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ORIGINAL ARTICLE

Enhancement of biogas yield during anaerobic digestion of *Jatropha curcas* seed by pretreatment and co-digestion with mango peels

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Abstract

The combustion of fossil fuels is accompanied with a number of alarming problems such as fossil fuel depletion, increase in their prices, and emission of greenhouse gases. Thus, the need for the alternative renewable biofuels was increased to replace non-renewable fossil fuels. The sustainable use of non-edible feedstocks and waste for production of biofuels is a potential approach for reducing dependency on fossil fuels and mitigating environmental pollution. In the current study, the effects of carbon to nitrogen (C/N) ratios on methane yield during anaerobic co-digestion of *Jatropha curcas* de-oiled seed kernel and mango peels were evaluated in continuous reactors. The biogas potential and effects of acid pretreatment on *J. curcas* fruit were also evaluated during anaerobic batch digestion. The methane yield of co-digested mango peels and seed kernel (1:4 weight ratio based on volatile solids) was 61%, 50%, 36%, and 25% higher compared with the methane yields of mango peels, seed kernel, mango peels/seed kernel (2:1 w/w), and mango peels/seed kernel (1:1 w/w), respectively. The methane yields of the co-digestion of mango peels and seed kernel at 1:4, 1:1, and 2:1 ratios were 52%, 39%, and 32% of the theoretical yields, respectively, illustrating the importance of adjusting C/N ratio with the right amounts of co-substrate. The biogas yield of pretreated fruit coat was 7%, 22%, 34%, 50%, and 74% higher than that of the seed kernel, fruit coat (non-pretreated), de-oiled kernel plus seed coat (pretreated) (1.7:1, by weight), seed coat (pretreated), and seed coat (non-pretreated), respectively. Additionally, pretreatment of fruit coat and seed coat resulted in 22% and 47% higher biogas yields compared with their non-pretreated counterparts. This study revealed key substrate selection and pretreatment methods for increasing methane production from common seed oil production and agricultural wastes.

Keywords Mono-digestion · Anaerobic digestion · Pretreatment · C/N ratio · Methane; Mango peels

Abbreviations

ANOVA	Analysis of variance
C/N	Carbon-to-nitrogen ratio
CHNS	Carbon, hydrogen, nitrogen, and sulfur
MP + JSK	Mango peels plus *Jatropha curcas* seed kernel
NARC	National Agriculture Research Council
NL	Normalized liter
NmL	Normalized milliliter
S K	+*Jatropha curcas* deoiled seed kernel mixed
SC(H_3PO_4)	with dilute acid treated seed coat
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

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1 Introduction

Lignocellulosic biomass is a potentially sustainable renewable resource for the production of biogas through anaerobic conversion processes. However, lignocellulosic biomass has a high resistance to biodegradation due to a variety of structural and compositional properties such as accessible surface area, cellulose crystallinity, degree of polymerization of cellulose, degree of hemicellulose acetylation, and presence of lignin and hemicellulose [1–5]. A possible solution is pretreatment before anaerobic digestion, with the goal of affecting the lignocellulosic structure of biomass to decrease crystallinity as well as to increase accessibility to bacterial enzymes. These changes to structure increase biodegradability, resulting in increased production of biogas [3, 6–8].

Jatropha curcas is a drought and pest-resistant shrub of the Euphorbiaceae family that typically grows to a height of 5 m and can reach up to 10 m under favorable conditions [9]. After planting, the shrub produces fruit from the second year onward, and, if properly managed, can produce 4–5 kg of seeds after the fifth year and over its life span of 40–50 years [10]. The seeds are a source of oil, which is released via an extraction step. During processing of seeds, de-oiled cake is produced. The *J. curcas* de-oiled cake is a potential environmental pollutant, and anaerobic digestion has been identified as a possible solution to this problem [11]. While it has been documented that the biogas yield of *J. curcas* seed cake is 60% higher than that of cattle dung [12], mono-digestion of *J. curcas* seed cake still results in a low biogas yield, because of the relatively low carbon to nitrogen (C/N) ratio of about 9:1 [11]. This suggests that co-digestion with other substrates having a high C/N ratio (47:1 for fruit and vegetable wastes) [13] is needed to achieve more appropriate C/N ratios (20:1–30:1) [14]. A possible co-digestion substrate is fruit waste, such as mango peels, with a reported C/N ratio of 45.4 [15]. Pakistan is at fifth position in mango production worldwide [16]. Mangoes are consumed in ripen as well as in green forms. As a result, a large quantity of mango peels are wasted as useless resource creating nuisance and environmental pollution. In order to increase their economic viability, they can be used as a valuable resource for biofuel production. To date, no study has been conducted on the co-digestion of mango peels with *J. curcas* to standardize the C/N ratios for a stable anaerobic digestion process. The mango peels have high C/N ratio. Therefore, in the current study, mango peels were used as a potential substrate to optimize the C/N ratios (20:1–30:1) of *J. curcas* seed.

