

Plant Seed-Based Bio-coagulant Development and Application for Fecal Sludge treatment and Biogas Production Improvement.

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Abstract

This work addresses the development and application of *Cucumeropsis mannii* and *Luffa acutangula* seed proteins as novel bio-coagulants for fecal sludge concentration to increase the biogas yield. The seed protein content was determined and extracted using 1M NaCl, isolated and precipitated by ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$, and dialyzed to obtain the purified proteins. Their coagulation/flocculation was compared to *Moringa oleifera* and pumpkin seeds as the primary treatment for the fecal sludge concentration. Optimal coagulant dosages of 20 mg/l at pH 7.25 increased the total solids from 0.4–2.8% and obtained 180 to 350 ml/l of fecal sludge volume per liter. The bio-coagulants effect on the supernatant water reduced 71.20% to 96.88% of turbidity, 76.48–88.70% of COD, and 81.33 to 95.30% of ammonia nitrogen. The BMP result has shown that plant seed-based bio-coagulant are effective and triple the methane yield result compared to the FS without bio-coagulant treatment with a specific methane yield of 192.8 NmL CH_4 /gVS, and FS with bio-coagulant pretreatment was increased up 350.5 to 465.3 NmL CH_4 /gVS, with a ratio 2:1 on VS basis.

Highlights

- *Cucumeropsis mannii* Seed Proteins based Bio-coagulant.
- 1M NaCl and 30-60% of ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ for proteins extraction.
- Dialysis for Protein purification.
- Jar test for coagulant/flocculant capacity.
- Biomethane Potential test for methane yield.

Introduction

The technology of biogas is more and more adopted due to many advantages of anaerobic digestion (AD), providing electricity, controlling the diffusion and pollution of waste (Jenicek, Koubova et al. 2010), abatement of GHG, promoting and production of organic fertilizer for modern agriculture (Burg, Bowman et al. 2018). AD process comprises hydrolysis, acetogenesis, acetogenesis, and methanogenesis to produce the methane CH_4 as the leading natural gas (Chen and Neibling 2014, Li, Chen et al. 2019). It's a promising and sustainable technology convenient for the management of organic waste (Holm-Nielsen, Al Seadi et al. 2009), considered the most environmentally friendly process in terms of energy consumption, low biomass production, and its high pathogen removal rate during the waste transformation into gas and digestate (Castro, Escalante et al. 2017, Logan and Visvanathan 2019). Effective biogas recovery from fecal sludge depends on the treatment process used. Fecal sludge concentrates from the coagulation process have proven to produce a suitable concentrate with a COD value ideal for the bioenergy recovery (Gong, Jin et al. 2015).

Plant-seeds-based bio-coagulants could provide adequate and affordable water and wastewater treatment solutions worldwide. Bio-flocculants generally have received attention recently due to their biodegradability strength and lack of secondary pollutants from their degradative intermediates. The term "Plant-seed based bio-coagulant" is related to water-soluble ionic or non-ionic polymers with various molecular weights and coagulant capacity from the organic plant-seed (Dezfooli, Uversky et al. 2016, Saleem and Bachmann 2019). Generally, plant-seed-based bio-coagulants are mainly identified through phytochemical tests on leaves, barks, roots, seeds, and even gums through the quantitative and qualitative primary or secondary metabolism tests to determine and extract the functional coagulating compounds (Croteau, Kutchan et al. 2000, Taiz and Zeiger 2006). These active coagulating compounds could be classified as polysaccharides (Shamsnejati, Chaibakhsh et al. 2015, Kurd, Fathi et al. 2017, Kebaili, Djellali et al. 2018), amino-polysaccharides (Lee, Chong et al. 2014), polyphenolics (Wang, Liang et al. 2013, Wang, Yu et al. 2014, Wang, Zhao et al. 2018, Parsaeian, Dadfarnia et al. 2022), proteins (Baraniak, Świeca et al. 2009, Liang and Garcia 2021, Yamaguchi, Cusioli et al. 2021), tannins, proteolytic enzymes (Liu and Smith 2021), or glycoproteins (Pathak, Devi et al. 2015, Alam, Vandamme et al. 2016, Qi, Zhu et al. 2019).

