



# Alkaline and co-digestion pretreatments: process optimization for enhancing the methane yield of *Jatropha* press cake

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## Abstract

Oil extraction and biodiesel production process produce a massive amount of by-products like *Jatropha* press cake (JPC) and crude glycerol (CG), which could be used as a potential substrate for methane production. However, the higher lignocellulosic and nitrogen content in the JPC act as a recalcitrant and inhibitor, respectively, for microbes that are involved in the anaerobic digestion (AD) process. Therefore, the present study aimed to enhance the methane yield of JPC by optimizing the alkaline pretreatment and co-digestion process conditions. The effects of NaOH concentration, incubation temperature, and retention time on methane and soluble chemical oxygen demand (sCOD) yields were evaluated and modeled by employing a response surface methodology coupled with central composite design (RSM-CCD). Moreover, a series of batch experiments with various feedstock concentrations (FCs) were tested to investigate the methane yield of JPC when co-digested with CG at different levels. The methane yields of all pretreated samples were significantly higher when compared with these of the untreated JPC. Pretreating the JPC using 7.32% NaOH at 35.86 °C for 54.05 h was the optimum conditions for maximum methane increment of 40.23% (353.90 mL g<sup>-1</sup> VS), while co-digesting 2% CG with JPC at 2 g VS L<sup>-1</sup> FC enhanced the methane yield by 28.9% (325.47 mL g<sup>-1</sup> VS). Thus, the methane yield of JPC was effectively increased by alkaline pretreatment and co-digesting with CG. However, the alkaline pretreatment was relatively more effective compared with the co-digestion process.

**Keywords** Co-digestion · Crude glycerol · *Jatropha* press cake · Methane yield · Optimization · Pretreatment

## Abbreviations

adj. $R^2$	Adjacent $R$ square	CG	Crude glycerol
APHA	American Public Health Association	°C	Degree Celsius
NH <sub>3</sub>	Ammonia	$E_i$	Energy input
NH <sub>4</sub> OH	Ammonium hydroxide	$E_o$	Energy output
AD	Anaerobic digestion	EPA	Environmental protection agency
ANOVA	Analysis of variance	$\eta$	Eta (Greek letter)
BCR	Benefit-cost ratio	FC	Feedstock concentration
BMP	Biochemical methane potential	GC	Gas chromatography
Ca(OH) <sub>2</sub>	Calcium hydroxide	g	Gram
CO <sub>2</sub>	Carbon dioxide	H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
C/N	Carbon-to-nitrogen ratio	ISR	Inoculum-to-substrate ratio
CCD	Central composite design	JPC	<i>Jatropha</i> press cake
		K	Kelvin
		kg	Kilogram
		kJ	Kilojoule
		kPa	Kilopascal
		L	Liter
		MRV	Measurement, reporting, and verification
		MJ	Mega joule
		m <sup>3</sup>	Meter cubic
		CH <sub>4</sub>	Methane

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MC	Moisture content
$R^2$	Multiple $R$ square
NIBIO	Norwegian Institute of Bioeconomy Research
pred. $R^2$	Predicted $R$ square
RSM	Response surface methodology
rpm	Revolution per minute
NaOH	Sodium hydroxide
sCOD	Soluble chemical oxygen demand
TCD	Thermal conductivity detector
TS	Total solid
VFA	Volatile fatty acid
VS	Volatile solid
wt	Weight
$\xi$	$\Xi$ (Greek letter)
HMF	5-hydroxymethylfurfural

## 1 Introduction

The demand for fuel energy sources and their actual consumption across the globe are increasing drastically [1]. Fossil fuel resources supplied almost 87% of the global energy demand [2]. However, three-fourths of the world's poor households consumed only 10% of the worldwide energy supply [3]. Most of these poor households are living in net oil-importing Sub-Sahara Africa, where Ethiopia is ranked at the bottom of the energy poverty index [4]. In Ethiopia, 91% of the energy demand is satisfied by the local available traditional biomass resources [5]. However, the utilization of these conventional energy sources has resulted in severe environmental problems like forest degradation and greenhouse gas emissions. Furthermore, the entire transport energy requirement of Ethiopia is dependent on imported petroleum, with the cost of 65–80% of the total export earnings [6]. For instance, in 2018/2019 alone, 3.99 million metric tons of petroleum products were imported; and the expense of this import was more than 2.4 billion US dollars [7]. The costs associated with port rent, along with petroleum price and fluctuation, have severely affected the trade balances of the country. As a result, the Ethiopian government has launched a strategy in 2007 to assist the production and utilization of biofuels (biodiesel, bioethanol, and biogas) from different non-edible oil seeds and agro-industrial and municipal solid organic wastes [8].

*Jatropha curcas* L. (*Jatropha* hereafter), castor bean (*Ricinus communis*), Croton (*Croton macrostachyus*), and Moringa (*Moringa stenopetala*) plant seeds have been identified as promising non-edible energy plants for biodiesel production [9]. *Jatropha* seed is characterized by high oil content [10]. The oil is dominated by monounsaturated and polyunsaturated fatty acids [11]. Although the seeds are suitable for biodiesel production, 65–70% of them reported being disposed of as *Jatropha* press cake (JPC) during oil extraction

[12], while 100 kg of biodiesel production generates 10–14 kg of crude glycerol (CG) [13]. Considering the availability of adequate *Jatropha* plantation in Ethiopia [4] and higher seed production per ha [14], biodiesel production would generate a substantial quantity of JPC (10.5–11.4 million tons) and CG (0.61–0.70 million tons) per year.

CG can be purified into high grade for various applications in cosmetics and pharmaceuticals. However, the high purification cost hinders its use in such industries and usually considered organic waste [2]. While JPC contains toxic chemicals like phorbol ester and curcumin [15], thus, the cake could not be directly used as animal feed or organic fertilizer [16]. As a result, managing and controlling these organic wastes become the most challenging issue for most biodiesel producers. Detoxification and anaerobic digestion (AD) were identified as promising pretreatment methods for utilizing JPC for animal feed and organic fertilizer, respectively. With regard to this, studies have tried to investigate the biochemical methane potential (BMP) of JPC [17, 18]. However, JPC contains a considerable amount of seed husks, which are enriched with lignocellulosic materials. Liang et al. [19] showed that JPC contains 19% lignin and 27% carbohydrates, while Kumar et al. [20] measured 14% and 28% cellulose and hemicellulose, respectively. Furthermore, the carbon-to-nitrogen ratio (C/N) of JPC was reported to be 9:1 [16], which is significantly lower compared with the optimum ratio of 20:1–30:1 [21]. The digestibility of lignocellulosic biomasses for methane production is limited mainly due to the rate-limiting hydrolysis stage caused by the complex cellulose-hemicellulose-lignin networks [22]. Higher nitrogen content may cause inhibition of the methanogenic activities [23] and subsequently leads to process collapse due to the ammonia accumulation [18]. In contrast, CG is very rich in carbon, which means the mono-digestion of CG for biogas production is impossible due to nitrogen deficiency for maintaining the microbial biomass growth [2].

Pretreating the JPC using proper techniques can improve the anaerobic degradation process by bringing a structural change. The structural change would help the microbes to convert the carbohydrate polymers into simple fermentable sugars [24], whereas, co-digestion could improve the methane yield by optimizing the required C/N ratio [2, 25]. Therefore, various techniques such as mechanical [26], thermochemical [24], and steam explosion [27, 28] pretreatments were commonly used for pretreating lignocellulosic biomasses. Furthermore, the biodegradability of feedstocks with lower C/N ratio could be improved by co-digestion, solid-state AD, bio-augmentation, or nutrient supplementation [21, 23].

In the present study, alkaline pretreatment and co-digestion process were selected as a potential technique for enhancing the methane yields of JPC due to various positive characteristics compared with other pretreatment methods. Briefly, thermo-acidic pretreatments are corrosive and cause degradation of carbohydrates and lignin [29]. Biological pretreatments

are expensive and time-consuming due to the slow processes, and finding a specific enzyme is complicated [21, 26, 30, 31]. Physical pretreatments are energy-intensive processes [21, 26]. The alkaline pretreatment may increase the pH of the feedstock due to the residual chemical after the pretreatment [32]. However, the remaining chemical is useful since the next step, the AD, requires an alkaline addition for controlling pH drop due to volatile fatty acid (VFA) accumulation. Moreover, alkaline pretreatment is highly effective in lignin removal by cleaving the lignin-carbohydrate linkages with minimal carbohydrate degradation [21].

Among different alkaline chemicals, NaOH is effective for enhancing the biogas yield of lignocellulose biomasses [21]. However, the efficiency of alkaline pretreatments is affected mainly by chemical concentration, temperature, and retention time [33–35]. The effect of these process variables in the alkaline pretreatment was studied by varying one variable at a time but keeping the other variables into a constant value. However, the synergistic effects from simultaneous changes of more than one process variable were not widely investigated. Failure in optimizing the effects of individual and interactive process variables would lead to insufficient hydrolysis of lignocellulosic materials or AD process inhibition due to the accumulation of degraded products [36]. To the best of our knowledge, no reports are found on the optimum condition of alkaline pretreatment, and the amount of CG that should be added for enhancing the methane yield of JPC. Thus, the present study was initiated to examine and model the effect of NaOH concentration, incubation temperature, and retention time in the alkaline pretreatment on the methane and soluble chemical oxygen demand (sCOD) yield of JPC.