J. curcas seeds are rich in oils and are used for the biodiesel production. Usually, the seeds when de-oiled for biodiesel purposes are being wasted. Therefore, in the current study, the de-oiled seed cake was used as a valuable resource for bioenergy production to increase its economic viability. The extracted oil can be used for biodiesel production. Studies have been conducted on the biogas potential of different parts of the *J. curcas* shrub, but to date, not much attention has been paid to improve the biogas yield of the *J. curcas* fruit and de-oiled seed kernel using acid pretreatment and optimization of the C/N ratio. The objectives of the present study were to (1) evaluate the biogas potential of different parts of *J. curcas* fruit, (2) determine the effect of dilute phosphoric acid pretreatment on biogas yield of these different parts, and (3) determine the impact of adjusting the C/N ratio on biogas yield by co-digestion of *J. curcas* de-oiled seed kernel and mango peels.

2 Materials and methods

2.1 Materials and chemical reagents

Jatropha curcas fruits and seeds were kindly supplied by Dr. Samiullah Khan from District Bannu, Khyber Pakhtunkhwa, Pakistan. *J. curcas* fruit was divided into three parts (de-oiled seed kernel, seed coat, and fruit coat) to evaluate their biogas potential and the effects of acid pretreatment. The oil was extracted from the seed kernel using an oil expeller. The seed kernels were pressed in the oil expeller using continuous friction and pressure of 30 MPa from the screw at about 120 rpm. This pressure heated the kernels to approximately 60–80 °C, causing release of kernel oil into the reservoir. The pressed cake was removed and the cycle repeated three times to obtain the maximum yield of oil. In all experiments, the fruit parts (de-oiled seed kernel, seed coat, and fruit coat) were mechanically pretreated in a fruit blender (Deuron Blender, Pakistan) to convert all substrates to powder form. To determine the effects of C/N ratios on methane yield, mono-digestion and co-digestion of mango peels and de-oiled seed kernel were carried out at various C/N ratios, as described below.

2.2 Inoculum

Effluent samples from a continuous-flow anaerobic digester (retention time of 60 days) at the Pakistan Agriculture Research Council (PARC), Islamabad, Pakistan, treating cattle manure slurry, and operated at ambient temperatures, were collected twice and used as inoculum in both batch and continuous anaerobic digestion. The inoculum was incubated at 37 °C for 2 weeks to minimize endogenous methane production and to acclimatize the microbial consortia at 37 °C. After incubation, total solids (TS) and volatile solids (VS) of the inoculum were measured as described previously [17] (Table S1).

2.3 Feedstock characterization

J. curcas fruit parts (de-oiled seed kernel, seed coat, and fruit coat) and fruit waste (mango peels) were used as substrates. The *J. curcas* seeds and fruits were purchased from a local merchant (pansari) in Peshawar Khyber Pakhtunkhwa, Pakistan. The different parts of *J. curcas* fruits were transferred in ziploc bags and kept at 4 °C. Mango peels were taken from the local market of Quaid-i-Azam University Islamabad and stored at − 20 °C until use. Carbon, hydrogen, nitrogen, and sufur (CHNS) analysis of de-oiled seed kernel and mango peels was performed using a CHNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany; access: varioEL cube V1.2.1 2009-1-27).

2.4 Anaerobic digestion

Anaerobic digestion of *J. curcas* fruit was carried out in continuous mode to assess the effect of C/N ratio and in batch mode to evaluate biogas potential. For continuous experiments for mono- and co-digestion, 2.5-L reactors with working volumes of 2 L were used. In the batch setup, the biogas potentials of de-oiled seed kernel, seed coat, fruit coat, and de-oiled seed kernel + seed coat (seed coat pretreated with 1% H_3PO_4) were determined. For batch experiments, 0.5-L reactors with working volumes of 0.4 L were used. The reactors were kept at 37 °C in both batch and continuous experiments.

