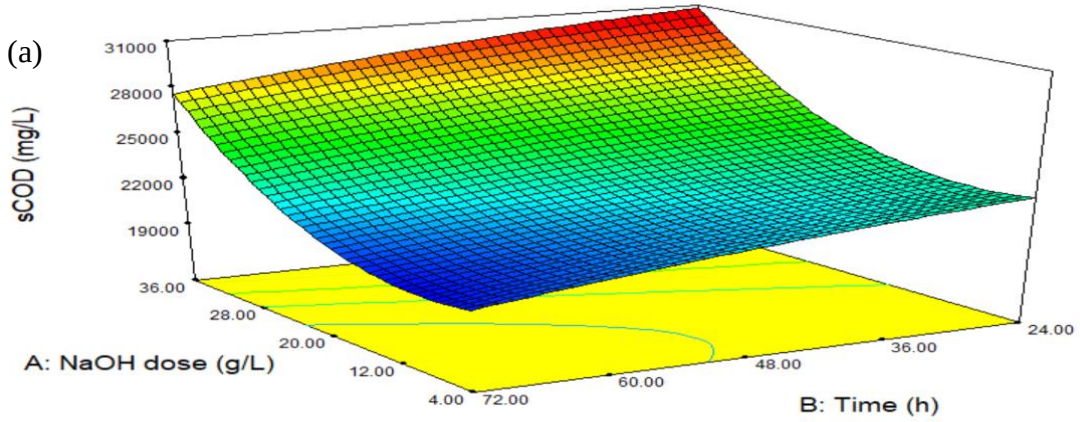
Where *A*, *B* and *C* symbolizes the coded values of NaOH dose, reaction time and temperature. The positive and negative sign in the model equation point towards the synergic and antagonistic effects.

4.6.4 Optimisation of pretreatment parameters for chemical treatment

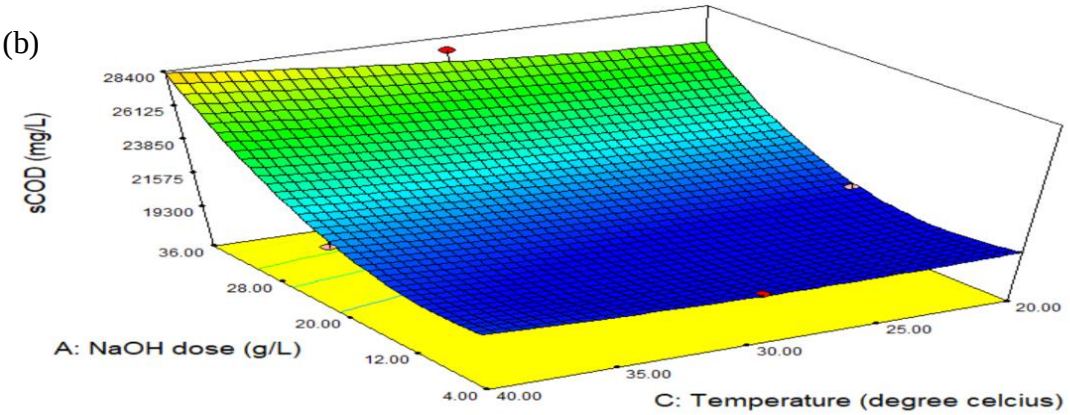
The interactive effect of three factors was observed by plotting 3D contour varying independent factors and keeping one factor constant at a time. The influence of NaOH loading and time on sCOD at the constant temperature of 36.05 ℃ is represented in Figure 4.17 (a). The sCOD content was increased with NaOH concentration (*A*) and reduced with time (*B*). NaOH increment might have caused the disorganization of cell wall structure of lignocellulosic waste present in OFMSW sample and caused breakdown of lignin-carbohydrate linkages, conversion of other complex matter into smaller compounds and hence resulted into increment of sCOD value. The declined value of sCOD with time is due to the formation of VFAs that is in accordance with results obtained from influence of same factors on VFA as shown in Figure 4.17 (d). The interactive effect of temperature and concentration for sCOD is shown in Figure 4.17 (b) at time 72 h. The sCOD was slightly increased with temperature. It might be due to increase in temperature responsible for enhanced the rate of hydrolysis. VFA concentration was also found to increase according to increasing temperature, as

shown in Figure 4.17(e).

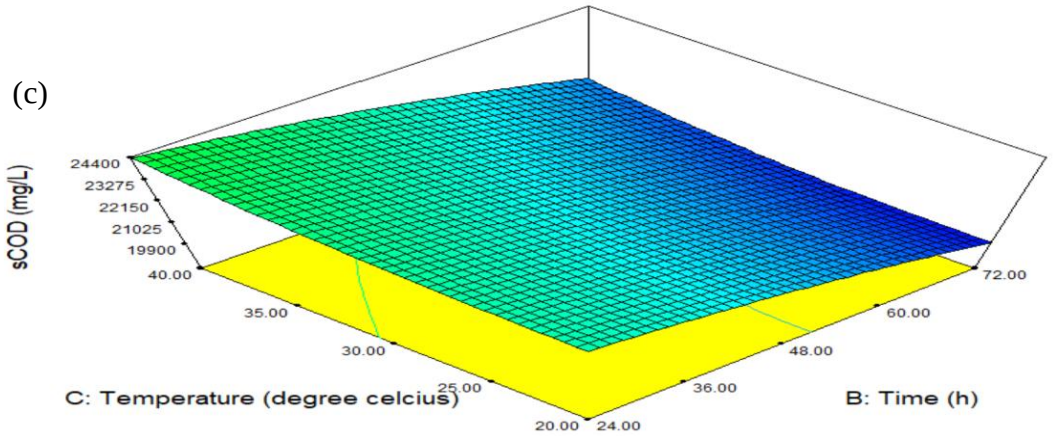
(a)



(b)



(c)



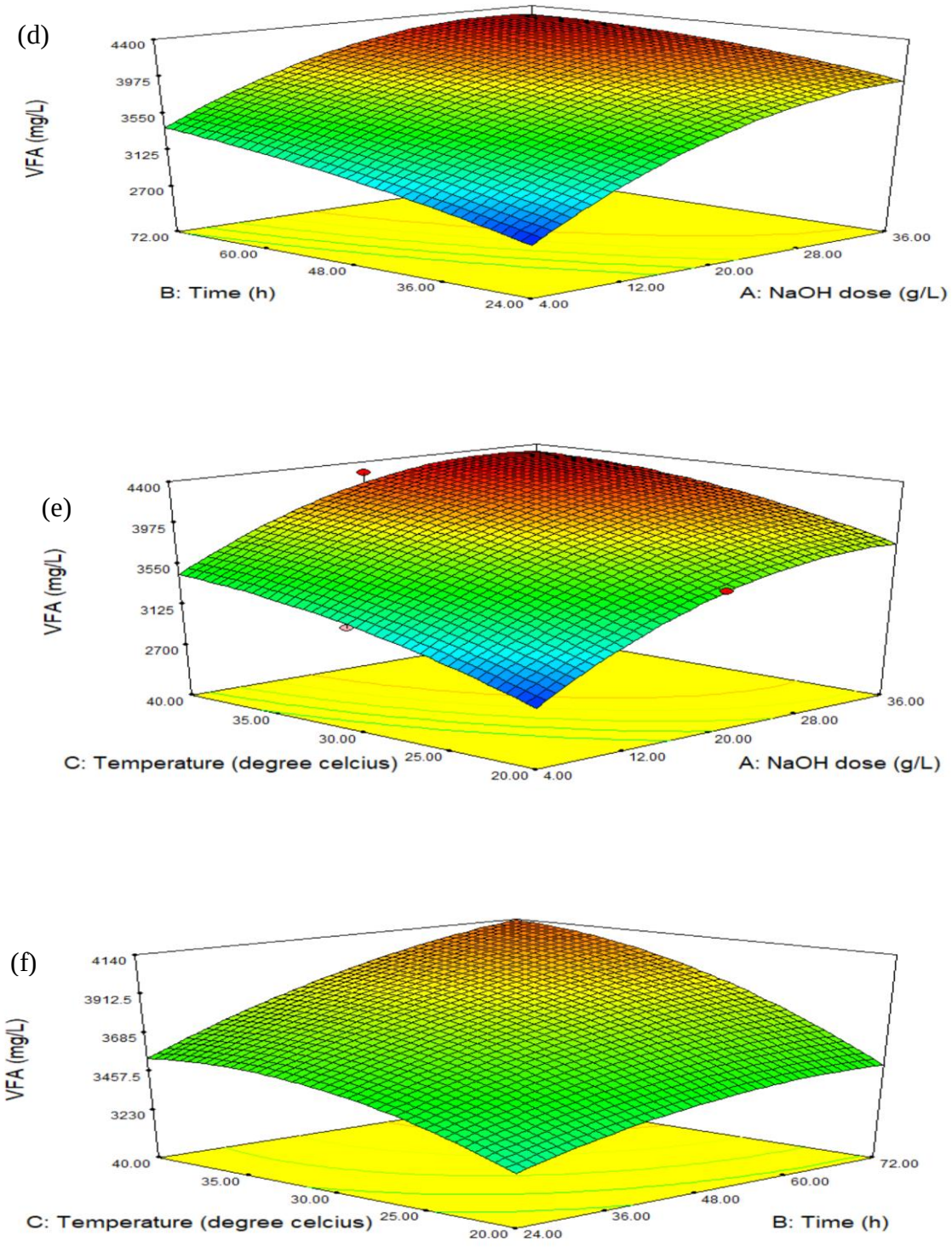


Figure 4.17 3D surface response for sCOD (a-c) and VFA (d-f) yield: (a) and (d) NaOH concentration vs. time, (b) and (e) NaOH concentration vs. temperature, c and (f) time vs. temperature of chemically treated OFMSW

The 3D contour represented in Figure 4.17 (c) indicated the significant dependency of sCOD with time. It was decreased with time whereas the plot in Figure 4.17 (f) signified the increment in VFA content with time. The optimum condition obtained from RSM-BBD design for maximum solubilisation of OFMSW was NaOH dose 18.41 g/L, reaction time 72 h and temperature 36.05 ℃.