## 2 Materials and methods

### 2.1 Source of raw materials

The JPC sample was kindly provided by YME Design and Manufacture Company located at Hawassa, Ethiopia. At the time of supply, the company exploded the oil from the *Jatropha* seed using a hydraulic press machine. The fresh JPC was dried at room temperature (27 °C) until its moisture content reduced to 10% on the dry weight basis. The dried JPC was then transferred into airtight zipped plastic and stored at 4 °C until further processing. The bacterial inoculum prepared from cow manure was collected from a biogas laboratory operated under mesophilic conditions. The dominant types of microbes living in the manure-based inoculum were identified by Ozbayra et al. [37]. *Firmicutes* and *Bacteroidetes* accounted for about 46% and 36% of the bacterial community, respectively, while the methanogenic community was dominated by methanomicrobia (65%), methanobacteria (25%), and methanoplasma (8%) archaea. The analytical grade of

NaOH for the alkaline pretreatment was purchased from VWR, Oslo, Norway. The CG that co-digested with JPC was purchased from biodiesel producer company, Perstorp AB, located in Sweden.

### 2.2 Co-digestion of *Jatropha* press cake with crude glycerol

The BMP of JPC that co-digested with CG at various levels (0, 1, 2, 3, 4 wt%, on VS basis) was investigated by following a completely randomized design [2]. The actual experimental layout for the AD experiment is shown in Table 1, and each treatment was duplicated into a total of 30 batch reactors.

### 2.3 Alkaline pretreatment

The alkaline pretreatment was conducted by following the method described by Zhu et al. [38], with some modifications. Accordingly, JPC was allowed to pass through a sieve with a mesh size of  $\leq 2.5$  mm [39]. Then, 100 g of sun-dried sample (with 8% moisture content) was transferred into 1-L beakers that contain 2.64%, 4%, 6%, 8%, and 9.36% of NaOH, based on the dry weight of JPC. The samples were then adjusted into a moisture content of 82% by adding the required amount of tap water (Eq. (1)) [40]. After adding the water, all samples were gently stirred with glass rod to homogenize the mixture. Then after, all beakers were covered with plastic films and fastened with plastic rings and incubated at 26.6 °C, 30, 35, 40, and 43.4 °C for 7.68, 24, 48, 72, and 88.82 h. Following the subsequent pretreatments, a subsample was taken from individual treatment and centrifuged at 6000 rpm for 20 min to separate the solid and liquid fraction for sCOD analysis. Finally, the solid/liquid mixture and liquid fractions of the samples were immediately transferred into zipped plastic bags and 20-mL vials, respectively. Both samples were stored in the freezer until the actual biogas production and sCOD analysis were started. For the control experiment, 100 g of JPC was diluted with the same amount of water without alkaline and incubated under room temperature for the maximum retention time of 88.82 h [29].

$$\text{MC required (\%)} = \left( 1 - \frac{\text{Dry weight}_{\text{JPC}}}{\text{Weight}_{\text{JPC}} + \text{water}_{\text{added}}} \right) \times 100 \quad (1)$$

where MC is moisture content and JPC is *Jatropha* press cake.

### 2.4 Biochemical methane potential assays

The effects of alkaline pretreatment and co-digestion of CG with JPC on the BMP were investigated using 57 and 32-

**Table 1** The experimental layouts for co-digesting the JPC with CG at different FC and CG levels

Feedstock concentrations	CG levels (%)	CG (g VS L <sup>-1</sup> )	JPC (g VS L <sup>-1</sup> )	Inoculum (g VS L <sup>-1</sup> )
2 g VS L <sup>-1</sup>	0.0	0.000	2.184	5.300
	1.0	0.022	2.184	5.300
	2.0	0.044	2.184	5.300
	3.0	0.066	2.184	5.300
	4.0	0.088	2.184	5.300
5 g VS L <sup>-1</sup>	0.0	0.000	5.002	5.300
	1.0	0.050	4.950	5.300
	2.0	0.099	4.901	5.300
	3.0	0.146	4.853	5.300
	4.0	0.190	4.822	5.300
7 g VS L <sup>-1</sup>	0.0	0.000	7.000	5.300
	1.0	0.070	6.930	5.300
	2.0	0.138	6.862	5.300
	3.0	0.205	6.795	5.300
	4.0	0.270	6.729	5.300

batch reactors, respectively. The total and working volume of the batch reactors for alkaline samples were 122 and 70 mL, respectively, while the bigger batch reactors with 530 and 350 mL of total and working volume, respectively, were used in the co-digestion process. The main reason for preferring the bigger batch reactor in the co-digestion process was the limited working volume from the smaller reactor for adding the required amount of CG. In contrast, the smaller reactors were used for the alkaline-pretreated samples due to the inoculum shortage. Moreover, the authors assumed that the effect of volume variation on methane yield is minimal since the inoculum-to-substrate ratio (ISR), incubation temperature, agitation rate, and reactor configuration were the same for all batch reactors.

The manure-based inoculum was further incubated at  $37 \pm 0.5$  °C under the anaerobic condition for 1 week, which helps the microbes to assimilate the environment and reduce the endogenous biogas production. Following the pre-incubation, the prepared reactors were filled with the required amount of inoculum (5 g VS L<sup>-1</sup>); then, 3.34 g VS L<sup>-1</sup> of untreated or pretreated JPC was added into all batch reactors. Thus, in this experiment, the ISR was kept to 1.5 on a VS basis [27]. However, the amount of VS added into the bigger batch reactor (530 mL) depends on the predefined FCs, i.e., 2, 5, and 7 g VS L<sup>-1</sup>. The respective working volumes of the reactors were then adjusted by diluting the inoculum and substrate mixture with tap water. For blank and positive control experiments, the same amount of inoculum was poured to each bottle, and tap water was added for required working volume: 1.5 g L<sup>-1</sup> pure cellulose (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)  $n < 20$  μm was added into the bottles reserved for positive control. For alkaline-pretreated JPC, triplicated bottles were prepared for samples pretreated under the corner and axial (star) points, whereas six bottles were

prepared for samples pretreated under the center points (Table 2). The batch reactors arranged for co-digestion experiments were duplicated, and a total of 30 bottles were utilized. All batch reactors were closed with a rubber stopper and aluminum crimps and then purged with pure nitrogen using a syringe for 5 min to make an anaerobic environment [41]. Finally, the reactors were incubated at  $37 \pm 0.5$  °C and continuously centrifuged to 90 rpm. The methane yield increment (MYI) due to the alkaline pretreatments and the co-digestion processes were estimated using Eq. (2) [42].

$$\text{MYI (\%)} = \left( \frac{\text{MYI}_{\text{pretreated}} - \text{MYI}_{\text{untreated}}}{\text{MYI}_{\text{untreated}}} \right) \times 100 \quad (2)$$

where MYI is methane yield increment.

## 2.5 Experimental design

The alkaline pretreatments were carried out for each combination of all variables (NaOH concentration, incubation temperature, and retention time) using RSM-CCD, and all pretreatments were carried out in random orders. A total of 20 treatments that containing eight factorial points, six axial points, and six replicates for the center value were applied. The values estimated from center points were used to determine the experimental error. The coded and real values were calculated using Design-Expert version 12 (State Ease Inc., Statistics Made Easy, Minneapolis, MN, USA). The lower and upper values of each factor (Table 2) were determined following the methods of Zheng et al. [43]. The data obtained from AD and hydrolysates were used to develop a second-order polynomial equation that relates the responses to the process variables (Eq. (3)). Both the experimental design

**Table 2** Coded and real values of each variable used in the RSM-CCD

Variable	Symbol	Levels				
		$-\alpha$ (-1.68)*	-1	0	+1	$+\alpha$ (1.68)
NaOH concentration (wt%)	$X_1$	2.64	4.00	6.00	8.00	9.36
Incubation temperature (°C)	$X_2$	26.6	30.00	35.00	40	43.40
Retention time (h)	$X_3$	7.68	24.00	48.00	72	88.32

\* $\alpha$  is the distance from the axial point to the center point calculated by  $2^{k(1/4)}$  ( $k$  is the number of independent factors being used in the alkaline pretreatment = 3)

and statistical analysis were also done using the same software. Furthermore, the second-order polynomial equation from RSM-CCD was used to define the optimum value of each process variable that could maximize the methane and sCOD yields. The linear, interactive, and quadratic effect of temperature, NaOH concentration, and retention time was evaluated using a multivariate analysis of variance (ANOVA). The contour and 3D response surface plots were drawn to depict the interactive effect between two process variables while keeping the third variable into its center value. Values for multiple  $R^2$ , adj.  $R^2$ , and pred.  $R^2$  were used to evaluate the fitted model.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (3)$$

where  $Y$  is the response (sCOD or methane yield);  $X_1$ ,  $X_2$ , and  $X_3$  are the coded independent variables; and  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ ,  $\beta_{11}$ ,  $\beta_{22}$ ,  $\beta_{33}$ ,  $\beta_{12}$ ,  $\beta_{13}$ , and  $\beta_{23}$  are the model coefficients calculated from the experimental value.