2.4.1 Continuous anaerobic digesters to evaluate the effect of C/N ratio

In continuous mode experiments, mono-digestion and co-digestion of de-oiled seed kernel and mango peels were investigated. The substrates were crushed to a fine size to avoid any hindrance during feeding, and stored at − 20 °C. During co-digestion, mango peels and de-oiled seed kernel were fed in 2:1, 1:1, and 1:4 ratios (by VS weight). The hydraulic retention time of all reactors was 20 days, and were fed daily until the reactor reached quasi-steady-state conditions, i.e., when the difference in biogas production was less than 5% during three consecutive readings. The effluent flow rate was 0.1 L/day, and the organic loading rate was 1.5 g VS/L day. The biogas produced was collected at room temperature (~ 26 °C) and measured using a water displacement method. The obtained biogas volumes were converted into normalized L at standard temperature and pressure (STP; 273.15 K and 101,325 Pa). To quantify methane content during steady state, a scrubbing solution (3 M NaOH) was used for the removal of CO_2 in the last few days of all continuous reactor runs. The volatile fatty acids (VFAs) and alkalinity of the effluent were measured at regular intervals. The actual biogas and methane yields obtained during co-digestion of mango peels plus *J. curcas* de-oiled seed kernel (MP + SK) at different ratios were compared with calculated yields. The mean values of actual yields from mono-digestion of each substrate (mango peels and de-oiled seed kernel) were used to determine calculated yields for co-digestion, using the same ratios as used in co-digestion.

Dilute acid pretreatment Fruit coat and seed coat were separately pretreated with 1% dilute H_3PO_4 to compare their biogas potential with non-pretreated fruit coat and seed coat, respectively. Moreover, the pretreated seed coat was also combined with de-oiled seed kernel (naturally present in the weight ratio 1.7:1; sample abbreviated as SK + SC(H_3PO_4). Pretreatment involved adding a specific amount of each substrate based on VS (Table 1) to 1% dilute H_3PO_4 solution in triplicate and autoclaving at 121 °C and 103,421 Pa (15 psi) for 20 min. The pH was then adjusted to 7 by the addition of either 6 M NaOH or 1 M HCl solutions (as needed). The non-pretreated samples along with controls were added to a volume of 50 mL distilled water.

2.4.2 Batch digestion: biogas potential and acid pretreatment

For batch anaerobic digestion, the substrates were incubated in 0.5-L bottles in triplicate with a 4:1 ratio (VS basis) of inoculum to substrate. The calculated amounts of inoculum and substrate were added to the reactors to a working volume of 0.4 L (Table 1). The reactors were incubated at 37 °C in a water bath, and biogas was collected in air-tight bags and measured with a syringe. The biogas measurements were converted into normalized mL (at STP). After filling the reactors with inoculum and the respective substrates, the reactors were flushed with nitrogen gas and then sealed with a rubber stopper. The incubation period was 60 days.

2.5 Statistical analyses

All of the yields in batch and continuous modes were presented as mean values ± standard deviation. Multiple mean comparisons using Bonferroni's and Scheffe's tests were used to check significant differences in batch and continuous modes, respectively, with level of significance at $p < 0.05$.

3 Results and discussion

3.1 Effects of carbon-to-nitrogen ratio on biogas yield of de-oiled seed kernel

Nitrogen present in organic substrate is used by microorganisms for synthesis of amino acids, proteins, and nucleic acids. A low amount or absence of nitrogen may cause the washout of microbial communities during anaerobic digestion, ultimately resulting in lower biogas yield or reactor failure. Organic nitrogen is converted to ammonia, a strong base that neutralizes the volatile acids produced by fermentative bacteria, thus maintaining pH conditions needed for the growth of microorganisms. However, an increase in total ammonia nitrogen (free ammonia nitrogen plus ammonium nitrogen) concentration to above 3 g/L will have toxic effects on methanogens and cause reactor failure [18, 19]. Thus, the appropriate concentration of nitrogen in the feedstock is needed to simultaneously avoid nutrient limitations and ammonia toxicity, and an imbalance in C/N ratios can have large effects on biogas yield and microbial activity [20]. The optimum

Table 1 Summary of batch biogas potential tests of different parts of *Jatropha curcas*

	Inoculum only	Inoculum + cellulose	Inoculum + fats	Seed kernel	Seed coat (without pretreatment)	Seed coat (pretreated)	Fruit coat (without pretreatment)	Fruit coat (pretreated)	Deoiled seed kernel + seed coat (pretreated) *
Inoculum (g)	400	397.1	397.1	396.8	395.3	395.3	396.3	396.3	396.03
De-oiled seed kernel (g)	0	0	0	3.13	0	0	0	0	2.53
Seed coat (g)	0	0	0	0	4.68	4.68	0	0	1.44
Fruit coat (g)	0	0	0	0	0	0	3.72	3.72	0
Cellulose (g)	0	2.90	0	0	0	0	0	0	0
Fats (g)	0	0	2.90	0	0	0	0	0	0
H_3PO_4 (1% w/v) (mL)	0	0	0	0		50	0	50	25
Water (mL)	50	50	50	50	50	0	50	0	25
Working volume (mL)	400	400	400	400	400	400	400	400	400
Total adjusted volume (mL)	450	450	450	450	450	450	450	450	450
Inoculum/substrate (VS/VS)	4:1	4:1	4:1	4:1	4:1	4:1	4:1	4:1	4:1