The *Cucumeropsis mannii* and *luffa acutangula* are members of the Cucurbitaceae family with multiple nutritional and medicinal benefits (Schaefer and Renner 2010). Their seeds are rich in protein and edible oil source (Kortse and Oladiran 2013). The *cucumeropsis mannii* seeds contain about 30–38% protein (Mbuli-Lingundi, Belitz et al. 1983, Houdegbe, Sogbohossou et al. 2016), and *luffa acutangula* kernel is potentially rich in protein and fat (39 and 44%), higher than those contained in many plant seeds (Shendge and Belemkar 2018). These proteins can be isolated, extracted, and used as a plant seed-based bio-coagulant. Their seed protein is healthy and safe to use (Ogunbusola, Fagbemi et al. 2012). However, using the plant-based bio-coagulant improves the sanitation system and wastewater treatment, as these plants do not affect human health and do not pollute the environment (Ahmad, Kurniawan et al. 2021).

Plant-seed-based bio-coagulants have been used as effective natural coagulants for sludge dewatering. *For example*, *Abelmoschus esculentus* (okra) can remove 98% turbidity and recover 68% of water during sludge dewatering (Lee, Chong et al. 2015). *Opuntia ficus Indica* (prickly pear cactus) juice has been used effectively for dewatering sewage sludge by adding (0.4 g/kg) as the optimum dosage with serum response factor (SRF) = $(0.13 \times 10^{12} \text{ m/kg})$ (Betatache, Aouabed et al. 2014). Their application protects human safety and preserves the environment.

This study assessed the analysis and extraction of *Cucumeropsis mannii* seed and *luffa acutangula* protein content as a bio-coagulant for the fecal sludge treatment or organic matter concentration to improve the biogas yield. Their coagulation/flocculation capacity compared to the existing bio-coagulants such as Pumpkin and *Moringa oleifera* seeds, allowing fecal sludge concentration and the biogas yield.

Materials And Methods

2.1 Materials

2.1.1 Seeds sample

The *Cucumeropsis mannii*, *Luffa acutangula*, *Moringa oleifera*, and pumpkin seeds were collected, cleaned, dried, ground, sieved, and the powder was used as raw material.

2.1.2 The wastewater (fecal sludge) sample

Wastewater samples were collected from public and private toilets from the Nanjing Academy of Resources and Ecology Science public toilet and farmer location in Nanjing (Jiangsu, China). FS samples were stored in a 20L bottle and transported to the Nanjing Academy of Resources and Ecology Science laboratory, where they were stored at 4 °C until analysis.

2.2 Methods

2.2.1 Qualitative analysis

10 ml of distilled water was added to 2g of the plant's seed powder and filtrated. The filtrated solution is considered an "extract solution."

- **Test for protein**

Biuret test: 1 ml of the extract solution was mixed with 1 ml of 4% copper sulfate. The color change (violet or pink) confirms the protein presence.

Million's Test: one part of mercury was digested with two parts of HNO₃, and the resulting solution was diluted with two volumes of water. 5 to 6 drops of water million's reagent were added to 1ml of extract solution. The white precipitates that turn to red color when heated show the protein's presence.

- **Test for oil and fats**

1ml of extract solution was mixed with 3 drops of 0.5N alcoholic potassium hydroxide and a depth of phenolphthalein and heated in a water bath for 2 hours. The presence of oil and fats is indicated by the development of soaps in the solution.

2.2.2 Quantitative analysis

The quantitative analysis was performed according to AOAC's approach (William 2000).