4.6.5 RSM model validation for chemical treatment of OFMSW

Optimisation of NaOH pretreatment for maximum solubilisation of OFMSW was performed by BBD design of RSM software. The optimal values of influencing factors obtained from predicted model were examined experimentally. The predicted values of response were in good agreement of experimental value indicating the adequacy and validity of RSM model (Table 4.13). There was only 1.63 % error in VFA yield and 1.35 % error in sCOD yield. The deviation of experimental result from predicted yield was very less suggesting adequacy of the model for hydrolysis process.

Table 4.13 RSM model validation for chemical treatment of OFMSW

	NaOH concentration (g/L)	Time (h)	Temperature (℃)	VFA (mg CH_3COOH/L)	sCOD (mg/L)
Predicted	18.41	72	36.05	4,094.33	20,786
Experimental	18.40	72	36	4,161	21,066

4.7 Experimental biogas evaluation

The observed experimental evaluation of biogas production from the anaerobic digestion of OFMSW hydrolyzed with different NaOH concentrations in batch reactor

is provided in Table 4.14. The cumulative biogas production for liquidised OFMSW hydrolyzed using 4, 20 and 36 g/L NaOH is shown in Figure 4.18. Amount of biogas generated after 20 days from NaOH hydrolysed slurry at 4, 20 and 36 g/L was 369.24, 435.24 and 327.84 NmL/gVS, respectively. During the first five days the amount of biogas production was almost independent of the concentration of NaOH, but later on the biogas generation from 20 g/L NaOH hydrolyzed sample occurs at higher rate as compared to biogas production from 4 g/L NaOH hydrolyzed sample. This is because with increase in NaOH concentration, breakdown of lignin and other complex materials occurs easily into smaller degradable volatile fractions which boost the biogas production. Low production of biogas at high concentration (36 g/L) might be due to the hindrance of bacterial growth by absorption of excessive sodium ions (Zhang et al., 2017). It is also reported in literature that at high NaOH concentration degradation of sugars takes place that might have reduced the yield of biogas (Jönsson and Martín, 2016). Also, phenolic toxicity might be the reason of microbial growth inhibition. The high NaOH doses lead to formation of high content of phenolic compounds from the degradation of lignin present in lignocellulosic portion of OFMSW.

OFMSW slurry after NaOH hydrolysis at optimum condition (NaOH dose 18.41 g/L, time 72 h and temperature 36.05 ℃) obtained from RSM analysis was followed by anaerobic digestion to examine the biogas production yield (Figure 4.18). Biogas production was in accordance with the hydrolysis results obtained at optimum pretreatment conditions. Biogas production rate increased continuously with some fluctuation up to 6 days of digestion period. It was then decreased and became almost constant after 13 days. The cumulative biogas yield obtained was 465.67 NmL/gVS after 20 days of digestion period.

4.8 Kinetic model evaluation for chemically treated OFMSW

4.8.1 First order model

First order model is used to fit the experimental data obtained by batch anaerobic digestion of liquidised OFMSW slurry hydrolyzed by NaOH. Plots between the logarithmic values of daily production of biogas versus time were plotted for different concentrations of NaOH using equation (3.20) mentioned in the chapter material and methods (Figure 4.19). A linear regression analysis is performed using microsoft excel 2010 version to obtain a straight line for the plotted data. The model parameters obtained are given in Table 4.15. The *k* values were found in the range of 0.143-0.182 d^{-1}. In a study of anaerobic digestion of food waste *k* values were obtained in the range of 0.056-0.364 d^{-1} (Browne and Murphy, 2013). Difference in *k* values may be due to different characteristics of substrates. The obtained model parameters are used to predict the cumulative biogas produced theoretically.

4.8.2 Modified Gompertz model

The theoretical prediction of the amount of biogas produced by batch anaerobic digestion of liquidised OFMSW slurry hydrolyzed by NaOH using modified Gompertz model is given by equation (3.21). The parameters such as the maximum biogas production (M_m), maximum biogas production rate (R_m) and lag phase period (λ) are obtained from experimental results. The values of the parameters are given in Table 4.15. The R_m values were found in the range of 42.13-55.45 N mL/gVS.d. The R_m values were recorded in the range of 0.62-24.47 for co-digestion study of activated sludge, anaerobic granular sludge, cow dung and food waste (Gaur and Suthar, 2017). The R_m value was more in present study because of better biodegradability of OFMSW sample studied in comparison to above mentioned case. The obtained parameters are used to predict the theoretical biogas produced cumulatively.

Table 4.14 Biogas produced from experimental results and model predictions of chemically treated OFMSW

Biogas yield (NmL/gVS)									
Time (d)	Experimental			First order model			Modified Gompertz model		
	NaOH concentration (g/L)								
	4	20	36	4	20	36	4	20	36
1	22.85	23.61	24.54	64.57	65.32	62.89	6.19	10.78	6.94
2	46.72	47.49	54.52	118.78	121.94	115.31	24.37	28.72	21.63
3	82.91	83.94	89.02	164.28	171.02	159.01	60.59	59.01	48.21
4	118.59	120.39	124.91	202.48	213.56	195.44	111.04	100.20	84.84
5	149.39	161.72	161.93	234.54	250.42	225.81	166.10	147.86	126.38
6	204.84	211	204.06	261.46	282.38	251.12	217.10	196.83	167.39
7	250.53	253.87	232.62	284.06	310.08	272.23	259.39	242.88	204.08
8	271.84	282.87	251.56	303.03	334.08	289.82	291.98	283.47	234.69
9	298.02	307.51	269.73	318.95	354.90	304.48	315.88	317.58	258.99
10	318.82	329.07	282.93	332.32	372.93	316.70	332.85	345.23	277.63
11	338.58	354.74	292.94	343.54	388.56	326.89	344.63	367.09	291.58
12	343.46	380.67	301.46	352.96	402.11	335.39	352.69	384.03	301.83
13	349.88	389.91	309	360.86	413.86	342.47	358.15	396.98	309.28
14	354.5	399.67	315.01	367.50	424.03	348.37	361.83	406.78	314.64
15	359.64	411.22	319.84	373.07	432.86	353.29	364.30	414.13	318.47
16	363.38	420.46	323.07	377.75	440.50	357.39	365.95	419.62	321.21
17	366.06	426.97	325.28	381.68	447.13	360.81	367.05	423.70	323.15
18	367.96	431.13	326.77	384.97	452.88	363.66	367.78	426.73	324.52
19	368.83	433.85	327.49	387.74	457.86	366.04	368.27	428.97	325.50
20	369.24	435.24	327.84	390.06	462.17	368.01	368.59	430.62	326.19

Table 4.15 Estimation of kinetic model's parameters for chemically treated OFMSW

Model	Parameters	NaOH concentration (N)			
		4	20	36	Optimised
First order	M_m (NmL/gVS)	402.21	490.25	377.94	539.70
	k (d^{-1})	0.175	0.143	0.182	0.145
Modified Gompertz model	M_m (NmL/gVS)	369.24	435.24	327.84	465.67
	R_m (NmL/gVS.d)	55.45	49.28	42.13	46.56
	λ (d)	2	2	2	2

4.8.3 Kinetic model comparison

The experimental and theoretical cumulative biogas yield for batch anaerobic digestion of liquidised OFMSW slurry hydrolyzed by 4, 20 and 36 g/L NaOH is shown in Table 4.14. The comparison of graphical results of experimental and theoretical methods is shown in Figure 4.18 (b). Root mean square error analysis was conducted to evaluate the compatibility of the theoretical biogas produced cumulatively by two different, first order and modified Gompertz models with the experimental ones by using equation (3.25). The theoretical result obtained by the first order model fitted the experimental data with an error of 0.6177, 0.6256 and 0.5019 for samples hydrolyzed by 4, 20 and 36 g/L NaOH, respectively. On the other hand the theoretical results obtained by the modified Gompertz model fitted the experimental data with an error of 0.2082, 0.1713 and 0.2543 for samples hydrolyzed by 4, 20 and 36 g/L NaOH, respectively.