*De-oiled seed kernel was mixed with seed coat (pretreated) in 1.7:1 ratio to mimic the actual composition of *Jatropha curcas* seed, when deoiled

range of C/N ratio for biogas production has been reported to be 20:1 to 30:1 in anaerobic digestion [11, 12]. Since the C/N ratio of de-oiled seed kernel of *J. curcas* is low (11), addition of a substrate with high C/N ratio (mango peels with a C/N ratio of 53) was investigated.

Co-digestion of high nitrogen containing substrates with high carbon containing substrates to achieve a more optimal C/N ratio is considered an effective strategy to enhance biogas yield. During mono-digestion, the steady state biogas and methane yields of seed kernel (Table 2) were 21.9% and 22.7% higher than those of mango peels. The biogas yield of de-oiled seed kernel was significantly higher ($p < 0.05$) than that of mango peels (Fig. 1a, b). During mono-digestion of de-oiled seed kernel, the process reached pseudo-steady state on day 23, with less than 5% variation in biogas production for the next eight days (Fig. 1b).

During mono-digestion of mango peels, steady state was reached on day 26 and continued for the next 10 days (Fig. 1a). The biodegradability of de-oiled seed kernel in continuous mode was higher than that of mango peels, indicated by the higher VS reduction. There were higher variations in the reactor pH of mango peels than for the de-oiled seed kernel during anaerobic digestion. The pH range (Table 2) during de-oiled seed kernel digestion (6.58–7.22) was closer to the normal range of 6.5–7.6 [21] than in the digestion of mango peels (5.91–7.04), indicating the relative stability of the reactor treating de-oiled seed kernel. The lower pH of the mango peels in the feed (4.7–4.8) affected digestion, resulting in more fluctuations in biogas yield of mango peels compared with that of the seed kernel (feed pH of 6.12–6.25). The pH in the reactor treating mango peels dropped below the normal range on days 26 and 32, and 2–3 g $NaHCO_3$ was added to bring the pH back to normal range. The pH during co-digestion of MP + SK at 1:4 was in the normal range (Table 2), while the pH was below the normal range during co-digestion of MP + SK at 1:1 and 2:1 (Fig. 1c, d). The pH

Table 2 Mono-digestion and co-digestion results of mango peels and de-oiled seed kernel during anaerobic digestion in continous mode

Conditions	Average daily biogas production rate (NL/L day)	% CH_4	Average daily CH_4 production rate (NL/L day)	VS reduction (%)	pH range	C/N ratio
Mango peels	0.37	67	0.25	52	5.91-7.04	53
De-oiled seed kernel	0.49	70	0.34	69	6.58-7.22	11
Mango peels + de-oiled seed kernel (2:1)	0.70	61	0.42	56	6.40-7.0	39
Mango peels + de-oiled seed kernel (1:1)	0.74	66	0.49	65	6.44-7.0	32
Mango peels + de-oiled seed kernel (1:4)	0.93	71	0.66	70	6.84-7.42	20