- **Crude protein content**

1g of *Cucumeropsis mannii* seeds powder was taken, and 6 ml of concentrated sulphuric acid was added and left to stand for 24 hours after 3.5 ml of H₂O₂ (30 %) was added slowly to the solution. When the violent reactions stopped, it was shaken and left in the rack. 3 g of accelerated reagent (a mixture of copper sulfate pent hydrate and anhydrous potassium sulfate) was added and left for 15 minutes, and digested. The mixture was stored at 37 °C for 4 hours.

After digestion, it was cooled in a hood on the rack, and 25 ml of distilled water was added to dissolve the residue; 25 ml of 40 % NaOH was added to the digested sample and placed in the distiller. 25 ml of boric acid (H₃BO₃) saturated solution and 25 ml of distilled water were added to the 250 ml conical flask, followed by three drops of methyl red. The solution was placed in the distiller, and 150-200 ml of distillate was taken after distillation and titrated with 0.1 M HCl. The protein amount was given using the formula.

$$\text{Protein (\%)} = \frac{\text{Titre value} \times 0.0014 \times 6.25}{\text{weight of sample}} \times 100$$

(1)

- **Fat content**

A cleaned flask was dried at 105°C for 1 hour, cooled, and weighed in the oven. 50 ml of petroleum ether was added to the flask. A thimble containing fat-free cotton and 2g of *Cucumeropsis mannii* seeds powder was added to the petroleum ether and heated at 80°C in the fat determination apparatus for 1 hour. The thimble was hung and heated at the same temperature for 2 hours, and then the solvent was recovered for 15 minutes. The flask was dried again in the oven at 90°C for 30 minutes, cooled, weighed containing the extract, and then calculated the fat extracted.

$$\text{W of fat} = \frac{\text{W flask after extraction} - \text{W flask before extraction}}{\text{W of sample}} \times 100$$

(2)

2.2.3 *Cucumeropsis mannii* seeds Coagulant preparation

2.2.3.1 Seeds preparation

The preparation is divided into three parts, and the first part consists of seed shell removal, cleaning, drying, powdering, sieving to obtain a uniform size, and oil removal. The second part includes the extraction of the protein using salt solution. The third part consists of protein purification using different centrifugation and dialysis techniques.

2.2.3.2 Oil removal

The *Cucumeropsis mannii* seeds oil was removed using hexane as solvent via Soxhlet extraction. 10 g of the seeds powder fed in electrothermal Soxhlet apparatus and added 180 ml of solvent, and the extraction was run for 6h in triplicate ($n = 3$). Therefore, *Cucumeropsis mannii* seed preparation or treatment generates some by-products such as shells removed with a knife or using the adapted machine could be converted into active carbon, the oil extracted from the seeds using electrothermal Soxhlet apparatus with organic solvent (hexane) to avoid the propagation of this oil in the treated water, can be transformed into edible oil, and the sludge produced can be used as a biofertilizer (Fig.1)

After the extracted oil yield was $36.5\% \pm 1.85\%$ representing a weight percentage (mean $\pm$ standard deviation), and the protein content of the defatted residues after the oil extraction was higher ($34.48\% \pm 2.03\%$ against $28.0\% \pm 1.45\%$) by evaluating the Nitrogen Kjeldahl (protein = $N (\%) \times 6.25$). This increase in protein content can help in the efficiency of *Cucumeropsis mannii* seeds powder solutions as bio-coagulant.

The oil extraction process can produce by-products that can easily be treated or recovered on a laboratory or industrial scale. The husk, the sludge from seeds grinding and sieving, can be converted into activated carbon, and the oil extracted also can be used as edible oil. This process is advantageous in managing this waste and contributes economically by selling these by-products.

2.2.3.3 Seeds proteins extraction

10% w/v or g of seed powder obtained after the oil extraction was mixed within 100 ml of 1M of NaCl solution, stirred for 15 minutes and stored at room temperature for 30 min.