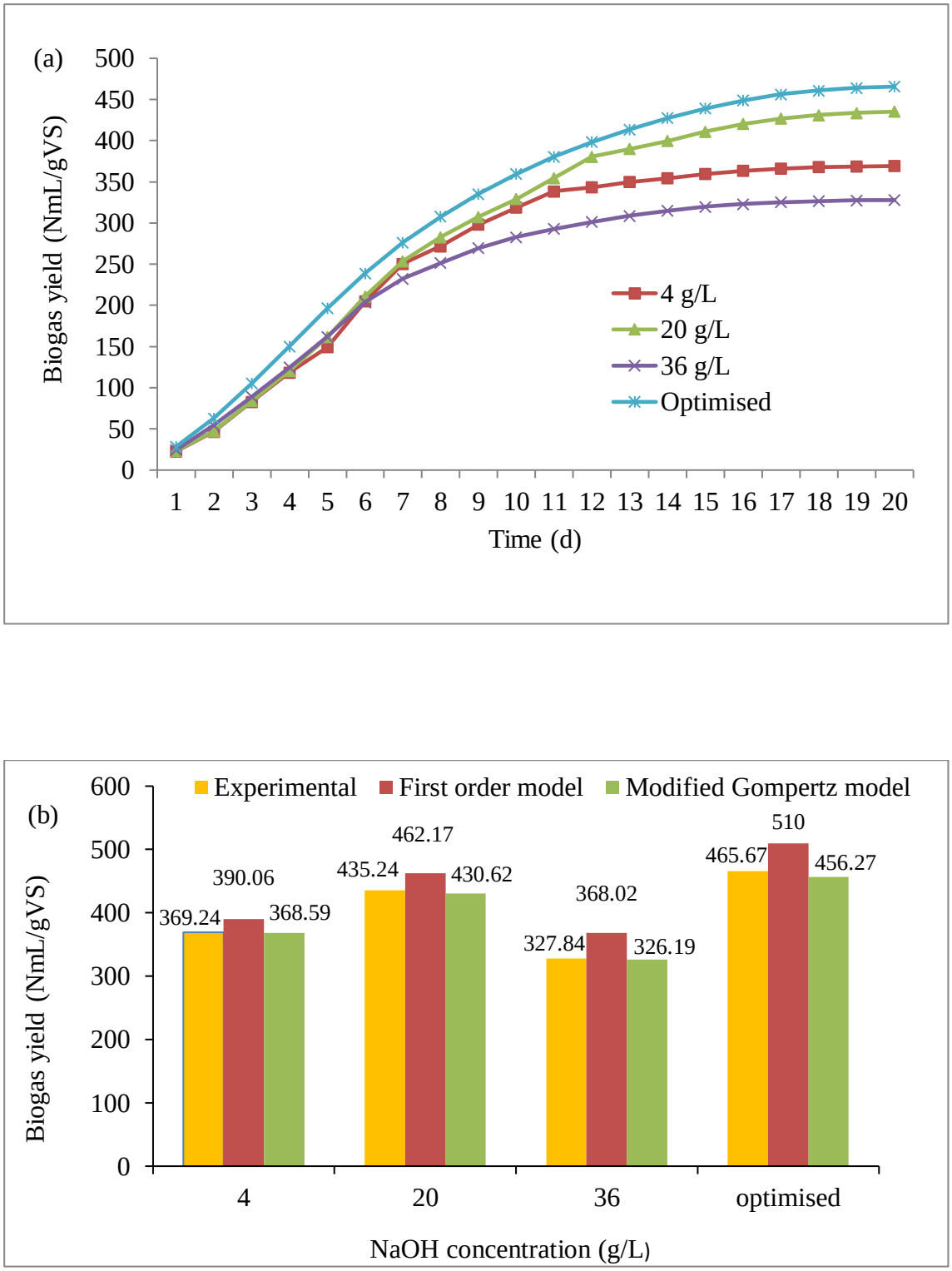


Figure 4.18 (a) Biogas yield at different NaOH concentration (b) comparison between experimental and theoretical biogas produced at 4, 20, 36 g/L and optimised condition of NaOH for chemically treated OFMSW

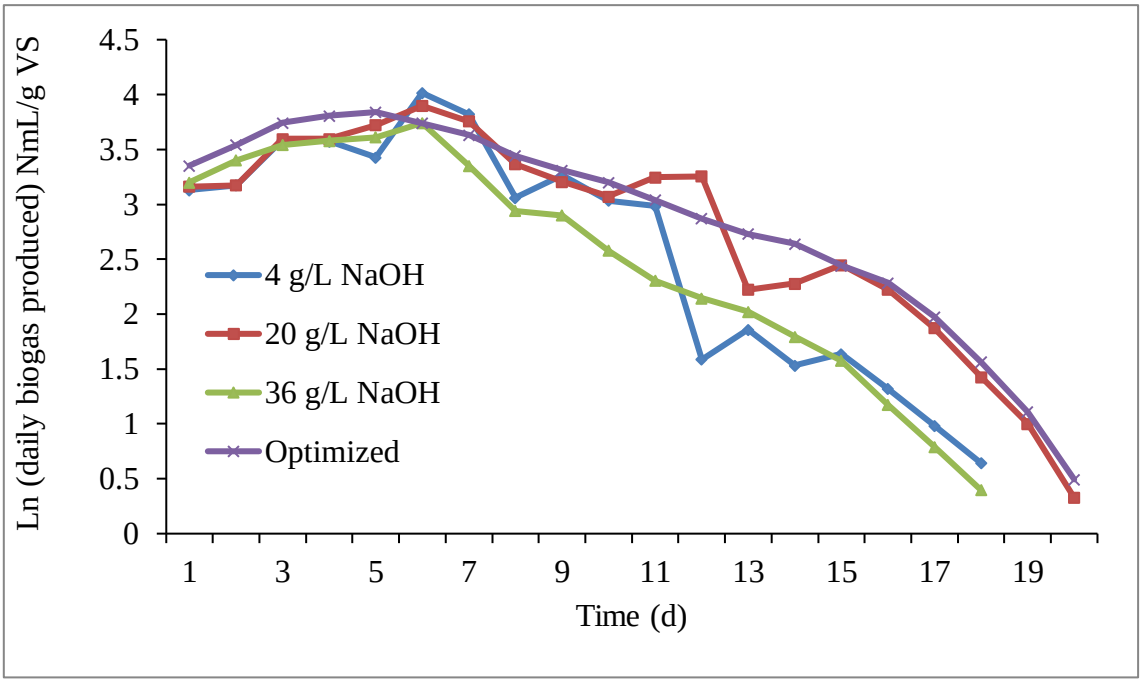


Figure 4.19 Logarithmic values of biogas production vs. time of operation for 4, 20, 36 and optimised 18.4 g/L NaOH treated OFMSW sample.

The obtained results are in accordance with the results of Kafle and Kim, (2013), where first order and modified Gompertz models were used for the kinetic analysis of anaerobic digestion of apple waste added to swine manure. First order model and modified Gompertz model were also applied on biogas production of optimised hydrolysed OFMSW slurry. Parameters of first order and modified Gompertz model are given in Table 4.15. The modified Gompertz model was found to be fitted well than first order model with the RMSE 0.2305 while the latter model had RMSE 0.5127.

4.9 Optimisation of thermo-chemical treatment of OFMSW

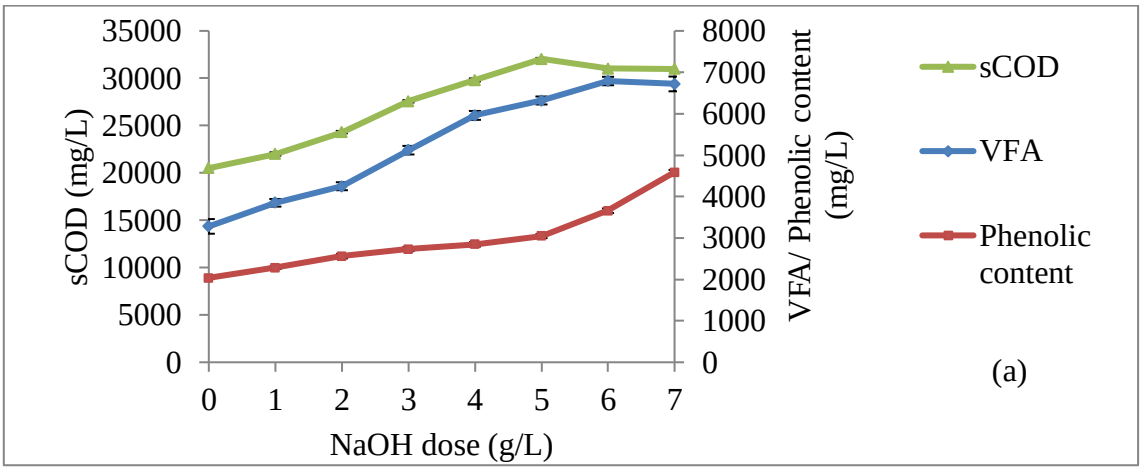
4.9.1 Effect of thermo-chemical treatment on OFMSW solubilisation

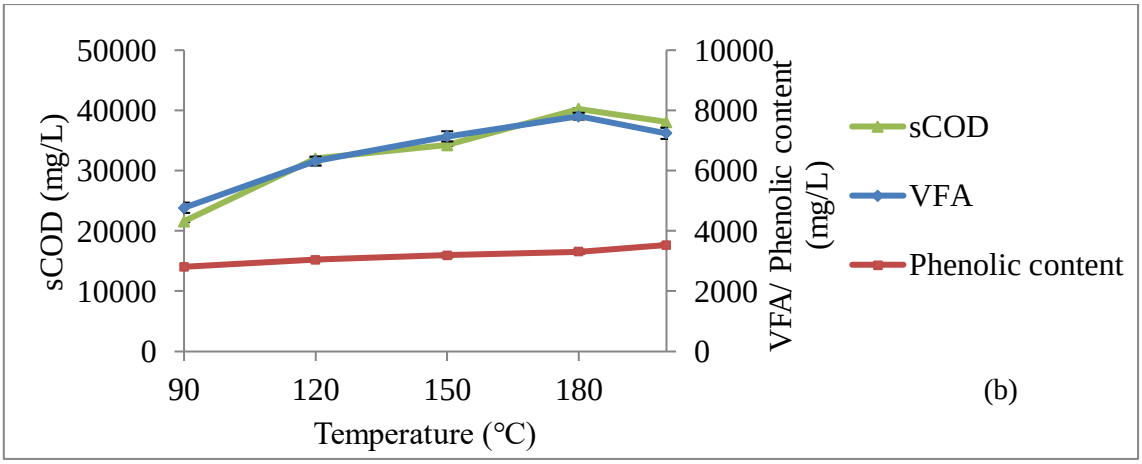
The NaOH dose significantly increased the sCOD, VFA and phenolic content, as shown in Figure 4.20 (a). The sCOD and VFA values were found to be approximately constant after 6 g/L NaOH dose. NaOH and thermal treatment carried out the conversion of

complex compounds to simpler one by changing the surface structure and dissolving the cell membrane. Elevated sCOD and VFA yield signified the effect of pretreatment. NaOH performed saponification and solvation reaction causing uronic acid substitution on the side chains of hemicelluloses and cleavage of main ether bonds in lignin, ester and glycosidic bonds in cellulose, hemicellulose and lignin (Xu et al., 2016). The occurrence of other reactions in alkaline pretreatment are break down of C-C bond, condensation and methylation. The C-C bond is very stable when present between aryl-aryl groups; it is breakable in aryl-alkyl or alkyl-alkyl and can reduce the size of the lignin molecule (Xu et al., 2016). Degradation of lignin led to the production of phenolic by-products. Therefore, the phenolic content was increased by raising the NaOH concentration.