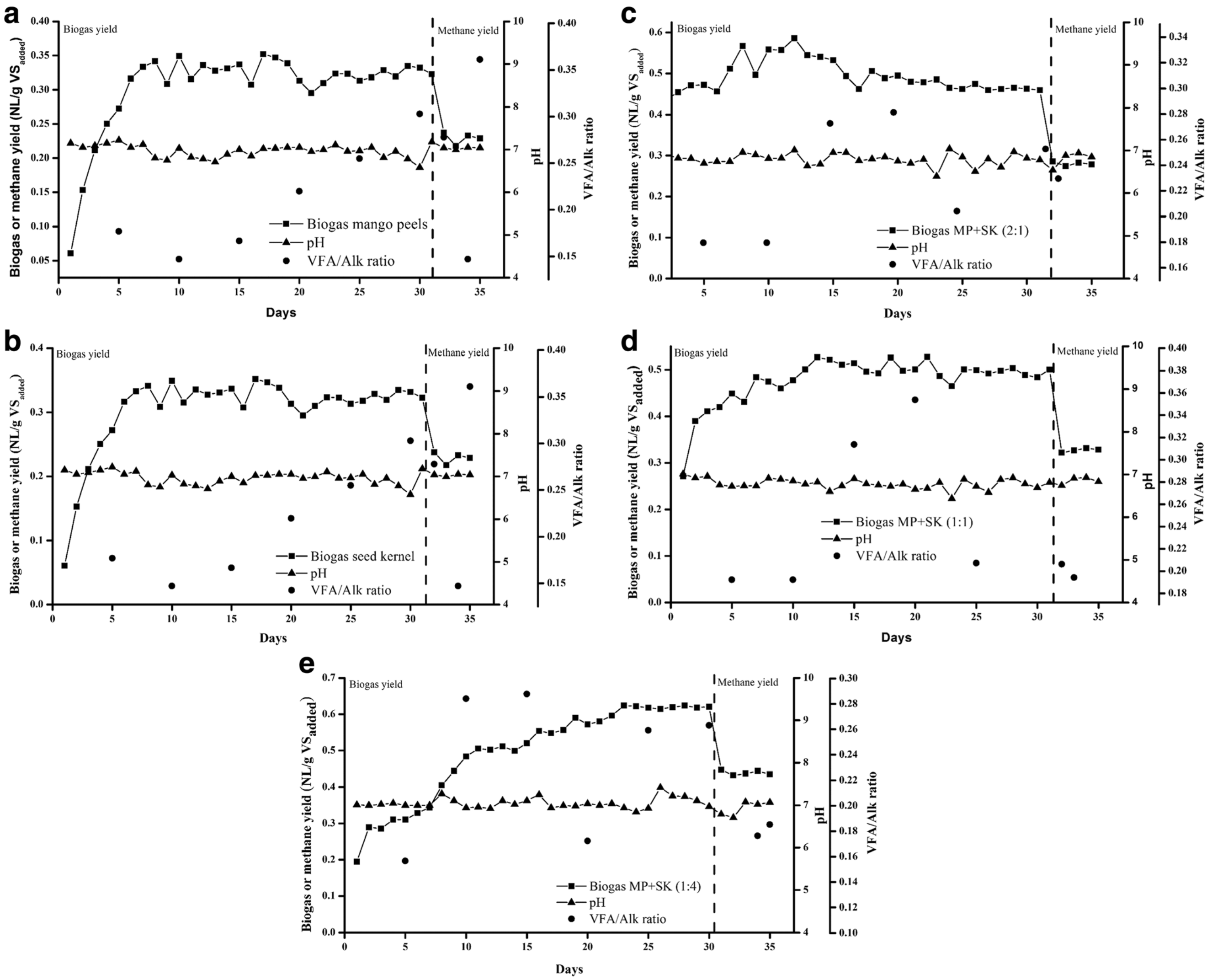


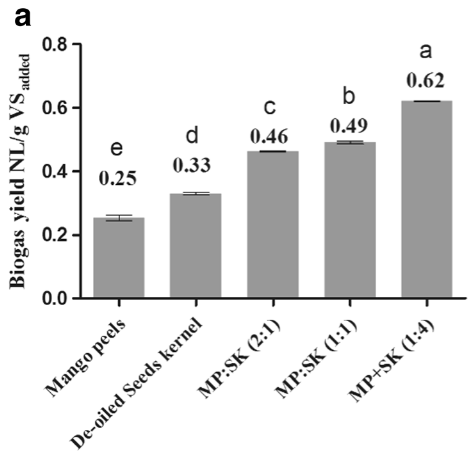
Fig. 1 Biogas and methane yield, normalized liter per gram VS, during continuous anaerobic mono- and co-digestion of mango peels and de-oiled seed kernel. **a** Mango peels, **b** seed kernel, **c** mango peels and de-oiled seed kernel (MP + SK) in 2:1 ratio, **d** mango peels and de-oiled seed kernel in 1:1 ratio, **e** mango peels and de-oiled seed kernel in 1:4 ratio. The methane yields are shown only during pseudo steady state (days 31–35)

during co-digestion of MP + SK at 1:4 was in the normal range (Table 2), while the pH was below the normal range during co-digestion of MP + SK at 1:1 and 2:1 (Fig. 1c, d). The VFA-to-alkalinity ratios of de-oiled seed kernel (Fig. 1b) and of MP + SK co-digestion 1:4 and 2:1 were in the normal range (0.1–0.2) (Fig. 1c, e) [22].