The presence of salt in the solution improves the ionic power and stimulates the high solubility of protein components (Garcia-Fayos, Arnal et al. 2016). The solution was centrifuged at 11000 rpm for 15 minutes and filtrated using a vacuum. Before the protein isolation, the filtrated solution was considered unpurified protein solution and stored in the refrigerator at 4°C. The solution was used as an "extract solution" for protein isolation.

2.2.3.4 Protein isolation

The protein isolation by adding 30-60% of (NH₄)₂SO₄ into 1M NaCl at room temperature and with a natural pH was used (Dezfooli, Uversky et al. 2016). 19,74g was poured slowly into 100 ml of 1M NaCl extract samples, gently mixed on magnetic stirred for 10 min, and stored in a temperature room to allow the protein precipitation reaction.

As a result, protein aggregates formed in the solution. The solution was centrifuged at 11,000 rpm for 15 minutes, and the protein agglomerates or heterologous white mater aggregates were collected and used for the purification process.

2.2.3.5 Protein purification by dialysis method

10 ml of Tris Buffered Saline (TBS 10x concentration) dissolved large protein aggregates. According to the amount of the protein sample, the dialysis membrane was put in 80°C tap water for 10 min and transferred to the buffer for 15 min before use.

The membrane was filled with the protein sample and placed in the buffer with continuous stirring for 8 hours. The buffer was exchanged each 2h. The interior of the dialysis membrane was expanded due to the alternation of the dialysate buffer and the sample buffer. After the complete dialysis, the proteins were purified and used in the jar test.

3 Jar test

The coagulation/flocculation test was performed using jar test apparatus to determine and evaluate the coagulant capacity and efficiency on turbidity, COD, ammonia-nitrogen removal, and the organic matter concentration.

The experiments were performed using the proteins purified by dialysis. The coagulant/flocculant test uses different dosages (4-20mg/l) of unpurified and purified proteins as bio-coagulants. The rapid mixing allows the coagulant and organic matter mixing (200 rpm - 2 min.), slow mixing for flocculation (45 rpm - 10 min.), clarifier mixing (20 rpm - 10min), and the organic matter setting up (sedimentation) (0 rpm-30min).

After the sedimentation, their bio-coagulant capacity was tested by comparing their effectiveness on turbidity, COD, and ammonia nitrogen removal. The volume of organic matter in each treatment process is evaluated to analyze the fecal sludge concentration using these bio-coagulants. The turbidity and pH were repeated 4 times, except the COD and ammonia nitrogen were tested in triplicates.

4 Bio-methane potential test

The biomethane potential (BMP) test was performed for 30 days commonly at 37°C and monitoring the production of biogas together with its composition throughout the test to determine the anaerobic biodegradability, or the biogas yield of the fecal sludge pretreated using the plant seed-based bio-coagulant. Three criteria were used to evaluate BMP anaerobic feedstock in the lab, and this includes feedstock characterization: This involves the test for chemical oxygen demand (COD), volatile suspended solids (VS), and total suspended solids (TS). Triplicates of each combination inoculum-substrate were used.

- 3 reactors as blanks (containing only inoculum and (optional) distilled water were used to analyze the amount of the biogas produced by the inoculums themselves and test the quality.
- 3 reactors with inoculum + substrate (samples)

- 3 reactors as positive controls (containing raw fecal sludge + inoculum) – to test the quantity of biogas produced by the fecal sludge without adding bio-coagulant.

The quantity of inoculum to substrate ratio was determined using the ratios of 2:1 (based on VS) (Tab.1).

The results obtained from the test can ascertain the concentration of organics in the pretreated fecal sludge that can be anaerobically converted to biogas or methane. This is then used to evaluate the potential efficiency of the anaerobic process for this specific substrate and the importance of the pretreatment.