## 2.6 Analytical method

### 2.6.1 Substrate analysis

The total solid (TS) and volatile solid (VS) contents of inoculum and JPC were determined based on the APHA standards [44]. The carbon, nitrogen, and hydrogen contents were estimated using an elemental analyzer (Thermo Flash 2000) from soil laboratory located at Norwegian University of Life Sciences, Ås, Norway. The crude protein content of JPC was estimated from its nitrogen content [45]. Karl Fisher titration was used for determining the moisture content of CG [45], and the TS was calculated by considering the moisture content. The VS content of CG was assumed to be the same with the TS value as nothing was left in the crucible when it was ignited at 550 °C for 2 h. The sCOD values for each treatment were estimated following DIN ISO 15705, which is analogous to EPA 4104 and APHA 5220 D [42]. Merck Spectroquant® COD kits with a measuring range of 500–10,000 mg L<sup>-1</sup> were used in the analysis of the sCOD yields. The caloric value and

crude fat content of the dried JPC were determined using a Bomb Calorimeter (IKA<sup>R</sup> C 200) [42] and conventional Soxhlet extraction method, respectively [18].

### 2.6.2 Biogas analysis

The BMP of both the alkaline pretreated and co-digested JPC with CG were regularly monitored for 61 and 56 days, respectively, until the last methane production in all flasks was less than 2% of the total amount. Briefly, the biogas pressure was measured using a digital manometer (GMH 3161 Reisinger Electronics, Germany), and the concentration of CO<sub>2</sub> and CH<sub>4</sub> in the biogas was determined using gas chromatography (3000 Micro GC, Agilent Technologies, USA), equipped with a thermal conductivity detector (TCD) using helium as a carrier gas [42]. After the successive biogas measurements, the excessive pressure was released by inserting the needle into the rubber stopper. All measured gas volumes were reported at 273 K and 101.3 kPa using Eq. (4), and the specific and cumulative methane volume was calculated using the normalized methane concentrations. The endogenous methane value produced from the blank controls (inoculum) was subtracted from the total methane yield. Besides CO<sub>2</sub> and CH<sub>4</sub>, the biological degradation of organic matter could generate hydrogen gas. However, considering its small fraction (5–10%) when compared with the total biogas yield [46], this study did not measure the hydrogen concentration during the AD process.

$$V_b = \frac{n \times R \times 273}{P_o} = \left( \frac{dp \times V}{R \times T} \right) \times \left( \frac{R \times 273}{P_o} \right) = \left( \frac{dp \times V \times 273}{T \times P_o} \right) \quad (4)$$

where  $V_b$  is the volume of biogas (L) at the standard condition of 273 K (0 °C), 1 atm total pressure;  $P_o$  is 1 atm;  $n$  is the number of moles;  $R$  is the ideal gas constant;  $V$  is the volume of headspace (L);  $T$  is the temperature in the incubator room (310 k); and  $dp$  is the overpressure measured in the bottle (atm) (1013.25 mbar = 1 atm).

### 2.6.3 Energy balance and economic feasibility

Energy balance and benefit-cost analysis are essential tools for examining the economic viability of various pretreatment techniques applied for enhancing the biogas production potential lignocellulosic material. Although all alkaline and co-digestion processes improved the methane yield of JPC, energy balance and cost analysis were done only for the optimum conditions identified for higher methane yields by using Eqs. (5) and (6) [47]. The benefit-cost ratio (BCR) analysis was performed based on the following assumptions: (1) the average market price of NaOH is assumed to be 412 € t<sup>-1</sup> [48] and (2) the estimated cost of methane sale is 0.57 € m<sup>-3</sup> [47]. Moreover, the optimum temperature (35.86 °C) identified in the alkaline pretreatment is nearly the same as the maximum ambient temperature (33 °C) of Hawassa City, Ethiopia, where the JPC sample was brought. Thus, we assumed that no heat energy input was needed for this alkaline pretreatment.

$$E_o = \Delta p \times \xi \times \eta \quad (5)$$

$$E_i = E_{\text{NaOH}} \times n \quad (6)$$

where  $E_o$  is energy output (kJ kg<sup>-1</sup> VS);  $E_i$  is energy input (kJ kg<sup>-1</sup> NaOH);  $E_{\text{NaOH}}$  energy needed for 1-kg alkaline production (7 MJ kg<sup>-1</sup>);  $\Delta p$  is net methane yield increment (m<sup>3</sup> g<sup>-1</sup> VS);  $\xi$  is methane heating value (35.8 kJ L<sup>-1</sup>);  $\eta$  is methane energy conversion efficiency (90%), and  $n$  is the amount of NaOH needed in the pretreatment process (kg).

### 2.6.4 Statistical analysis

The interactive effect between FC and CG levels on the biogas and methane yield of JPC was tested using a two-way ANOVA via R software (version 3.6.2), while the linear, interactive, and quadratic effects of NaOH concentration ( $X_1$ ), incubation temperature ( $X_2$ ), and retention time ( $X_3$ ) were analyzed using Design-Experts (version 12) as discussed in Section 2.5. All ANOVA was carried out using Tukey's test. Thus, there were statistically significant variations at  $p \leq 0.05$  between mean with different letter groups, or the mean differences were considered statistically significant if the  $p$  value is  $\leq 0.05$ .

## 3 Results and discussion

### 3.1 Substrate composition

The physical and chemical constituents of JPC, CG, and inoculum characterized using the methods described in Section 2.6.1 are shown in Table 3.

**Table 3** Physicochemical characteristics of substrates being used in the AD process

Parameters	Types of substrate		
	JPC	Inoculum	CG
TS (%)	90.00 ± 0.01	4.40 ± 0.28	85.00 ± 0.03
VS (%)	93.36 ± 0.08	59.90 ± 0.34	–
sCOD (g L <sup>-1</sup> )	717.43 ± 3.19	–	1399.33 ± 6.99
Nitrogen (%)	3.99 ± 0.09	–	–
Carbon (%)	50.40 ± 2.07	–	–
Hydrogen (%)	7.17 ± 0.23	–	–
C/N ratio	13.10 ± 0.88	–	–
Crude protein (%)	24.90 ± 0.28	–	–
Crude fat (%)	9.00 ± 20	–	–
Calorific value (kJ kg <sup>-1</sup> )	19.87 ± 0.62	–	–

### 3.2 Impact of feedstock concentration and CG level on BMP of JPC

Various studies indicate that FC has a detrimental effect on the AD process and the subsequent methane recovery process [49–51]. Moreover, the higher protein, lipid, and long-chain free fatty acid concentration present in the JPC could also inhibit the activity of microbes in the digester unless the steady-state condition is maintained. In the present study, the feedstock concentration (FC) is defined as the amount of JPC and CG in VS bases added into the working volume of the batch digester (g VS L<sup>-1</sup>). Significant variations in cumulative and specific methane yields were observed among treatments operated under different FCs (2, 5, and 7 g VS L<sup>-1</sup>) and CG levels (0, 1, 2, 3, 4%). Most of the batch digesters run without CG produced lesser biogas and methane when compared with the treatments containing both CG and JPC (Table 4). The lower methane yield might be attributed to the higher content of lignocellulosic materials along with lower C/N ratios. The availability of higher nitrogen in the JPC could generate non-ionized and/or ammonium ion in the AD process [52]. Then, the ammonium reacts with water and forms ammonium hydroxide (NH<sub>4</sub>OH), which alkalizes the bulk solution of the digester. The alkalized bulk solution could inhibit the anaerobic process by affecting the activity of *Archaea methanogenesis* [53]. Moreover, variation in trends and peaks of daily methane yield also noted among treatments (Fig. 1a, b, and c). Co-digesting 2% CG with JPC at 2 g VS L<sup>-1</sup> FC showed a fast degradation rate, which needed only 3 days to attain the maximum methane peak value (48.9 mL g<sup>-1</sup> VS day<sup>-1</sup>) when compared with all other treatments (Fig. 1a).