The biogas yield during co-digestion of MP + SK in 1:4 was significantly ($p < 0.05$) higher than all other treatments in continuous mode (Fig. 2a). The biogas yield of mango peels plus de-oiled seed kernel (MP + SK at 1:4) was 25.8% and 30.3% higher, respectively, than the yields at 1:1 and 2:1 ratios, and 51.5% and 62.1% higher than the biogas yields of seed kernel and mango peel mono-digestion, respectively. Similarly, the methane yield of MP + SK at 1:4 was also significantly higher ($p < 0.05$) (25 and 36.4% higher, Table 2) than the co-digestion at 1:1 and 2:1 (Fig. 2b), respectively.

The methane yield also showed the same pattern; the methane yields during co-digestion at 1:4 were 25%, 36.4%, 50%, and 61.4 % higher than the 1:1, 2:1, seed kernel (mono-digestion), and mango peels (mono-digestion) methane yields, respectively (Fig. 3b).

The actual biogas yield of MP + SK in 1:4 (Fig. 3a) was significantly higher ($p < 0.05$; 50% increase) than the corresponding calculated yield. Similarly, the actual biogas yield of MP + SK in 2:1 was significantly (41.7%) higher than the corresponding calculated biogas yield. However, the actual biogas yield of MP + SK in 1:1 was 6.5% lower than the corresponding calculated yield. The actual methane yields of MP + SK in 1:4, 1:1, and 2:1 ratios were significantly higher



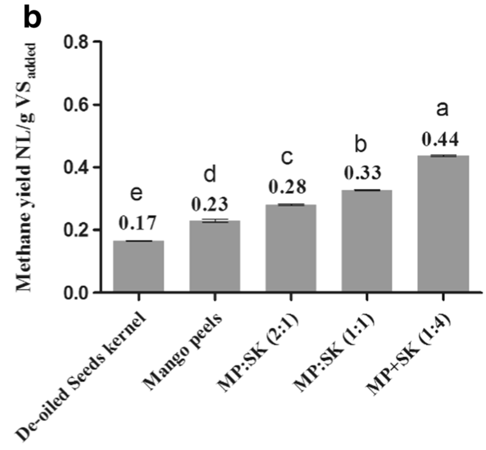


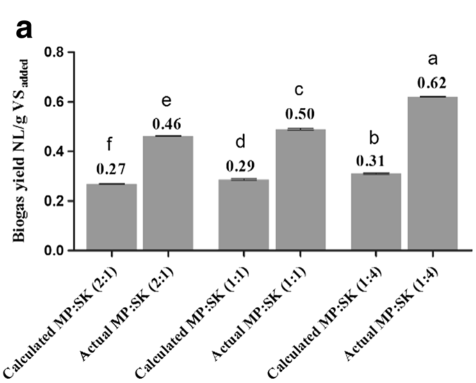
Fig. 2 Comparison of biogas and methane yield of mango peels and de-oiled seed kernel in mono-digestion and co-digestion in continuous setup. **a** Biogas yield of mango peels and de-oiled seed kernel mono-digestion and co-digestion (MP/SK; 2:1, 1:1, and 1:4). **b** Methane yield of mango peels and de-oiled seed kernel mono-digestion and co-digestion (MP/SK; 2:1, 1:1, and 1:4). MP mango peels, SK de-oiled seed kernel. Data are means ± SD. For comparisons of all treatments, one-way ANOVA, followed by Scheffe post-test, was used. Different letters on the bars show significant differences ($p < 0.05$) between treatments

($p < 0.05$) with 50%, 39.4%, and 32.1% increases, respectively, compared with the calculated corresponding methane yields (Fig. 3b). The results show that as the C/N ratio increases from 20:1 to 39:1, the normalized methane yield increases, consistent with previous studies [11, 12, 23]. C/N ratios of the mono-substrates tested here (11:1 and 53:1) were too low or too high, respectively. These findings underscore using the appropriate range of C/N ratios to maximize methane production in anaerobic co-digestion. The higher actual methane yields compared with calculated yields based on mono-digestion yields and proportional mixes may indicate synergistic impacts of co-digestion that cannot be predicted by single-substrate digestion studies. These again may be based on more optimal C/N ratios, but may also indicate other positive interactive effects on the microbial populations involved.

The VS reduction was higher during the anaerobic co-digestion of MP + SK in 1:4 compared with those at 1:1 and 2:1 (Table 2), again showing that more optimal C/N ratios lead to higher VS reduction and consequently higher methane production [24]. These results clearly indicate that for a stable biogas reactor with efficient biogas and methane yield, the C/N ratio must always be in the range of 20–30.