Beside the cleavage of lignocellulosic complex, NaOH might have caused denaturation of protein into small peptides and amino acids. In alkaline medium, the stability of the tertiary structure of protein might have decreased that led to break down of intermolecular hydrogen bonding. The hydrolysis of the peptide bond might happen due to the presence of high temperature and NaOH. Also, the lipids are converted into fatty acids during pretreatment.

The temperature was also found to have an important role in solubilising the OFMSW slurry. The sCOD and VFA yields were increased up to 180 °C, and then it was slightly decreased (Figure 4.20 (b)). The peeling and hydrolysis reactions of NaOH takes place at high temperature (>140 °C) that causes swelling of the substrate and degrade carbohydrates into small sugar moieties. The reduction of sCOD and VFA beyond 180 °C is associated with thermal decomposition and caramelisation process at high temperature that can result in sugar degradation and less solubilisation yield (Alvarez-Gallego et al., 2015).





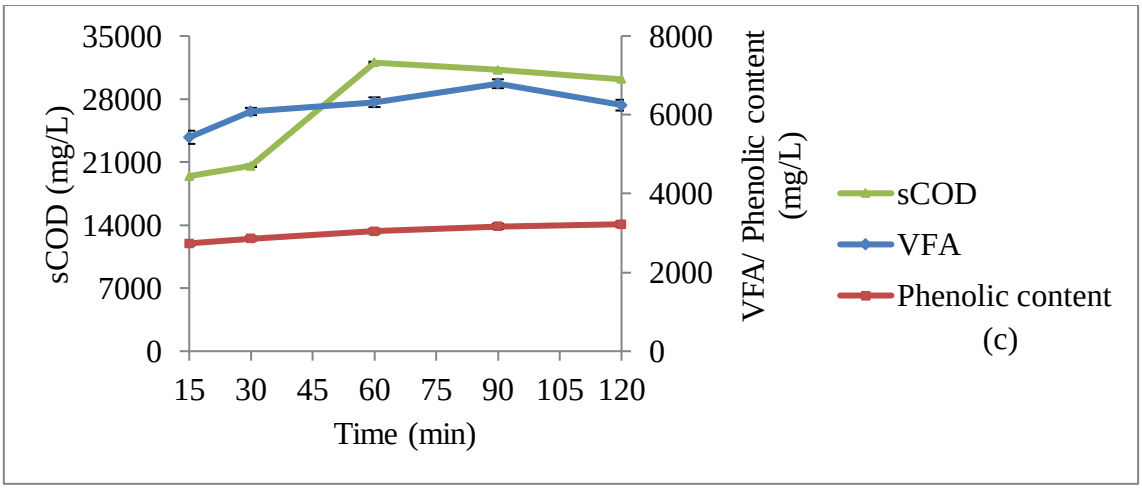


Figure 4.20 Effect of thermo-chemical treatment (a) NaOH dose (b) temperature and (c) time on OFMSW solubilisation in terms of sCOD, VFA and phenolic content

Effect of the time period of treatment is represented in Figure 4.20 (c). The decrease of sCOD and VFA occurred at higher time period due to reduced efficiency of NaOH at longer times. The phenolic compounds increased slightly concerning NaOH concentration, temperature and time period.

Solubilisation yield of OFMSW was better in thermo-chemical treatment in comparison to biological treatment. Therefore, thermo-chemical treatment was optimised for better results. The effect of pretreatment factors on solubilisation parameters was concluded, and their narrow range was decided for RSM optimisation. The range selected was NaOH dose (3-6 g/L), temperature (150-180 °C) and time (30-90 min).

4.9.2 Box-Behnken design for thermo-chemical treatment of OFMSW

sCOD and VFA are important parameters to evaluate the pretreatment effect. During the hydrolysis process, the conversion of complex compounds into simpler molecules takes place that raises the sCOD and VFA concentration. These molecules are utilised by microorganisms for their metabolic needs and biogas formation. Therefore, increased solubilisation yield can be associated with increased sCOD and VFA values. Also, the increased solubilisation of lignin causes the formation of phenolic compounds into hydrolysate. Phenolic compounds are considered as inhibitory to microbial growth (Wirth et al., 2015). Therefore, to optimise the thermo-chemical treatment sCOD, VFA and phenolic compounds were taken under consideration.

The Box-Behnken design was generated by considering NaOH dose, pretreatment temperature and time as potential influencing factors with the help of Design-Expert version 7.0 software. Maximisation of sCOD and VFA yields and minimisation of phenolic compounds were taken as an optimal response. Summary of obtained responses according to the 17 experiments suggested by BBD design are depicted in

Table 4.16. The experiments were done in duplicates, and the average value was reported in the design matrix.

Table 4.16 Box-Behnken design matrix for thermo-chemical treatment of OFMSW

Run	C: NaOH dose (g/L)	T: Temperature (°C)	t: Time (min)	sCOD (mg/L)	VFA (mg/L)	Phenolic content (mg/L)
1	4.5	180	30	38965	8635	3276
2	4.5	165	60	33780	8143	3547
3	4.5	150	30	19205	7021	2914
4	6	165	90	40310	8657	4747
5	4.5	165	60	31010	8010	3512
6	4.5	180	90	35210	8367	3798
7	3	165	90	38704	6787	3750
8	3	150	60	35208	5423	3012
9	3	165	30	28620	6315	3171
10	3	180	60	43530	6511	3213
11	4.5	165	60	29880	8078	3572
12	6	180	60	47048	8816	4256
13	4.5	165	60	29930	7987	3505
14	6	150	60	41320	6416	3714
15	4.5	165	60	28990	7915	3576
16	6	165	30	35000	8451	4275
17	4.5	150	90	34230	7260	3284

4.9.3 Model fitting and ANOVA analysis

Experimental data was fitted into a quadratic model. C (NaOH dose), T (temperature) and t (time) are the coded influencing factors of pretreatment. The correlation equations for responses obtained were:

$$sCOD\ \left(\frac{mg}{L}\right) = 30718 + 2202\ C + 4348.75\ T + 3333\ t - 648.5\ CT - 1193.5\ Ct - 4695\ Tt + 7407.25\ C^2 + 3651.25\ T^2 - 2466.7\ t^2 \quad (4.3)$$

$$VFA\ \left(\frac{mg}{L}\right) = 8026.6 + 913\ C + 776.13\ T + 81.13\ t + 328\ CT - 66.5\ Ct - 126.75\ Tt - 751.68\ C^2 - 483.43\ T^2 + 277.58\ t^2 \quad (4.4)$$

$$Phenolic\ content\ \left(\frac{mg}{L}\right) = 3542.4 + 480.75\ C + 202.38\ T + 242.88\ t + 85.25\ CT - 26.75\ Ct + 38\ Tt + 337.05\ C^2 - 330.7\ T^2 + 106.3\ t^2 \quad (4.5)$$

Model adequacy was checked by ANOVA analysis. The value of regression coefficients, R^2 for sCOD, VFA, and phenolic content were 0.9693, 0.9857 and 0.993, respectively (Table 4.17). The high R^2 values signified the adequate model fitting. Also, a model is considered as significant if its P value is less than 0.05 (Saha et al., 2018). The P value for all the responses was less than 0.05, indicating the statistical acceptance of the model. The F-value is the test comparing the source's mean square to the residual mean square. The F-values for sCOD, VFA and phenolic content were 24.55, 53.53 and 110.33, respectively. The significant terms obtained for sCOD were C, T, t, Tt, C^2, T^2 and t^2. Values greater than 0.1 considered as non-significant terms. C, T, CT, C^2, T^2 and t^2 were the significant terms for VFA and C, T, t, CT, C^2, T^2 and t^2 were the significant terms for phenolic content. The value of predicted R^2 was in good agreement with adjacent R^2 for all the responses. The values of predicted R^2 for sCOD, VFA and phenolic content were 0.7884, 0.7984 and 0.9052 and the values of adjacent R^2 were

0.9298, 0.9673 and 0.9840, respectively. Adequate precision indicates the signal to noise ratio. Its value greater than 4 is desirable (Zhang et al., 2011). The value of adequate precision 20.787, 24.344 and 38.230 for sCOD, VFA and phenolic content, respectively indicated the model adequacy. The CV_r value is the standard deviation expressed as a percentage of the mean. Its value less than 10 % is considered significant. The CV_r value for sCOD, VFA and phenolic content was 5.08, 2.39 and 1.68, respectively signified the model accuracy.