A two-way ANOVA and Tukey post hoc test were conducted to investigate the statistical variation in biogas and methane

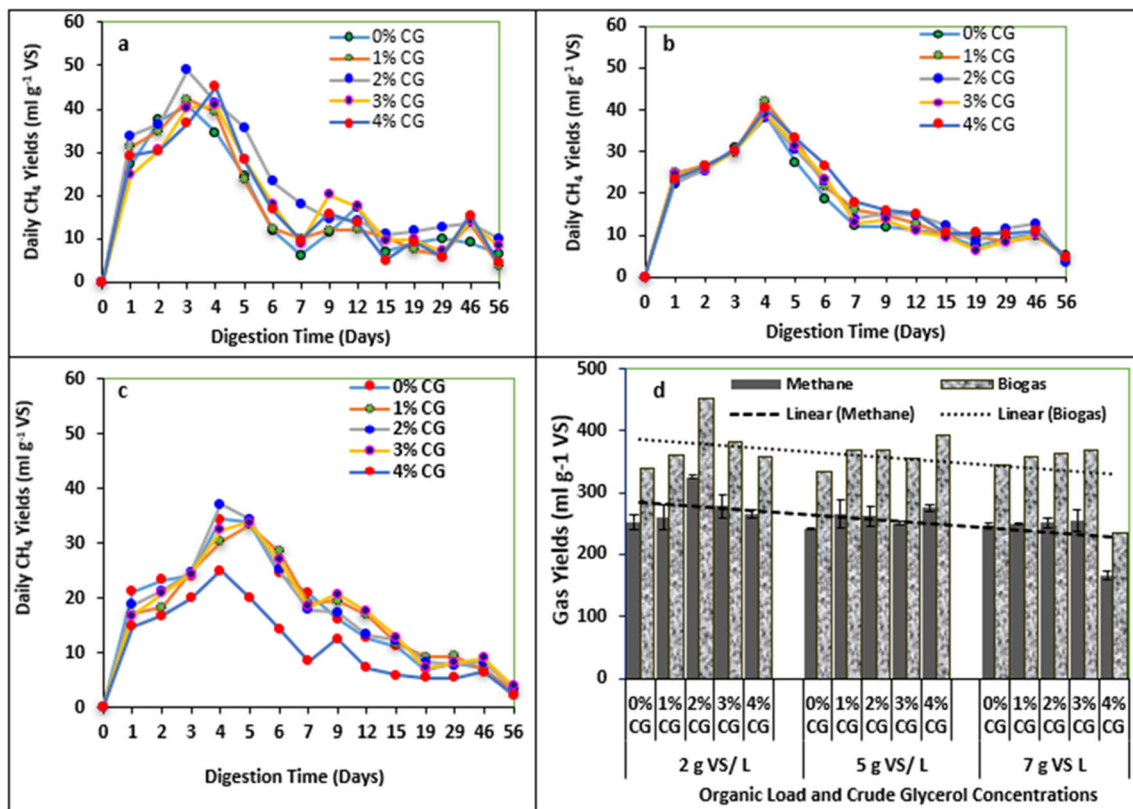
**Table 4** The cumulative biogas and methane production (mL g<sup>-1</sup> VS) of JPC co-digested with CG at different conditions

	Crud glycerol levels (%)	Feedstock concentrations		
		2 g VS L <sup>-1</sup>	5 g VS L <sup>-1</sup>	7 g VS L <sup>-1</sup>
Biogas (mL g <sup>-1</sup> VS)	0	339.91b	334.50b	344.24b
	1	360.24b	369.96b	358.42b
	2	451.43a	369.13b	364.20b
	3	382.74ab	355.54b	369.49b
	4	358.95b	391.96b	234.89c
Methane (mL g <sup>-1</sup> VS)	0	252.41b	240.11b	247.98b
	1	259.49b	264.33b	249.68b
	2	325.47a	261.97b	251.11b
	3	278.46ab	250.43b	254.19b
	4	265.23b	275.69b	165.86c

The difference in lowercase letters showed a statistical mean difference due to the interactive effect between FC and CG levels. The statistical variation of means in the same row was due to the FC effect, while the mean difference in the same column was due to the CG level effect

yields among digesters operated at various co-digestion conditions. The smaller *p* value ( $< 2.2 \times 10^{-16}$ ) of the ANOVA model indicates a statistically significant variation among anaerobic digesters in estimated cumulative biogas and methane yields (Table 4). The cumulative methane yield of JPC slightly

declined as increasing of both the FC and CG levels (Fig. 1d). The average cumulative methane yield of digester run by adding various concentrations of CG to JPC under 2 g VS L<sup>-1</sup> FC ranging between 252.41 to 325.47 mL g<sup>-1</sup> VS, while the methane yield obtained from 5 g VS L<sup>-1</sup> and 7 g VS L<sup>-1</sup>



**Fig. 1** The daily methane yield at FC of 2 g VS L<sup>-1</sup> (a), 5 g VS L<sup>-1</sup> (b), and 7 g VS L<sup>-1</sup> (c) and the cumulative biogas and methane yields resulted from the various FC and crude glycerol (CG) levels (d)

FCs varied from 240.11 to 275.69 and 165.87 to 254.19 mL g<sup>-1</sup> VS, respectively, depending upon the amount of added CG levels.

The highest cumulative methane yield (325.47 mL g<sup>-1</sup> VS) was obtained from digester working under 2 g VS L<sup>-1</sup> that contains 2% CG, while less methane yield (165.87 g<sup>-1</sup> VS) was recorded from digester operated under 7 g VS L<sup>-1</sup> and enriched with 4% CG. The methane increment recorded from the former treatment was 28.9% when compared with the samples containing no CG at the same FC. However, the methane yield obtained from treatment that co-digested 4% CG with JPC at 7 g VS L<sup>-1</sup> FC declined by 96.23% when compared with the maximum methane yield. Similar results were reported by Schwingel et al. [2], in which the biogas and methane yield from laying hen waste were strongly dependent on the amount of added CG. Oliveira et al. [54] noted that co-digesting 2% CG with sargassum resulted in 18% more methane as compared with the control treatment, while Schwingel et al. [2] showed that co-digesting 2.6% glycerol with laying hen manure produced 21.83% more methane than the manure without glycerol. Veroneze et al. [55] indicated that inhibition of methane production from swine manure when more than 5% of glycerol was added.

The increment in methane yield might be attributed to the higher biodegradability and enhancement of the C/N ratio due to the added CG. In contrast, the lower methane yield might be associated with organic overloading, fast production of VFAs, or acidification of the digester [43]. The rapid production of VFA due to the fast hydrolysis of CG may inhibit the methanogenesis bacteria [38]; however, its effect seemed small since variation in methane concentrations among reactors was not statistically significant. Organic overloading could stimulate the microbial production of biosurfactants and mostly resulted in partial degradation of organic matter [56], which subsequently causes lower methane yield (Fig. 1d). The VS content of the digestate after 61 days of AD was higher for batch reactors operated at higher FC. The effect of overloading on methane yield could be higher since JPC contains less biodegradable protein [50]. Moreover, stable small bubbles (foams) were observed in the batch reactors run at higher FC. The foaming incidence might be associated with the surfactant nature of protein and lipid available in the JPC [57]. Thus, the foaming incidence could reduce the methane yield by producing a gas-liquid film at the top of the digester.

Variation in average cumulative methane yield was also investigated for samples with the same FC but contained different levels of CG using a single-factor ANOVA. The *p*-values of digesters working under 2, 5, and 7 g VS L<sup>-1</sup> FCs were 0.02, 0.18, and <0.01, respectively, which indicate that the effects of added CG levels were statistically significant at lower and higher FCs; however, their effects were negligible at 5 g VS L<sup>-1</sup> FC. Besides the cumulative methane yield, the

average methane concentrations were calculated for all samples and the values ranging from 70.7 to 73.9%. The ANOVA model showed an insignificant variation in methane concentration among treatments. Therefore, considering the economic gain of using lower FC for higher cumulative methane production, co-digesting 2% CG with JPC at 2 g VS L<sup>-1</sup> FC was identified as the optimum condition for higher methane production. The optimum condition obtained in the present study was comparable with conditions suggested by Haitl et al. [58]; however, relatively higher methane yield (1058.9 mL g<sup>-1</sup> VS) was reported by co-digesting 2.5% CG with hen laying manure [2].

### 3.3 Modeling the degree of solubilization (sCOD)

The effect of alkaline pretreatment on the compositional change of JPC was assessed using the sCOD yields as a primary indicator [41]. A regression model was fitted to determine the optimum values of the selected process variables for higher sCOD production. By referring Eq. (3), the second-order polynomial equation obtained from the experimental data was taken in terms of coded and actual value to evaluate the correlation between predictors ( $X_1$ ,  $X_2$ , and  $X_3$ ) and the response (sCOD) variables (Eqs. (7) and (8)). Thus, the linear, interactive, and quadratic effects of  $X_1$ ,  $X_2$ , and  $X_3$  on the degree of solubilization were investigated using RSM-CCD. As shown in Eq. (7), all coded linear and two interaction terms had a positive effect, while all quadratic terms and the interaction between  $X_2$  and  $X_3$  adversely affected the degree of solubilization.