3.2 Batch anaerobic digestion of different parts of *Jatropha curcas* fruit

The pretreated fruit coat was more easily biodegradable than the rest of the substrates. The biogas yield of fruit coat (pretreated with 1% phosphoric acid) was significantly ($p < 0.05$) higher than de-oiled seed kernel, fruit coat (without pretreatment), de-oiled seed kernel plus seed coat (acid-pretreated), seed coat



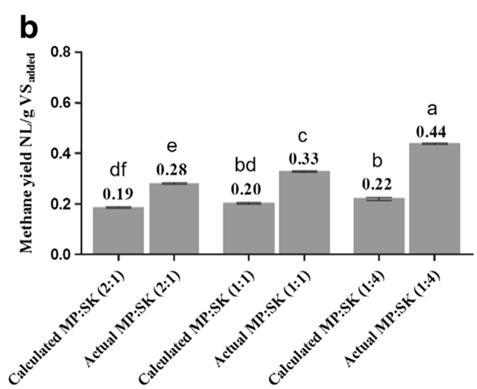


Fig. 3 Comparison of actual and calculated biogas and methane yield of mango peels and de-oiled seed kernel co-digestion in different ratios. **a** Mango peels and de-oiled seed kernel co-digestion at different ratios. **b** Mango peels and de-oiled seed kernel co-digestion at different ratios. Data are means ± SD. For comparisons of all treatments, one-way ANOVA, followed by Scheffe post-test, was used. The alphabetical annotation shows significant ($p < 0.05$) and non-significant difference between calculated and actual yields of each substrate in same ratios

(acid-pretreated) and seed coat (without pretreatment), by 7%, 22%, 34%, 50%, and 74%, respectively (Fig. 4a). Pretreatment with acid also improved biodegradability. The pretreated fruit coat and seed coat had 22% and 47% higher biogas yields, respectively, than their non-pretreated counterparts. Phosphoric acid hydrolysis of hemicellulose and cellulose containing organic biomass causes release of more sugars compared with other acids [25] that are directly available for microbial communities for use. Dilute sulfuric acid pretreatment is also effective, but may lead to competition between sulfate-reducing bacteria and methanogens [26]. The biogas yields (Fig. 4a) show that fats and cellulose had the highest yields (normalized per g VS added) indicating that the inoculum used contained the necessary consortia required for methanogenesis. The biogas yield of fats and cellulose (Fig. 4a) was below the theoretical methane yield of carbohydrates and lipids, which have been reported to be 0.415 L/g VS_{added} and 1.014 L/g VS_{added}, respectively [27].

The calculated biogas yield of SK + SC (H_3PO_4) was 219.44 NmL/g VS_{added}, which is 16.7% higher than the actual yield. It is evident that the seed coat (either pretreated or non-pretreated) reduced the co-digestion biogas yield potential of the de-oiled seed kernel. The seed kernel is rich in fibrous proteins and lipids, while the seed coat in seed cake is mainly composed of hemicellulose followed by cellulose and lignin [28, 29]. With acid hydrolysis, hemicellulose releases sugars which are sometimes subsequently converted to inhibitory products such as furfural, hydroxymethyl furfural, and acetic acid, which can be inhibitory to microorganisms. In addition, the subsequent conversion of sugars to these inhibitors also causes sugar loss which leads to decrease in biogas yield [25, 30, 31]. The high concentration of lignin (49.4% content in the seed coat) may also be the reason for the low biogas yield, since lignin prevents microbial access to cellulose and sugars [32]. *Jatropha curcas* seeds are composed of a number of toxic compounds such curcin, lectin, flavonoids, vitexine, trypsin inhibitors, isovitexine and phorbol esters, tannins, steroids, saponin, phenolics, glycosides, and volatile oils [33, 34]. Some of these phytochemicals play role in plant defense against microbial attacks. Being phytochemically rich, *J. curcas* has been traditionally used to treat various diseases and infections such dysentery, infertility, coated tongue, gonorrhea, hemorrhoids, skin infections, and inflammations [35]. These compounds are also well known for their antimicrobial effects and may inhibit different enzymes and inocula used for anaerobic digestion process. Therefore, pretreatment or their extraction is considered one of the most feasible solutions to reduce the toxicity of *J. curcas* seed and increase its biogas yield. Moreover, as these phytochemicals are known for their antimicrobial effects, it will be interesting to evaluate in the future their inhibitory effects on the different steps of anaerobic digestion process and the microbial communities involved in anaerobic digestion using metagenomic approaches. In addition, the biogas yield of *J. curcas* seed and process stability may also be increased by extracting these antimicrobial phytochemicals.