4.9.4 Effect of influencing factors on OFMSW solubilisation

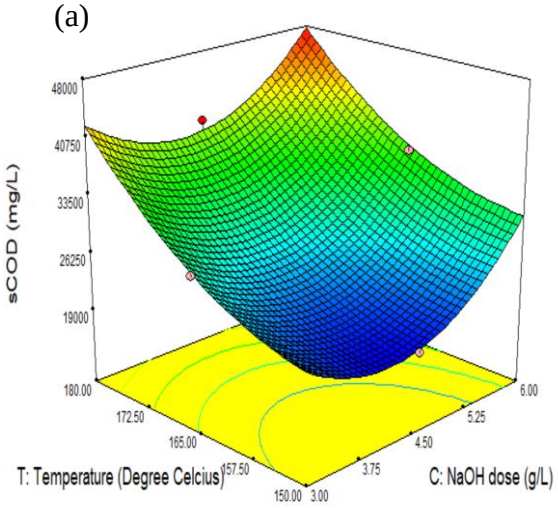
The interacting effect of three variables NaOH dose, temperature and time were studied on multiple responses (sCOD, VFA and phenolic content) using RSM methodology. The 3-D contours were generated to compare the effect of factors and optimise the pretreatment conditions. Figure 4.21 is representing the effect of influencing factors on response. The sCOD, VFA and phenolic content yields were increased with respect to NaOH dose and time. The rise in NaOH dose and temperature induced the break-down of complex compounds raising sCOD, VFA and phenolic content. The interacting effect of temperature and time on responses is represented in Figure 4.21 (d), (e) and (f). The sCOD and VFA values were found to be more dependent on temperature in comparison to time. The sCOD and VFA content was increased up to 60 min and then further increment in time reduced their yield. The low phenolic content was desirable, and it was found at treatment done at low temperature for less time period. The interacting effect of NaOH dose and time is shown in Figure 4.21(g), (h) and (i). The sCOD value was found maximum in two zones (Fig 4.21 (g)), i.e. it was increased for low NaOH dose and the increased time period and in the second case at high NaOH dose and less time period. Increasing the time period at high concentration might have induced the

formation of VFA and other compounds that resulted in less sCOD yield. VFA and phenolic content were increased by increasing the NaOH dose and time period.

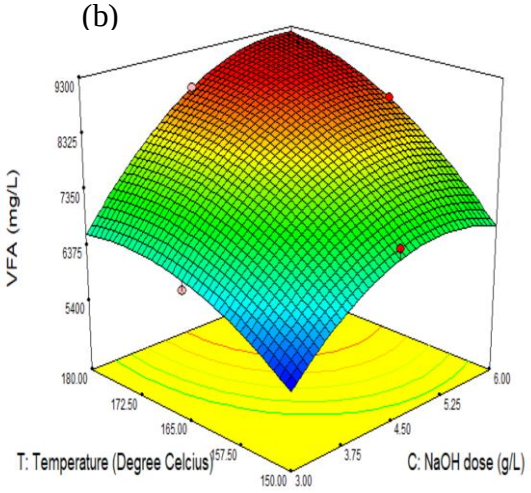
Table 4.17 Model fitting and ANOVA analysis of thermo-chemical treatment

ANOVA parameter	sCOD	VFA	Phenolic content
Model suggested	Quadratic	Quadratic	Quadratic
F value	24.55	53.53	110.33
p-value	0.0002	0.0001	0.0001
Significant terms	C, T, t, CT, C^2, T^2, t^2	C, T, CT, C^2, T^2, t^2	C, T, t, CT, C^2, T^2, t^2
R-squared	0.9693	0.9857	0.9930
Adj R-squared	0.9298	0.9673	0.9840
Pred R-squared	0.7884	0.7984	0.9052
CV_r	5.08	2.39	1.68

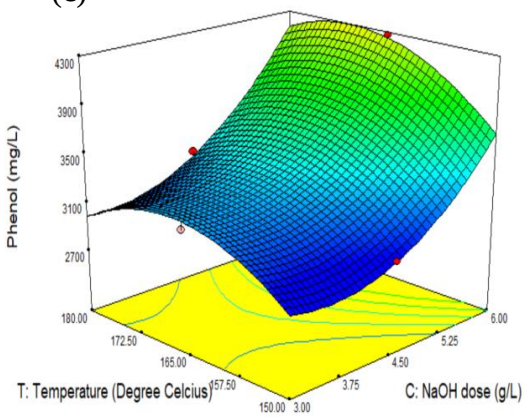
(a)


sCOD (mg/L)
48000
40750
33500
26250
19000
180.00
172.50
165.00
157.50
150.00
T: Temperature (Degree Celcius)
3.00
3.75
4.50
5.25
6.00
C: NaOH dose (g/L)

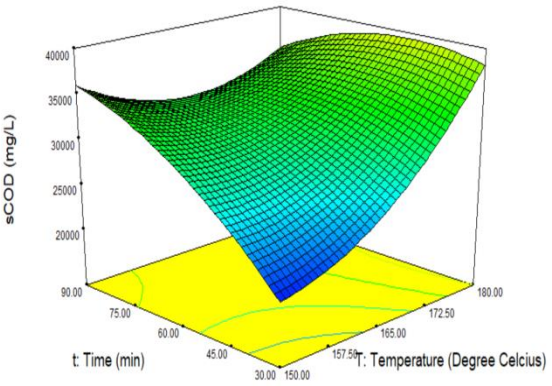
(b)


VFA (mg/L)
9300
8325
7350
6375
5400
180.00
172.50
165.00
157.50
150.00
T: Temperature (Degree Celcius)
3.00
3.75
4.50
5.25
6.00
C: NaOH dose (g/L)

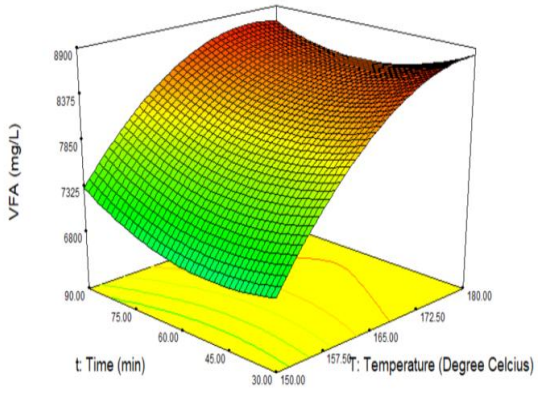
(c)


Phenol (mg/L)
4300
3900
3500
3100
2700
180.00
172.50
165.00
157.50
150.00
T: Temperature (Degree Celcius)
3.00
3.75
4.50
5.25
6.00
C: NaOH dose (g/L)

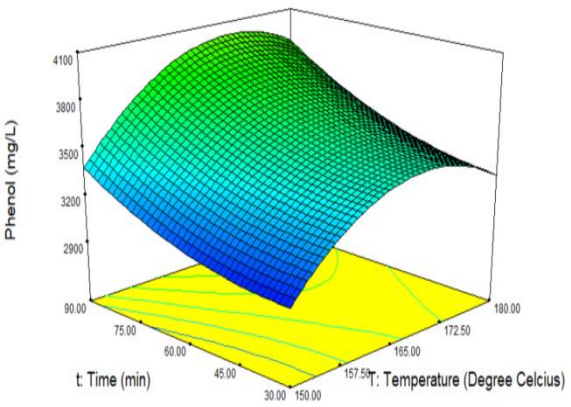
(d)


sCOD (mg/L)
40000
35000
30000
25000
20000
90.00
75.00
60.00
45.00
30.00
t: Time (min)
150.00
157.50
165.00
172.50
180.00
T: Temperature (Degree Celcius)

(e)


VFA (mg/L)
8900
8375
7850
7325
6800
90.00
75.00
60.00
45.00
30.00
t: Time (min)
150.00
157.50
165.00
172.50
180.00
T: Temperature (Degree Celcius)

(f)


Phenol (mg/L)
4100
3800
3500
3200
2900
90.00
75.00
60.00
45.00
30.00
t: Time (min)
150.00
157.50
165.00
172.50
180.00
T: Temperature (Degree Celcius)