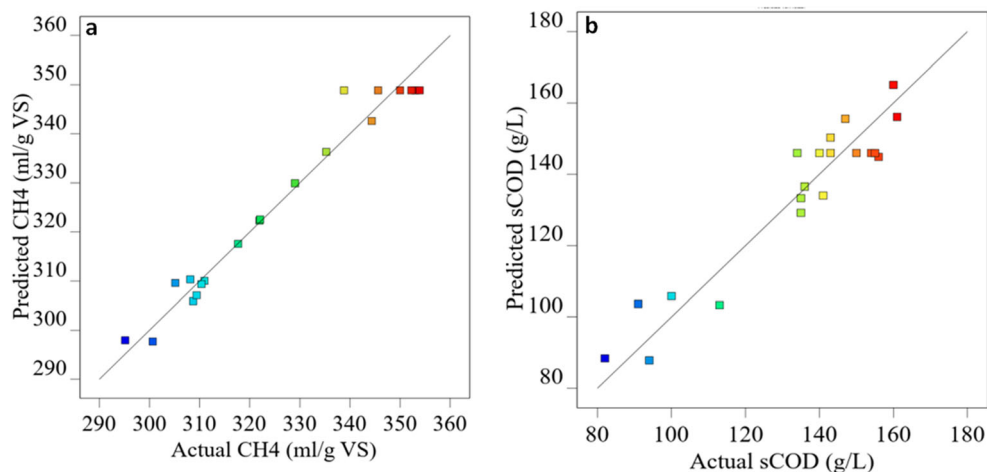
$$\begin{aligned} \text{sCOD yield (coded)} = & 145.96 + 12.25X_1 + 6.26X_2 \\ & + 20.13X_3 - 7.67X_1^2 - 2.19X_2^2 - 8.38X_3^2 \\ & + 3.00X_1X_2 \\ & + 0.25X_1X_3 - 4.5X_2X_3 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{sCOD yield (actual)} = & -183.31 + 18.39X_1 + 7.39X_2 \\ & + 3.52X_3 - 1.92X_1^2 - 0.09X_2^2 - 0.01X_3^2 \\ & + 0.30X_1X_2 \\ & + 0.01X_1X_3 - 0.04X_2X_3 \end{aligned} \quad (8)$$

The adj.  $R^2$  value of the quadratic model (0.81) was higher as compared with the values estimated for linear (0.68) and two-factor interaction (0.63) models; hence, the second-order polynomial equation was the preferred model in this study [59]. The strong correlation between experimental and model-predicted sCOD values further exhibited the suitability



**Fig. 2** Correlations between the actual and model-predicted values of methane (a) and sCOD (b)



of the model (Fig. 2b). This value lies between  $\pm 4.3$ , in which all estimated values were close to the model-predicted value. The value for the coefficient of determination ( $R^2$ ) was 0.90, which assured the applicability of the model for investigating the effect of these process variables in the alkaline pretreatment process. Furthermore, the  $p$  value  $> 0.05$  indicates that there was no evidence on the model's lack of fit relative to the pure error, which further elaborated the strength and adequacy of the model to depict the correlation between predictor and response variables.

The multivariate ANOVA for the second-order polynomial equation was also done to test the strength of the model. The results suggested that the second-order polynomial equation could better describe the relationship between sCOD productions and operational variables. As shown in Table 5, the  $p$ -values  $\leq 0.05$  indicate the significant linear impact of NaOH concentration ( $X_1$ ) and retention time ( $X_3$ ), as well as the

quadratic effects from the same variables ( $X_1^2$  and  $X_3^2$ ). Even though the  $p$  value of temperature was slightly greater than 0.05, it was included in the model by considering as important process variables in the solubilization of the JPC. This finding was in agreement with the report by Monlau et al. [60].

### 3.3.1 Effect of independent processing parameters on the degree of solubilization

Depending on the impact of various process variables, the sCOD yield increments ranging between 200 to 488% compared with the untreated JPC (Table 6). The increased sCOD yields were comparable with the reported value for greenhouse crop waste after the alkaline  $H_2O_2$  pretreatment [61]. The effect of three independent process variables on the sCOD yield of JPC is shown in Fig. 3b, d, and f. The impact of individual process variables on the degree of solubilization was examined by maintaining the other two variables into their center value.

The lowest sCOD yield ( $82 \text{ g L}^{-1}$ ) was obtained when the JPC was pretreated by adding 6% of NaOH and incubated at  $35^\circ \text{C}$  for 7.6 h. In comparison, the highest value of  $161 \text{ g L}^{-1}$  was recorded at the same NaOH concentration and incubation temperature but retained for 88.4 h (Table 6). Figure 3b shows that the sCOD yield from JPC was increased by 60% when the NaOH concentration increased from 2.64 to 6% by keeping the incubation temperature and retention time to their center value. However, with a further increase in NaOH concentration from 6 to 9.36%, the rate of sCOD yield increment showed a declining trend; only 6.85% improvement was observed. The catalyst (NaOH) was highly effective in attacking the binding bond between lignin and hemicellulose [62]. Moreover, NaOH is also effective for cleaving the ester and carbon-to-carbon bonds in lignin molecules (ferulic acid) and resulted in the release of soluble compounds from carbohydrate and lignin components. In the alkaline pretreatment, the

**Table 5** ANOVA for the model regression representing sCOD

Source	Sum of squares	df	Mean square	F value	p value
Model	10,051.77	9	1116.86	10.14	0.001*
$X_1$	2049.87	1	2049.87	18.61	0.002*
$X_2$	534.71	1	534.71	4.86	0.052
$X_3$	5531.94	1	5531.94	50.23	$< 0.001^*$
$X_1X_2$	72.00	1	72.00	0.65	0.438
$X_1X_3$	0.50	1	0.5000	0.006	0.948
$X_2X_3$	162.00	1	162.00	1.47	0.253
$X_1^2$	848.19	1	848.19	7.70	0.019*
$X_2^2$	69.23	1	69.23	0.63	0.446
$X_3^2$	1011.76	1	1011.76	9.19	0.013*
Residual	1101.23	10	110.12		
Lack of fit	751.23	5	150.25	2.15	0.211
Pure error	350.00	5	70.00		

\*Statistically significant effect of process variable on the sCOD yield at 5% errors

**Table 6** RSM-CCD's experimental layout and its corresponding experimental and model-predicted sCOD values ( $\text{g L}^{-1}$ )

Run order	Catalyst (%)	Temperature ( $^{\circ}\text{C}$ )	Time (h)	Actual value	Predicted value	sCOD in <sup>a</sup> . (%)
Unt. JPC <sup>b</sup>	0.00	0.00	88.60	27.40	–	0.00
1	6.00	43.40	48.00	143.00	150.30	423.00
2	6.00	35.00	48.00	134.00	145.90	390.00
3	4.00	30.00	24.00	94.00	87.80	244.00
4	6.00	35.00	48.00	140.00	145.90	412.00
5	8.00	30.00	24.00	100.00	105.80	265.00
6	4.00	30.00	72.00	136.00	136.60	397.00
7	6.00	35.00	7.68	82.00	88.40	200.00
8	8.00	40.00	72.00	160.00	165.10	485.00
9	9.36	35.00	48.00	156.00	144.90	470.00
10	6.00	35.00	48.00	143.00	145.90	423.00
11	6.00	35.00	48.00	150.00	145.90	448.00
12	2.64	35.00	48.00	91.00	103.70	233.00
13	8.00	30.00	72.00	147.00	155.70	437.00
14	6.00	35.00	48.00	155.00	145.90	467.00
15	8.00	40.00	24.00	135.00	133.40	393.00
16	6.00	26.60	48.00	135.00	129.20	393.00
17	4.00	40.00	72.00	141.00	134.10	415.00
18	4.00	40.00	24.00	113.00	103.30	313.00
19	6.00	35.00	88.32	161.00	156.10	488.00
20	6.00	35.00	48.00	154.00	145.90	463.00

<sup>a</sup> Increased soluble chemical oxygen demand after the alkaline pretreatments (%). <sup>b</sup> Untreated JPC