Initially, all of the different parts of *J. curcas* fruit exhibited an increase in biogas production rate (Fig. 4), with the pretreated fruit coat followed by non-pretreated fruit coat having the highest rates of biogas production. After 5 days, the biogas production in both these substrates slowed down and became stable. On the other hand, the biogas production from de-oiled seed kernel kept increasing after a slow start, eventually reaching the second highest biogas production rate by ~

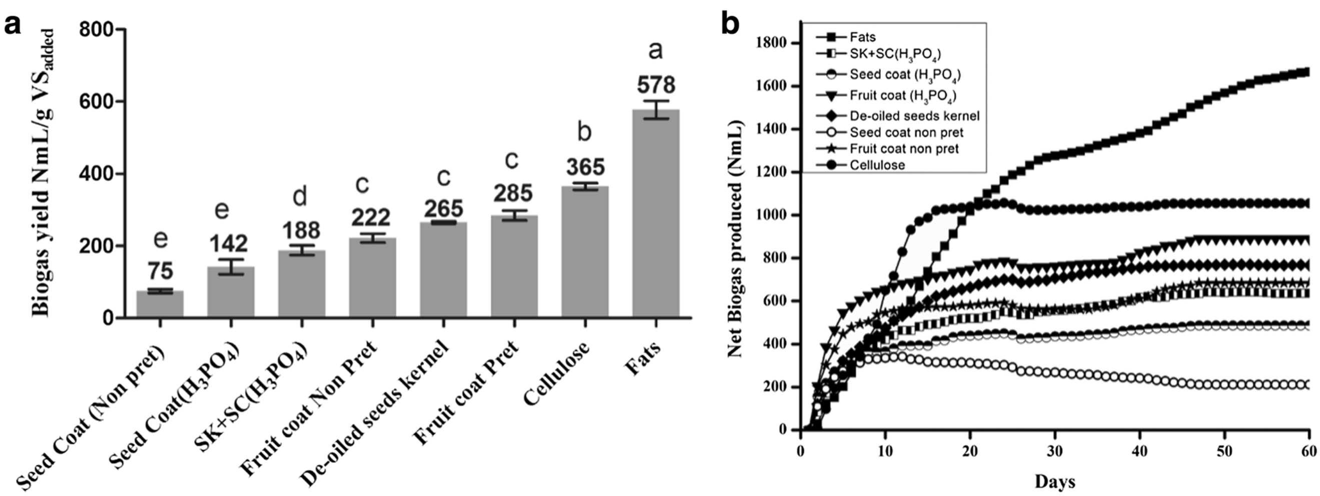


Fig. 4 Evaluation of biogas potential of different treatments of *J. curcas* fruit during batch anaerobic digestion setup. **a** Biogas yield presented as NmL/g VS_{added}. SK + SC (H_3PO_4) (co-digestion of *J. curcas* de-oiled seed kernel and seed coat (pretreated) mixed in 1.7:1 ratio and the seed coat was pretreated with 1% phosphoric acid); H_3PO_4 means pretreated samples. Data are means ± SD. For comparisons of all treatments, one-way ANOVA, followed by Bonferroni's post-test, was used. The alphabetical annotation shows significant ($p < 0.05$) and non-significant ($p > 0.05$) difference among the treatments. The identical alphabets on bars show non-significant, and the different alphabets shows significant difference among them. **b** Net biogas produced is presented as normalized mL (at standard temperature 273.15 K and pressure 1.013 bar) by different parts and combinations of parts of *J. curcas* fruit. The reactors were incubated at 37 °C for 60 days

15 days. Typically, the biodegradation of fats starts slower than that of sugar or protein-containing compounds [36]; this was also shown by the biogas production in the control (fats). The biogas production rate of fats was initially slow, but later had the highest biogas production rate (Fig. 4b).

4 Conclusions

To increase the biogas and methane yield compared with the calculated yield in large-scale reactors, the co-digestion of mango peels with de-oiled seed kernel of *J. curcas* is suggested. A C/N ratio close to 20:1 is very important for enhanced biogas yield and stability of reactor. This can be achieved by a 1:4 ratio of mango peels to de-oiled seed kernel. Moreover, dilute phosphoric acid (1% H_3PO_4) pretreatment of lignocellulosic materials enhanced biogas yield compared with non-pretreated biomass during anaerobic digestion. An optimized C/N ratio and dilute H_3PO_4 pretreatment are highly recommended for a more efficient anaerobic digestion process.

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Author contributions AH and MB designed this study. AH performed the experiments. AK, SK, AAS, FH, FdlR, and SA participated substantially in discussion and modifications. All authors read and approved the final manuscript.

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Data availability All relevant data are included in this manuscript.

Compliance with ethical standards

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

Code availability Not applicable.

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