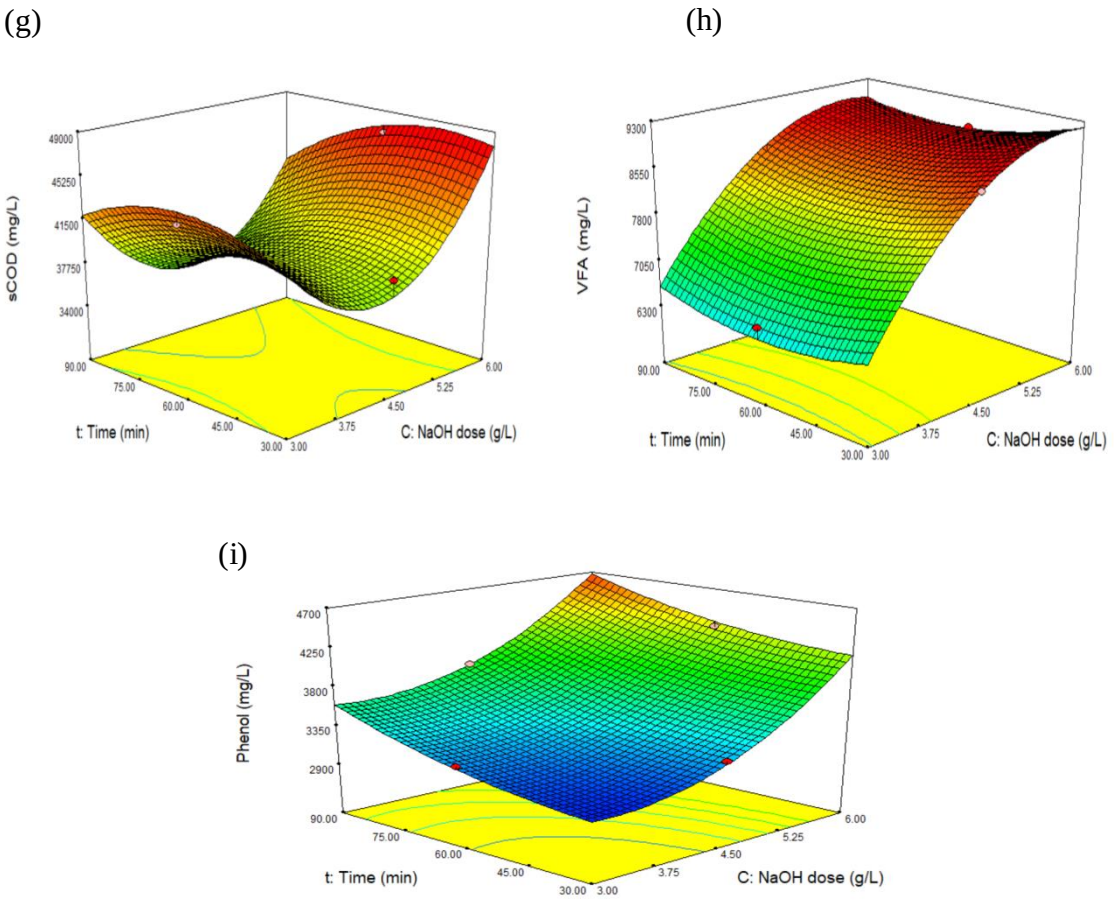


Figure 4.21 RSM optimisation of thermo-chemical treatment of OFMSW, (a), (b), (c) effect of NaOH dose and temperature, (d), (e), (f) effect of temperature and time and (g), (h), (i) effect of NaOH dose and time on sCOD, VFA and phenolic content

4.9.5 Model validation for thermo-chemically treated OFMSW

The optimised condition for thermo-chemical treatment of OFMSW using RSM was NaOH dose: 4.72, temperature: 180°C and time: 30.3 min. The experiment at this condition was done in duplicates to verify the accuracy of the model. The experimental results were compared with predicted values of responses, and the error % is summarised in Table 4.18. The model was fitted with an error of 0.6, 1 and 0.7 % for sCOD, VFA and phenolic content, respectively.

Table 4.18 RSM model validation of thermo-chemical treatment

	NaOH dose (g/L)	Temperature (°C)	Time (min)	sCOD (mg/L)	VFA (mg/L)	Phenolic content (mg/L)
Predicted	4.72	180	30.3	38221.6	8816	3336.46
Experimental	4.72	180	30	38451	8728	3360
Error (%)	-	-	-	0.6	1	0.7

4.10 Anaerobic digestion and kinetics of thermo-chemical treatment of OFMSW

4.10.1 Biogas production of thermo-chemically treated OFMSW

The results of biogas production are represented in Figure 4.22. The results of biogas production are in accordance with the solubilisation of OFMSW. The presence of lignin in OFMSW makes the microorganism inaccessible to the substrate. Thermo-chemical treatment at optimised condition (OTCT) resulted in an increment of 149.5 % biogas production as compared to untreated OFMSW. Thermo-chemical treatment (TCT) resulted in efficient solubilisation of organic matter present in OFMSW in terms of sCOD, VFA and phenolic content and significantly augmented the biogas production rate and cumulative biogas yield. The biogas production occurred with the fast rate in the first 10 days of digestion period in treated samples. The digestion period of anaerobic digestion of thermo-chemically treated samples was also reduced due to break down of complex material during the pretreatment step. The methanogenesis rate is dependent on the hydrolysis of the substrate. Pretreatment caused the dissolution of organic matter and increased the accessibility of microorganisms towards the substrate

that resulted in better substrate utilisation and better biogas yield. The cumulative biogas production from untreated OFMSW was 212 N ml/g VS. Biogas production from TCT, and OTCT samples resulted in 417 and 529 N ml/g VS biogas, respectively. The biogas production was increased by more than 50 % of control at 170 °C with 4 g NaOH/100g MSW (Wang et al., 2009). Methane concentration was found in the range of 66-70 % for thermo-chemically treated sample.

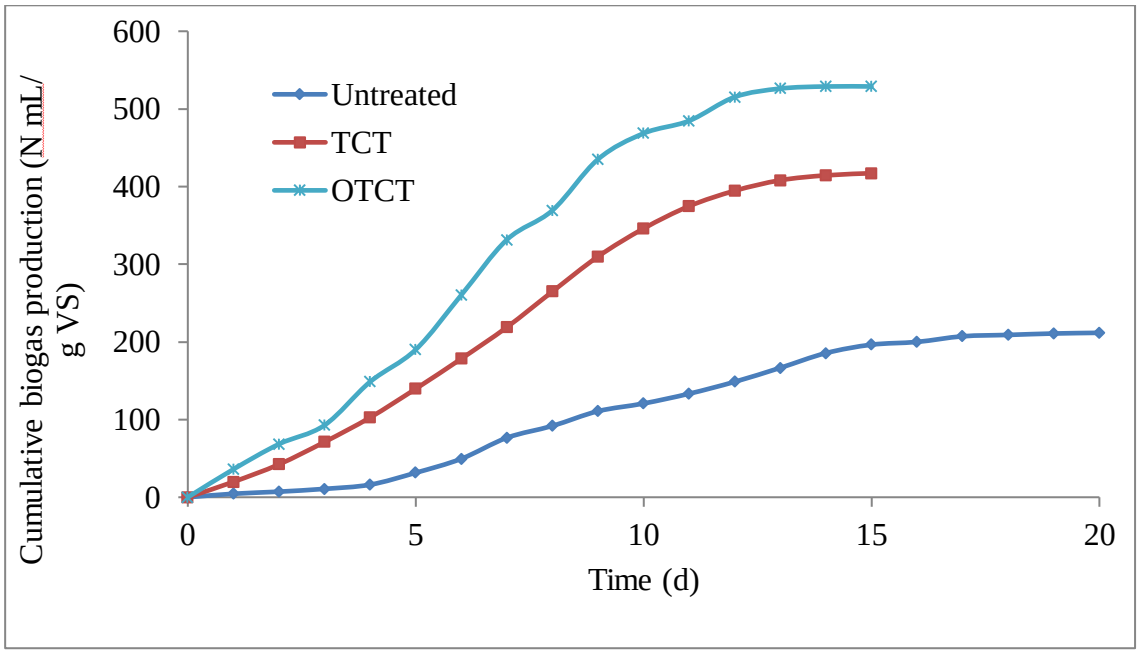


Figure 4.22 Biogas production from treated and untreated OFMSW

4.10.2 Kinetic modelling of thermo-chemical treatment

The first-order and modified Gompertz model was applied on anaerobic digestion of optimised thermo-chemical treatment. The kinetic parameters are depicted in Table 4.19. The value of M_m and rate constant was 0.127 d^{-1} and 670.87 N mL/g VS. The cumulative biogas from first order model and modified Gompertz model was 557.50

and 495.84 N mL/g VS, respectively. The Gompertz model was fitted well than first order model with a less RMSE of 0.1383.

Table 4.19 Kinetic modelling of anaerobic digestion at optimised thermo-chemical pretreatment of OFMSW

Model	Parameters	Value	Cumulative biogas yield (N mL/g VS)
Experimental	-	-	529
First order	M_m (N mL/g VS)	670.87	
	k (d^{-1})	0.127	557.50
	RMSE	0.3119	
Modified Gompertz model	M_m (N mL/g VS)	529	
	R_m (N mL/g VS.d)	55.94	495.84
	λ (d)	1	
	RMSE	0.1383	

4.11 Co-digestion Results

4.11.1 Physico-chemical characterisation of activated sewage sludge

The activated sewage sludge (ASS) contained moisture content of 96.4 %. The total solid and volatile solid were 3.6 and 2.55 %, respectively (Table 4.20). The pH of the sludge was 7.4. The carbon and nitrogen content found as 41.61 and 7.88 %,

respectively. The C/N ratio was 4.6. The sewage sludge should digest with other co-substrate to balance the C/N ratio for process stability. The combination of OFMSW and sewage sludge proved to be the efficient substrate for biogas production in literature review. The high C/N ratio of OFMSW and low C/N ratio of sewage sludge has been maintained by various researchers to increase the biogas production.

Table 4.20 Physico-chemical characterisation of sewage sludge

Parameter	Value
TS (%)	3.6±0.4
VS (%)	2.55±0.3
Moisture (%)	96.4±1.4
Ash (%)	0.94±0.2
pH	7.4±0.2
C (%)	41.61±3.6
H (%)	7.88±1.3
N (%)	9.1±0.5

4.11.2 C/N ratio selection for co-digestion

The C/N ratio was determined by mixing the OFMSW and ASS in different proportions. The C/N ratio near to 25 is considered as optimum for anaerobic digestion (Shetty et al., 2017). The C/N ratio was found to decrease with increasing the ASS concentration. It was found in the range of 24.2-9.8 by increasing the ASS amount in the range 20-70 %, respectively. The 4/1 ratio was selected for further experiments as it

contained the C/N ratio of 24.2. C/N ratios of different mixture of OFMSW/SS are represented in Table 4.21.