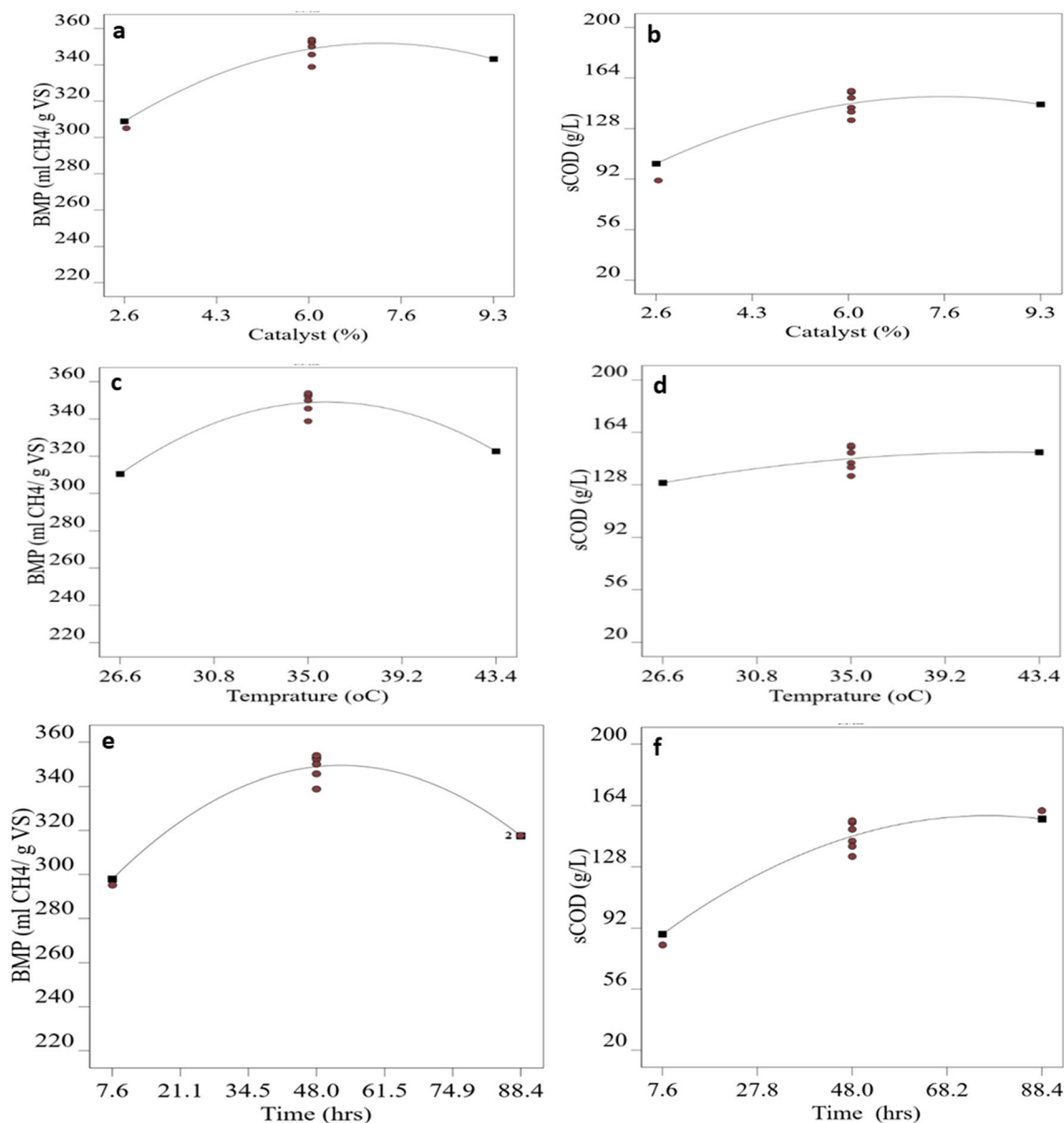
NaOH would dissociate into two  $\text{Na}^+$  and  $\text{OH}^-$  ions, and as the  $\text{OH}^-$  concentration increases, the rate of hydrolysis increases consequently [63].

The incubation temperature at all levels slightly increased the degree of solubilization from JPC (Fig. 3d). The sCOD yield increased by 11.11% when the incubation temperature increased from 26.6 to 43.4  $^{\circ}\text{C}$ , which implies that the alkaline pretreatment was effective at mild temperatures. However, the effect of temperature on sCOD yield was relatively lower when compared with the other pretreatment variables. The weaker impact of temperature on the degree of solubilization might be associated with the lower temperature ranges (26.60–43.40  $^{\circ}\text{C}$ ) applied in the pretreatment processes. In the same way, the effect of retention time on sCOD yield was highly significant in its lower range (Fig. 3f). For instance, maintaining the NaOH concentration and reaction temperature to their center value and then increasing the retention time from 7.6 to 48 h resulted in 78% more soluble compounds, but a further increase of the retention time caused for deterioration of the sCOD yields. The possible reason for the enhancement of sCOD for most levels of the process variables may be attributed to higher hydrolysis of the lignocellulosic materials found in JPC due to the optimum pretreatment conditions [64].

### 3.3.2 Effect of interactive factors on the degree of solubilization (sCOD)

Although the interaction effects among all variables were not statistically significant (Table 5), the sCOD yields were increased when the value of each process variable increased. More specifically, the amount of sCOD increased when the JPC was pretreated using higher NaOH concentration at the higher temperatures (Fig. 4b). However, a further increment of catalyst concentration ( $\geq 8\%$ ) and incubation temperature ( $\geq 40$   $^{\circ}\text{C}$ ) showed a declining trend in sCOD yields. Furthermore, as it is noted from the response surface curve, the effect of the catalyst was more powerful when compared with the temperature at constant retention time (48 h). On a similar vein, the degree of solubilization was increased with increasing of the NaOH concentration from 4 to 8% and retained up to 72 h (Fig. 4d). However, a further increase in NaOH concentration and retention time caused a slight reduction of sCOD yields. Figure 4f shows the amount of sCOD from the JPC increased with the corresponding increment of retention time and incubation temperature at all levels.

The increment in sCOD was associated with the solubilization of cellulose, hemicellulose, and lignin components at the optimum pretreatment conditions. In contrast, the possible reason for lower sCOD yield at severe pretreatment conditions

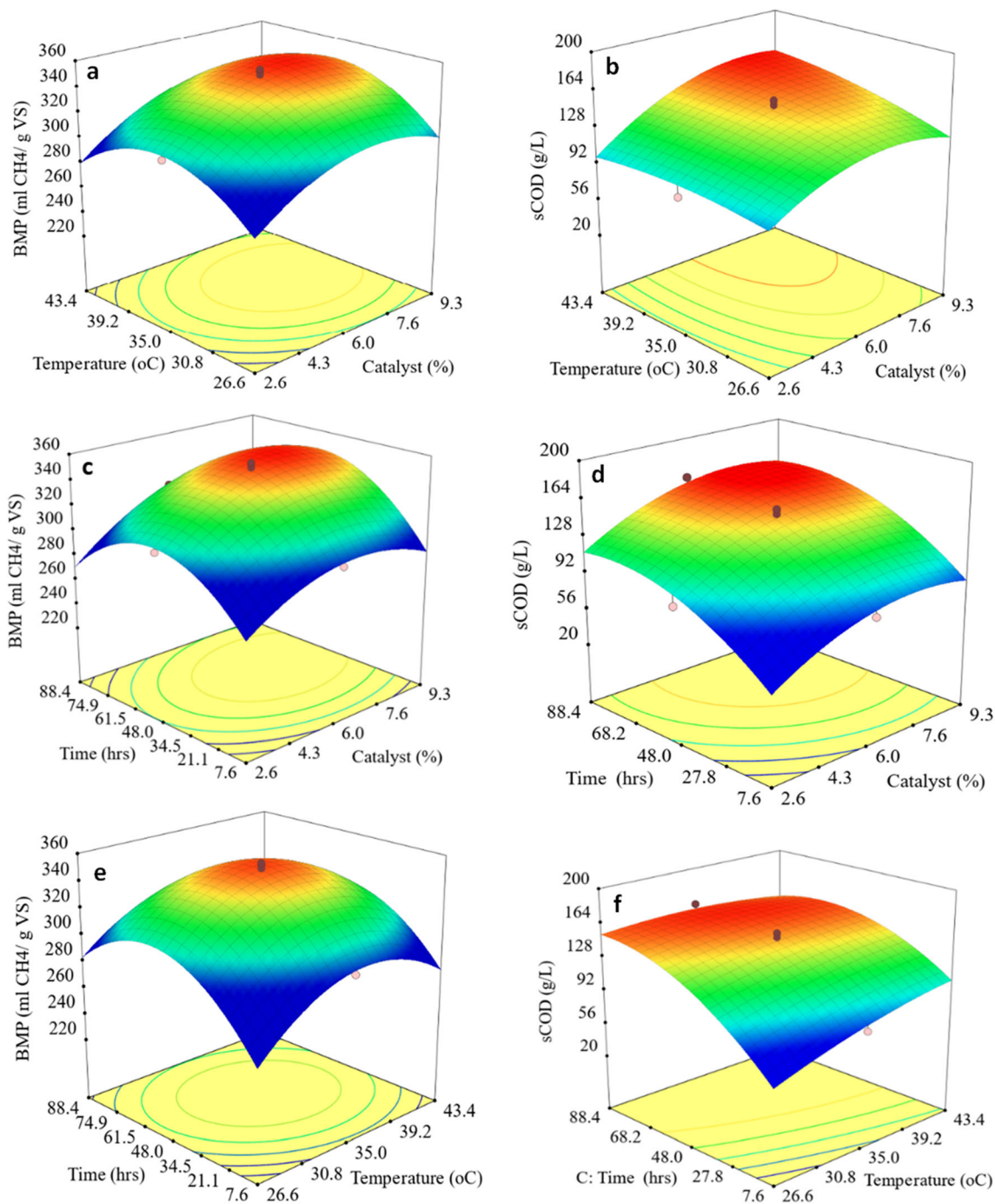


**Fig. 3** Effect of individual process variable: NaOH concentration (a, b), incubation temperature (c, d), and retention time (e, f) on methane and sCOD yields. One parameter was varied while the rests were maintained to their center points

might be due to condensation and precipitation of the solubilized components [65]. Furthermore, the lower sCOD yield could also be associated with instability and formation of complex non-soluble compounds at severe pretreatments [66]. Strong alkali concentration leads to a loss of carbohydrates [67]. The loss of carbohydrates is mainly due to the peeling and hydrolytic reactions, followed by the formation of lower molecular compounds [68]. Moreover, the degradation of these lower molecular compounds at severe pretreatment conditions triggers the loss of organic carbon in the form of carbon dioxide, which could subsequently result in lower sCOD yields. Loss of soluble compounds from cellulose and hemicellulose would increase with increasing NaOH concentration and retention time [35]; this leads to lower sCOD

recovery. Furthermore, at a higher temperature, the cross-link between sodium ion and lignin molecule would further decrease the solubilization of lignin during the alkaline pretreatment. As a result, the optimum operational variables for higher sCOD yield were investigated from the model developed using the actual value (Eq. (8)). According to the optimum value suggested by the RSM-CCD, pretreating the JPC with 7.84% NaOH at 37.54 °C for 64.84 h would result in the maximum predicted sCOD value of 163.31 g L<sup>-1</sup>.