Table 4.21 C/N ratio of different OFMSW-ASS mixture

OFMSW/ASS ratio	C/N ratio
4/1	24.2
7/3	21.5
3/2	18.7
1/1	14.1
2/3	11.4
3/7	9.8

4.11.3 RSM optimisation of process parameter of co-digestion

The process parameters of co-digestion were optimised by RSM for enhanced biogas production. The influencing parameters selected were substrate to inoculum ratio (S/I ratio), pH and temperature of anaerobic digester. The response was determined on biogas production. The pH and temperature crucially affect the microbial growth and biogas yield (Calicioglu et al., 2018). The S/I ratio is the food to microbe ratio that also essentially affects the biogas production. The efficient range of S/I ratio of 0.5-2 g VS $_{substrate}$/g VS $_{inoculum}$ has been reported by many researchers (Raposo et al., 2009; Kawai et al., 2014; Zhang et al., 2019; Vats et al., 2019). Many researchers worked on optimisation of S/I ratio for anaerobic digestion of different wastes. Also, the temperature was found to be effective in mesophilic (35-40 ℃) and thermophilic (45-70 ℃) ranges (Komemoto et al., 2009; Tian et al., 2018; Watanabe et al., 2017; Kim et al.,

2017). pH was found to be effective in the range of 6.5-7.5 for anaerobic digestion of different waste (Begum et al., 2018; Mao et al., 2017). A narrow range of S/I ratio (0.3-1.5), pH (6.5-7.5) and temperature (30-60 ℃) was determined according to the literature study.

4.11.4 Box-Behnken design of co-digestion

A box-Behnken design was generated by considering three influencing factors S/I ratio (0.3-1.5), pH (6.5-7.5) and temperature (30-60 ℃) and cumulative biogas production as response. Total 17 experiments with five central replicates were given by BBD design. The experiments were performed and summarised in BBD matrix as shown in Table 4.22.

4.11.5 Statistical analysis by ANOVA for co-digestion

ANOVA analysis was carried out for analysing the adequacy of model fitting and computing the significance. The model suggested was quadratic model. The ANOVA terms and their respective values are depicted in Table 4.23. The model was significant with p-value less than 0.0001 and coefficient of regression (R^2) 0.9758. The predicted R^2 was in good agreement with adjacent R^2. The significant terms were B, A^2, B^2 and C^2. The CV_r value was less than 10 % that demonstrated the adequacy of model. The data correlation is as follows:

$$Biogas\ yield\ (N\ mL/g\ VS) = 608.6 - 7.25\ A + 12.75\ B - 8.75\ C + 2.25\ AB - 1.75\ AC + 4.75\ BC - 33.92\ A^2 - 43.92\ B^2 - 57.43\ C^2 \quad (4.6)$$

Where, A, B, C are S/I ratio (gVS/gVS), pH and temperature (℃), respectively.

Table 4.22 Box-Behenken design for optimisation of co-digestion process parameters

Run	S/I ratio (g VS/ g VS)	pH	Temperature (°C)	Biogas (N ml/g VS)
1	1.5	7.5	45	537
2	0.3	7	60	510
3	0.9	7	45	595
4	1.5	6.5	45	507
5	0.3	7	30	536
6	0.9	7.5	30	518
7	0.9	7	45	601
8	0.9	7	45	608
9	0.9	6.5	30	502
10	0.3	6.5	45	529
11	0.9	7	45	617
12	0.9	7	45	622
13	0.9	7.5	60	522
14	0.3	7.5	45	550
15	1.5	7	60	495
16	1.5	7	30	528
17	0.9	6.5	60	487

Table 4.23 ANOVA Analysis of co-digestion

ANOVA parameter	Value
Model suggested	Quadratic
F value	31.41
p-value	<0.0001
Significant terms	B, A^2, B^2 and C^2
R-squared	0.9758
Adj R-squared	0.9448
Pred R-squared	0.8287
CV_r (%)	1.96

4.11.6 3D plots and optimisation for co-digestion

The 3D plots were generated to study the interactive effect of S/I ratio, pH and temperature on biogas production and condition of optimisation was achieved for maximum biogas yield. The all factors significantly affected the biogas yield. It can be concluded from the 3 D plot shown in Figure 4.23 (a) that biogas production is more pH dependent than S/I ratio. The biogas production was increased by increasing both S/I ratio and pH but to a certain limit. The cumulative biogas yield was decreased after further increasing the S/I ratio and pH. The low and very high pH inhibited the methanogens growth and hence caused less biogas yield. At low pH hydrolytic activities increases that results in high VFA production which inhibit the methanogen population (Mao et al., 2017)

The Fig 4.23 (b) depicted the interactive effect of temperature and S/I ratio on biogas production. The biogas production was increased by increasing the temperature to a certain limit and then followed a decreasing trend by further increment in temperature.

The S/I ratio also synergistically increased the biogas yield with temperature. The diminution of biogas at high temperature could be due to denaturation of proteins, enzymes etc required for microbial metabolism and hence inhibited their growth. The S/I ratio is associated with reversible and irreversible acidification. In the starting phase of anaerobic digestion accumulation of VFA occurs that reduces the pH. In methanogenesis step VFAs are consumed by methanogens to produce biogas. This is known as reversible acidification. In irreversible acidification consumption of acid is less than its production and hence causes inhibition of the process. By increasing the S/I ratio lag phase was increased due to accumulation of VFA and decrease in pH (Kawai et al., 2014; Yoon et al. 2014).

The third plot, depicted in Figure 4.23 is effect of temperature and pH on biogas production. The pH and temperature also significantly affect the biogas yield. By increasing the pH and temperature biogas yields were improved but very high pH and temperature found to be lethal to microbial growth and hence low production. The optimum condition achieved from RSM was S/I ratio 0.84 g VS/ g VS, pH 7.07 and temperature 43.97 ℃.

4.11.7 Model validation for co-digestion

The experiment was done in duplicates at the condition achieved by RSM. The predicted biogas at optimised condition was 610.15 N mL/g VS. The experimental production was 626 N mL/g VS. The model was found valid with the error of 2.55 % as shown in Table 4.24.

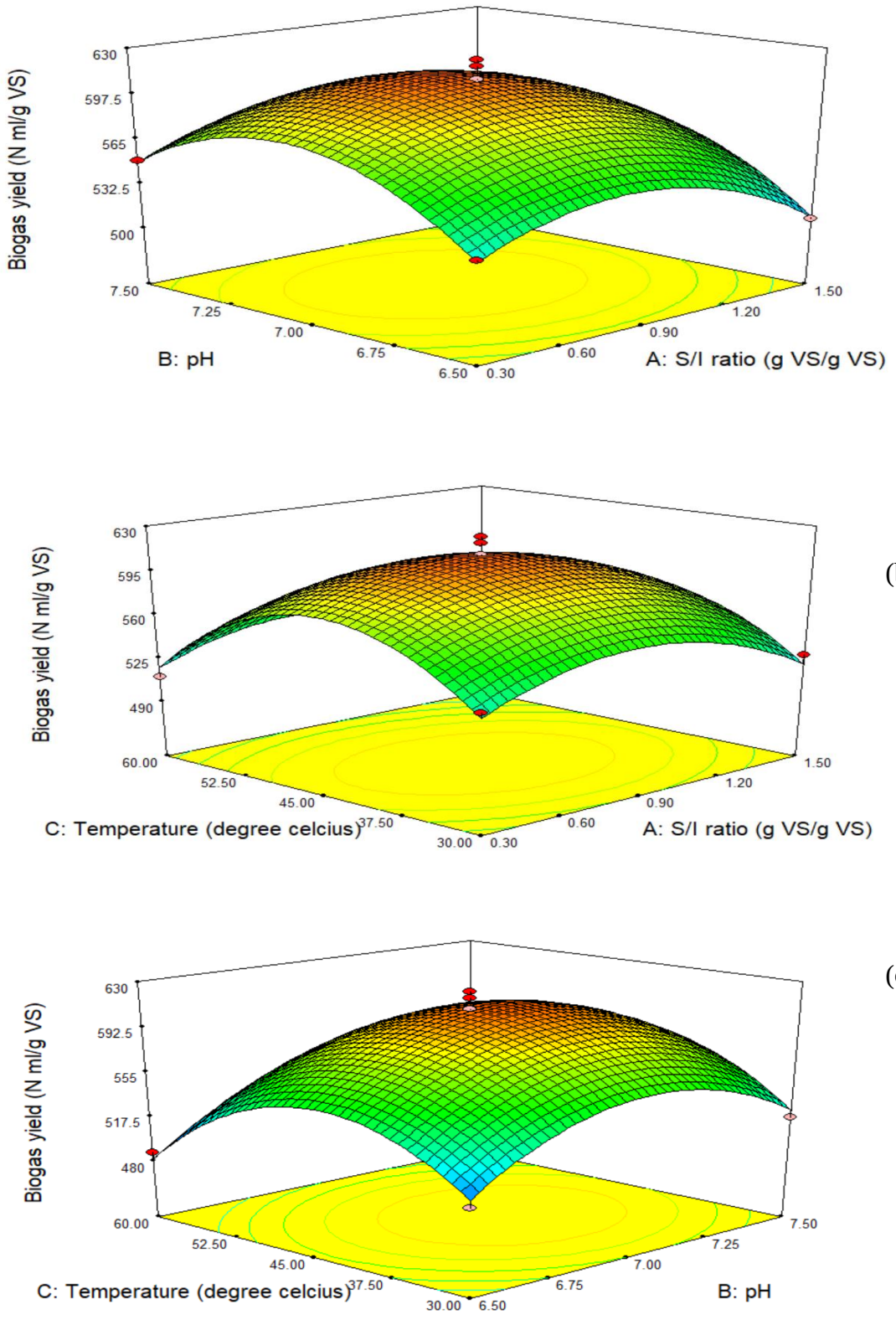


Figure 4.23 3D plots of co-digestion depicting the effect of influencing parameters on biogas production

Table 4.24 RSM model validation for co-digestion process parameter optimisation

	A: S/I ratio (gVS/gVS)	B: pH	C:Temperature (°C)	Biogas yield (NmL/gVS)
Predicted	0.84	7.07	43.97	610.15
Experimental	0.84	7.07	44	626
Error (%)	-	-	-	2.55

4.11.8 Characteristics of different digesters during anaerobic digestion

The working volume, lag phase, digestion time and biogas production were noted in all the digesters. The substrate was fixed and inoculum was varied to maintain the S/I ratio. The working volume was more in the digester with low S/I ratio. The lag phase was found to decrease by decreasing the S/I ratio and increasing the pH and temperature. The lag phase increased by increasing the S/I ratio because high S/I ratio lead to accumulation of VFA and drop in pH. Therefore, with decreased pH and increased S/I ratio increased the lag phase because of low consumption of VFA to produce biogas. The digestion time was decreased with increasing the temperature. The highest lag phase of 8 d was observed in the digester with S/I ratio 1.5, pH 6.5 and temperature 45 °C. The highest digestion time of 24 d was recorded for the digester with S/I ratio 0.9, pH 6.5 and temperature 30 °C as shown in Table 4.25.

Table 4.25 Characteristics of different digesters for co-digestion

Run	S/I ratio (g VS/ g VS)	pH	Temperature (°C)	Working volume (ml)	Lag phase (days)	Digestion time (days)	Biogas production (NmL/gVS)
1	1.5	7.5	45	196.3	7	21	537
2	0.3	7	60	415.5	1	13	510
3	0.9	7	45	236.2	4	18	595
4	1.5	6.5	45	196.3	8	22	507
5	0.3	7	30	196.3	2	20	536
6	0.9	7.5	30	236.2	5	23	518
7	0.9	6.5	30	236.2	6	24	502
8	0.3	6.5	45	196.3	2	16	529
9	0.9	7.5	60	236.2	4	16	522
10	0.3	7.5	45	415.5	1	15	550
11	1.5	7	60	196.3	6	18	495
12	1.5	7	30	196.3	7	25	528
13	0.9	6.5	60	236.2	5	20	487

4.11.9 Kinetic modelling of anaerobic co-digestion at optimised condition

The experimental data of co-digestion was fitted in First- order model and modified-Gompertz model. The RMSE was also measured to identify the best fit model. The value of kinetic parameters for each model is representing in Table 4.26. The plots were drawn in between the logarithmic value of daily biogas production vs. time (Figure 4.24) and value of k and M_m were calculated as 0.151 and 765.46, respectively. The value of cumulative biogas yield from First-order model was 657.96 NmL/gVS. The Figure 4.25 is depicting the comparison of experimental and model values.

The values of R_m and λ were 78.74 NmL/gVS and 3 days, respectively, noted from experimental result. The cumulative biogas production from modified Gompertz model was 572.70 NmL/gVS.

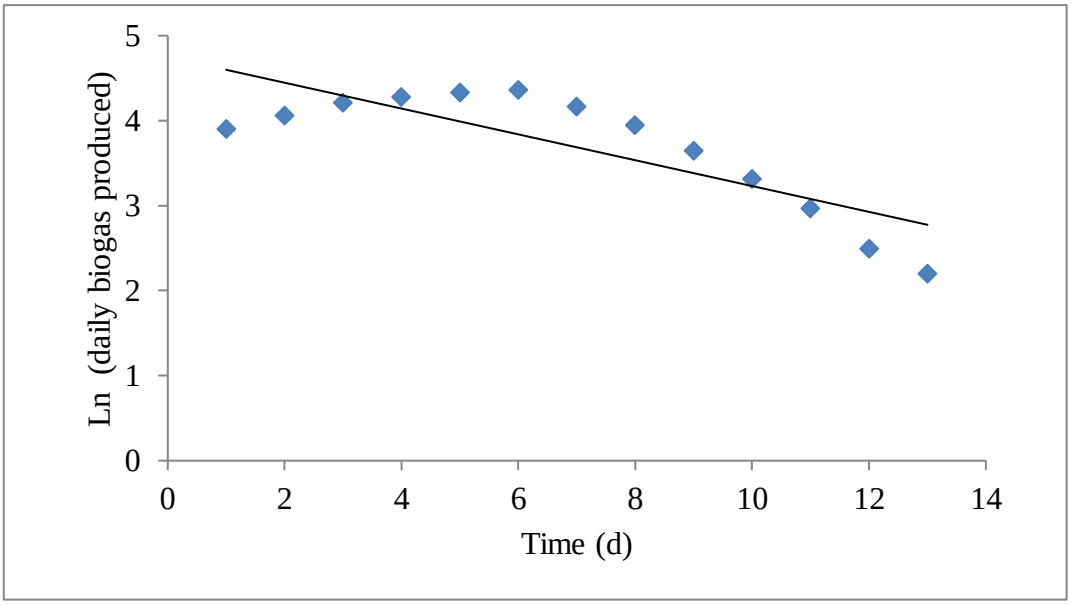


Figure 4.24 First-order model of co-digestion

Table 4.26 kinetics modelling parameters of co-digestion

Model	Parameters	Value	Cumulative biogas yield (NmL/gVS)
Experimental	-	-	626
First order	M_m (NmL/gVS)	765.46	
	k (d^{-1})	0.151	657.96
	RMSE	0.453	
Modified Gompertz model	M_m (NmL/gVS)	626	
	R_m (NmL/gVS.d)	78.74	572.70
	λ (d)	3	
	RMSE	0.508	

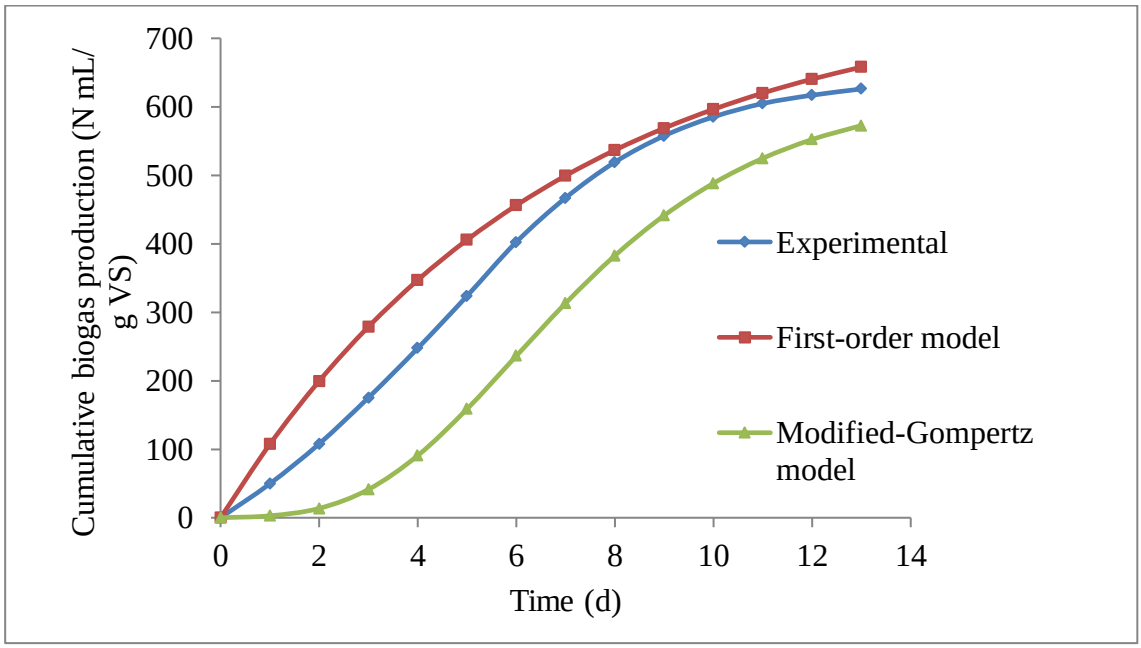


Figure 4.25 Biogas production at optimised condition and kinetic modelling for co-digestion

The RMSE value was 0.453 and 0.508 for first-order and modified Gompertz model, respectively. Therefore, in this case the first-order model was found to be best fit to experimental data.

4.11.10 Intermediate variation and end product characterisation

The variation in the value of parameters sCOD, VFA, phenolic content, and pH was noted in liquid hydrolysate of anaerobic digester at optimised condition. The sCOD, VFA and phenolic content followed an increasing trend upto 6 days and then it decreased afterwards as illustrated in Table 4.27. The initial pH was 7. It was decreased to 6.24 in 4 days and then followed an increasing trend. The final pH at the end of the process was 7.8.

The methane concentration was varied in the range of 65.53-15.78 % and CO_2 concentration was varied in the range of 27.31-30.7 %. The manure was characterised for nitrogen and potassium content as 4.4 % and 2.4 % respectively.

Table 4.27 Intermediate variation and end product characterisation

	Parameters	Time (d)					
		0	4	6	9	12	14
Liquid	sCOD (mg/L)	7952	8528	10217	7280	7216	6624
	VFA (mg/L)	7361	7750	7956	6727	6022	5425
	Phenolic content (mg/L)	622.56	711.04	944.78	863.04	833.92	818.24
	pH	7	6.24	6.52	6.87	7.45	7.8
Gas	CH_4 (%)		60.27	64.09	65.53	59.31	15.78
	CO_2 (%)		27.31	28.19	29.54	30.6	30.7
Solid (Manure)	Nitrogen (%)						4.4
	Potassium (%)						2.4