The optimum condition predicted for higher sCOD yields may not be suitable for higher methane production. The possible reasons could be the generation of degraded products like 5-hydroxymethylfurfural (HMF), furfural, and phenolic compounds at severe pretreatment conditions, which all are



**Fig. 4** The response surface plot mapped for BMP and sCOD yields; the interactive effects between NaOH and temperature (**a**, **b**), NaOH and retention time (**c**, **d**), and incubation temperature and retention time (**e**, **f**)

inhibitor and toxic for microbes involved in the AD process. Furfural and HMF at low levels ( $\leq 1.4 \text{ g L}^{-1}$ ) could enhance the biogas production [69]; however, at higher concentrations ( $\geq 2.0 \text{ g L}^{-1}$ ), they could disrupt the methanogenic activity [70, 71]. Although alkaline pretreatment is better in carbohydrate preservation as compared with acid pretreatment, the peeling reactions may lead to degradation of polysaccharides with the formation of acids like saccharinic, lactic, formic, and

different dihydroxy and dicarboxylic acids [72]. In general, the effect of time was higher when it interacts with a catalyst and temperature, while the impact of the catalyst was powerful when it was combined with temperature. The lower effect of temperature might be attributed to its narrow ranges supplied in the pretreatment process. These temperature ranges were kept intentionally to ensure low energy pretreatment demand, while the lower temperature value ( $26.6 \text{ }^{\circ}\text{C}$ ) is the real

ambient temperature in Ethiopia where *Jatropha* is abundantly growing.

### 3.4 Modeling for biochemical methane potential

In addition to the sCOD, the catalyst concentration ( $X_1$ ), incubation temperature ( $X_2$ ), and retention time ( $X_3$ ) were also selected as main process variables that could affect the methane yield of JPC. The RSM-CCD was employed to investigate the interaction between these process variables and the methane yields obtained after the alkaline pretreatments. Furthermore, RSM-CCD was used to predict the optimum pretreatment conditions for maximum methane production after 61 days of AD. Thus, using Eq. (3) as a generalized model, the actual multiple regression models were developed from the coded and actual experimental data, and the models indicated that a second-order polynomial equation was the best model that describes the relationship between these process variables and the methane yield obtained from JPC (Eqs. (9) and (10)).

$$\begin{aligned} \text{BMP (coded)} &= 348.85 + 9.81X_1 + 3.64X_2 \\ &+ 5.83X_3 - 8.04X_1^2 - 11.45X_2^2 - 14.52X_3^2 \\ &+ 1.02X_1X_2 + 2.62X_1X_3 - 1.48X_2X_3 \end{aligned} \quad (9)$$

$$\begin{aligned} \text{BMP (actual)} &= -392 + 22.83X_1 + 32.77X_2 \\ &+ 2.77X_3 - 20.01X_1^2 - 0.46X_2^2 - 0.03X_3^2 \\ &+ 0.10X_1X_2 + 0.05X_1X_3 - 0.01X_2X_3 \end{aligned} \quad (10)$$

where BMP is the biochemical methane potential.

Form Eq. (9), it is clearly shown that the linear effect of NaOH concentration ( $X_1$ ), incubation temperature ( $X_2$ ), and retention time ( $X_3$ ), and the two interaction terms ( $X_1X_2$  and  $X_1X_3$ ) has a positive effect, while all quadratic terms ( $X_1^2$ ,  $X_2^2$ , and  $X_3^2$ ) and the interaction between  $X_2$  and  $X_3$  inversely correlated with the methane yield of JPC. Moreover, a multivariate ANOVA was carried out to test the strength of the model that predicted the effect of linear, interactive, and quadratic terms of the  $X_1$ ,  $X_2$ , and  $X_3$  applied during the alkaline pretreatment process. The value for multiple correlation coefficients of  $R^2$ , adj.  $R^2$ , and pred.  $R^2$  was 0.97, 0.94, and 0.90, respectively, in which the higher  $R^2$  value (0.97) indicates that the regression model could not explain only 3% of the total variation in methane yields. The closed correlation between all experimental and model-predicted values indicates the reliable predictive power of the model (Fig. 3a). The effect of linear terms from  $X_1$ ,  $X_2$ , and  $X_3$  and the quadratic terms of the three process variables, i.e.,  $X_1^2$ ,  $X_2^2$ , and  $X_3^2$ , on methane production of JPC were statistically significant (Table 7).

**Table 7** ANOVA for the model regression representing the methane yields

Source	Sum of squares	df	Mean square	F value	p value
Model	7015.89	9	779.54	34.10	< 0.001*
$X_1$	1314.39	1	1314.39	57.50	< 0.001*
$X_2$	180.97	1	180.97	7.92	0.018*
$X_3$	464.90	1	464.90	20.34	0.001*
$X_1X_2$	8.38	1	8.38	0.3668	0.558
$X_1X_3$	55.07	1	55.07	2.41	0.152
$X_2X_3$	17.55	1	17.55	0.7679	0.401
$X_1^2$	932.23	1	932.23	40.78	< 0.001*
$X_2^2$	1890.31	1	1890.31	82.69	< 0.001*
$X_3^2$	3039.80	1	3039.80	132.98	< 0.001*
Residual	228.60	10	22.86		
Lack of fit	61.57	5	12.31	0.3686	0.851
Pure error	167.03	5	33.41		
Cor total	7244.49	19			

\*Statistically significant effect of process variable on the methane yield at 5% errors

#### 3.4.1 Effect of independent processing variables on methane yield of JPC

The estimated cumulative methane yield from untreated JPC was 252.41 mL g<sup>-1</sup> VS, which is relatively higher than the reported methane yield of sunflower oil cake [60]. Even though the methane yield of pretreated JPC varied across different pretreatment conditions, all values were higher than the methane yield obtained from untreated JPC (Table 8). The increment of cumulative methane yields due to the alkaline pretreatments were ranging from 16.9% (295.10 mL g<sup>-1</sup> VS) to 40.2% (353.90 mL g<sup>-1</sup> VS). The lower methane yield (295.1 mL g<sup>-1</sup> VS) obtained from the alkaline-pretreated JPC was comparable with the literature value of 281 mL g<sup>-1</sup> VS obtained after the thermochemical pretreatment [18]. However, in addition to longer digestion time (61 days) requirement, all methane yields were lower than the report by Chandra et al. [73]. The lower methane yield after alkaline pretreatment might be associated with a lack of inherent bacteria that could digest the JPC in the AD process. This effect is strongly supported by Chandra et al. [73] in which maximum methane yield was obtained from JPC within 30 days of digestion time using active bacterial inoculum synthesized from pongamia oil seed cake.

The present study showed that the methane yield of JPC increased by 14% when the concentration of NaOH increased from 2.6 to 6% at constant incubation temperature and retention time of 35 °C and 48 h, respectively (Fig. 3a). However, at the same temperature and retention time, with further increment NaOH concentration to 9.4%, the methane yield of

**Table 8** The RSM-CCD experimental layout and the corresponding experimental and model-predicted values of methane yields (mL g<sup>-1</sup> VS)

Run order	NaOH (wt%)	Temperature (°C)	Time (h)	Actual value	Predicted value	MYI (%) <sup>a</sup>
1	2.64	35.00	48.00	305.20	309.60	20.90
2	9.36	35.00	48.00	344.30	342.60	36.40
3	6.00	35.00	48.00	349.90	348.90	38.60
4	8.00	40.00	72.00	335.30	336.30	32.80
5	4.00	40.00	72.00	310.40	309.40	22.90
6	4.00	30.00	24.00	300.56	297.70	19.10
7	6.00	35.00	88.32	317.70	317.60	25.90
8	8.00	30.00	72.00	329.00	329.90	30.30
9	6.00	35.00	48.00	353.90	348.90	40.20
10	8.00	40.00	24.00	321.90	322.30	27.50
11	6.00	35.00	48.00	352.20	348.90	39.50
12	6.00	35.00	7.60	295.10	297.90	16.90
13	4.00	30.00	72.00	309.40	307.10	22.60
14	8.00	30.00	24.00	310.90	310.00	23.20
15	6.00	35.00	48.00	338.80	348.90	34.20
16	6.00	35.00	48.00	345.60	348.90	36.90
17	4.00	40.00	24.00	308.70	305.90	22.30
18	6.00	43.40	48.00	322.10	322.60	27.60
19	6.00	26.60	48.00	308.10	310.30	22.10
20	6.00	35.00	48.00	353.00	348.90	39.90
Unt. JPC <sup>b</sup>	–	–	–	252.40	–	–

<sup>a</sup> Methane yield increment after pretreatments (%)<sup>b</sup> Untreated *Jatropha* press cake

pretreated JPC declined by 1.33%. Similarly, Zheng et al. [43] indicated that adding 6% NaOH into the corn stover achieved 48.5% more biogas yield when compared with the untreated sample. Lower concentration of catalyst during the pretreatment condition leads to a slow rate of lignocellulosic biomass hydrolysis, which subsequently could result in less accessibility to microorganisms during the AD process [60]. Alkaline pretreatment is highly effective in the dissolution of lignin and hemicellulose [62]. However, the hydrolysate at higher NaOH concentration may contain soluble compounds from lignin that may not be metabolized by microbes in the AD process and leads to lower methane yields.

The effect of incubation temperature on methane yield from JPC is shown in Fig. 3c. Soaking the JPC with 6% NaOH and retaining it for 48 h at an incubation temperature of below or above 35 °C resulted in a slight reduction of the methane yields. The methane yield was increased by 13.24% when the applied temperature rose from 26.6 to 35 °C, but it again declined by 8% when the incubation temperature arises from 35 to 43.4 °C. The optimum temperature recorded in this study was in line with Chandra et al. [73], in which the wheat straw pretreated with 4% NaOH (g g<sup>-1</sup> TS) at 37 °C for 5 days achieved 112% more methane when compared with the untreated straw.

The effect of retention time on methane yield was investigated at constant NaOH (6%) and incubation temperature (35 °C). The effect of retention time was highly significant at its lower range when compared with the upper values (Fig. 3e). The methane yield was increased by 18.22% when the retention time increased from 7.6 to 48 h. However, when the retention time further increased to 88.4 h, the methane yield increased by only 9.83%. Higher retention time may give a chance of effective hydrolysis of the lignocellulosic component of the press cake and thus leads to fast anaerobic degradation, and then followed by higher methane production [38]. However, retaining the JPC for more extended hours may result in the accumulation of inhibitor compounds such as furfural, HMF, organic acidic, and phenolic compounds, as discussed in Section 3.3.2.

### 3.4.2 Effect of interactive factors on methane yield of JPC

The methane yield of JPC was increased as a result of increasing the value of pretreatment conditions from their lower to the corresponding center point values (Fig. 4a, c, and e). More specifically, in the lower pretreatment conditions, the methane yield of JPC was increased when both the catalyst concentration and reaction temperature increased (Fig. 4a). However,

when the amount of NaOH and incubation temperatures exceed approximately above 8% and 40 °C, respectively, the methane yield started to decline at a slower rate. Similarly, in the lower pretreatment conditions, the methane yield was increased as increasing of NaOH concentration and retention time (Fig. 4c). However, a further increment of NaOH concentration and retention time above 8% and 60 h, respectively, resulted in a decline of methane yields.

The interactive effect between incubation temperature and retention time is also shown in Fig. 4e. The curve from the response surface plot indicates that the methane yield was increased as the incubation temperature rose from 26.4 to 35 °C with the corresponding increment of retention time from 7.6 to 48 h at 6% of NaOH. When the incubation temperature and retention further increased, the methane yields started to decline at a slower rate (Fig. 4e). Comparable with the present study, Sukri et al. [74] indicated that the degradation of carbohydrates due to the higher NaOH concentration (5.25%) and retention time (90 min). In general, maximum methane yields were obtained from JPC at moderate pretreatment conditions. Therefore, the optimum conditions in an alkaline pretreatment were predicted using Eq. (10). The model indicates that pretreating the JPC using 7.32% NaOH at 35.86 °C for 54.05 h would result in maximum methane production of 353.90 mL g<sup>-1</sup> VS, which the methane yield was increased by 40.23% as compared with the untreated JPC. The methane yield of JPC obtained at this optimum condition was further evaluated against the literature values, as described in Table 9.

### 3.5 Energy balance and economic feasibility

Regardless of the costs for biogas plant and pretreatment tank installation, the energy balance and preliminary economic feasibility of the alkaline pretreatment and co-digestion processes were studied. Moreover, the optimum temperature utilized in the alkaline pretreatment was taken as normal temperature for the area from which the sample was brought (Hawassa, Ethiopia). The net increases in methane yields ( $\Delta P$ ) from the alkaline and co-digestion pretreatment processes were estimated to be 101.50 and 73.07 L kg<sup>-1</sup> VS, respectively. Accordingly, the energy outputs ( $E_o$ ) calculated from these extra methane yields were 3270.3 and 2354.3 kJ kg<sup>-1</sup> VS, respectively. In contrast, the energy input ( $E_i$ ) required for the alkaline pretreatment was estimated to be 548.8 kJ kg<sup>-1</sup> VS, and there was no external energy needed for the co-digestion experiments. Thus, the extra energy obtained after alkaline pretreatment was almost 5.96 times higher as compared with the energy demanded by the NaOH pretreatment.

The BCR analysis was done by considering the unit price of inputs and outputs supplied in the alkaline pretreatment. The cost for one tone of NaOH was estimated to be 412 € t<sup>-1</sup> [48], while selling of 1 m<sup>3</sup> methane can earn 0.57 € [47]. Thus, pretreating one tone VS of JPC using 7.32% alkaline needed 0.078 t of NaOH, which costs 32.3 €. One tone VS from JPC can produce 101.5 m<sup>3</sup> extra methane when compared with the untreated JPC, which prices 57.86 €. Therefore, the BCR obtained by dividing the total methane selling price with NaOH market cost was estimated to be 1.79, which was positive and encourage the application of alkaline

**Table 9** Effect of alkaline pretreatment on various lignocellulosic biomass as compared to the present study

Lignocellulosic biomasses	The experimental optimum pretreatment conditions	AD conditions	Gas increase (%)	References
Wheat straw	4% NaOH, 37 °C, 120 h	Batch, 37 °C, 35 days	111.60 <sup>a</sup>	[73]
Extruded grass	7.5% Ca(OH) <sub>2</sub> , 10 °C, 20 h	Batch, 37 °C, 30 days	37.00 <sup>a</sup>	[75]
Wheat straw	14.2% NH <sub>3</sub> , 51 °C, 27 h	Batch, 30 °C, 4 weeks	56.25 <sup>b</sup>	[76]
Greenhouse crop waste	1% H <sub>2</sub> O <sub>2</sub> , 50 °C, 6 h, 7% TS	Batch, 35 °C, 64 days	77.60 <sup>a</sup>	[61]
Rice straw	6% NaOH, 35 °C, 8 days	Batch, 38 °C, 30 days	157.48 <sup>b</sup>	[61]
Pinewood	8% NaOH, 100 °C, 10 min	Batch, 37 °C, 45 days	181.00 <sup>a</sup>	[33]
Sugarcane bagasse	8.5% Ca(OH) <sub>2</sub> , RT, 4 h	Batch, 37 °C, 35 days	33.00 <sup>a</sup>	[34]
Corn stover	2% NaOH, 6% CaO, 50 °C, 0.5 h	Batch, 37 °C, 60 days	54.00 <sup>b</sup>	[35]
Jatropha press cake	7.32% NaOH, 35.86 °C, 54.05 h	Batch, 37 °C, 61 days	40.23 <sup>a</sup>	Present study

RT room temperature, TS total solids

<sup>a</sup> Methane

<sup>b</sup> Biogas

pretreatment at a larger scale. The same positive BCR results were obtained by You et al. [35] and Romero-Güiza et al. [47] after alkaline pretreatment of corn stover and wheat straw, respectively. The major challenge of this alkaline pretreatment was the recovery of the added NaOH. Thus, our future research may focus on the recovery of the alkaline chemicals found in hydrolysate using various methods like electrokinetic cells. The recovery of the chemical could further improve the BCR of the alkaline pretreatment.

## 4 Conclusions

Alkaline pretreatment and co-digestion processes were identified as effective strategies for enhancing the methane production potential of the JPC and CG. However, the efficiency of the alkaline pretreatment was significantly affected by NaOH concentration, incubation temperature, and retention time. Moreover, the methane yield in the co-digestion process was influenced by FC and CG levels. Both the methane and sCOD yields were declined at severe alkaline pretreatments. The optimum conditions predicted for higher methane and sCOD yields were significantly varied. Variation in optimum conditions defined for maximum methane and sCOD yields could be used as an indicator of process inhibition, probably due to the accumulation of degraded products at severe alkaline pretreatment. The positive energy balance and higher BCR inspire the application of the alkaline pretreatment at a larger scale for enhancing the methane yield of JPC. To reduce the downstream processing cost and effective utilization of the catalyst, the future research work in this area should focus on the recovery of NaOH from the hydrolysate using different techniques.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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