Table 1. Preparation of the substrates and reactor

Bio-coagulants	TS (%)	VS (%)	Amount of the inoculum	Amount of substrate (FS)
Luffa acutangula	2.85	2.3	161.40 g	238.6g
Cucumeropsis mannii	2.5	2.07	151.37g	248.62g
Pumpkin	2.6	2.16	155.39g	242.35g
Moringa oleifera	2.6	2.2	157.14g	242.85g
Raw fecal sludge	0.4	0.4	42g	357.85g

Results And Discussion

5.1- Phytochemical test

Table 2
Phytochemical qualitative constituents

Test	Cucumeropsis mannii seeds	Luffa Acutangula seeds	Moringa Oleifera seeds	Pumpkin seeds
1- Test for protein and Amino-acids	+++	++	+++	++
(a) <i>Million's test</i>	++	+	+	+
(b) <i>Biuret test</i>				
2- Test for oil and fats	++	+++	+++	++
+++ = Highly present; ++ = Moderately present; + = Presence in trace amount.				

Table 3
Phytochemical qualitative constituents

	Cucumeropsis mannii seeds	Luffa Acutangula Seeds	Pumpkin Seeds	Moringa oleifera seeds
Parameters	Percentage DMB			
Protein	36.00 ± 0.24 ^a	35.83 ± 0.17 ^a	34.23 ± 0.1 ^a	36.73 ± 1.21 ^a
Fats	44.00 ± 0.58 ^b	37.93 ± 1.4 ^b	36.44 ± 0.3 ^b	38.22 ± 0.17 ^b

Values are means (n = 3),

DMB Dry matter base ¹: Carbohydrate, ^{a-b}: Values within a row with the same superscript means that there are no significant differences, but those with different superscript show significant differences (p < 0.05) by the Tukey's multiple comparison test; S.D.: Standard deviation

The seed protein obtained was similar to 36.00 ± 0.24 obtained by (Badifu and Ogunsua 1991), identical to Moringa oleifera 36.7%, and higher than luffa acutangula and pumpkin seeds protein content with 35.83 ± 0.17 and 34.23 ± 0.1 respectively. The fat (lipid) contents obtained 44.00 ± 0.56 were comparable to (Loukou, Gnakri et al. 2007), and (Anhwange, Ikyenge et al. 2010) result higher than the 42.29% obtained by (Abiodun and Adeleke 2010), while higher than that contained Moringa oleifera, Luffa acutangula and Pumpkin with 38.22 ± 0.17, 37.93 ± 1.4 and 36.44 ± 0.3 respectively.

Table 4. Protein content after oil removal

Plant seed	Proteins quantitative analysis	
	Before oil removal	After oil removal
Cucumeropsis mannii seeds	36.00±0.24 ^a	38.00±2.28
Luffa Acutangula seeds	35.83±0.17 ^a	37.43±1.18
Pumpkin	34.23±0.1 ^a	36.55±1.10
Moringa oleifera	36.73±1.21 ^a	38.63±1.36

The protein content of the seeds before and after oil extraction was determined by analysis of Nitrogen Kjeldahl (protein = N (%) × 6.25). Before extraction, the seed's protein content was (mean ± standard deviation, n = 3), and the protein content of the defatted residues after extraction was increased. This increase in the protein content should favor the coagulant effect of Moringa oleifera solutions.

5.2 Bio-coagulant selection result.

Table 5
The amount of ammonium sulfate to prepare the required percentage of a saturated solution.

Percentage of saturation	0–30%	30–60%	60–80%	80–100%
Amount of ammonium sulfate (for 100 ml solution)	17,83	19,74	14,18	15,36

The bio-coagulant test with 30–60% of (NH₄)₂SO₄ has better performance than others. Therefore in our research, the bio-coagulant with 30–60% of (NH₄)₂SO₄ was selected for each plant seed-based bio-coagulant.

5.3 Jar test result

Table 6
Fecal sludge characteristic

pH	TS (%)	VS (%)	Turbidity (NTU)	COD (mg/L)	TN (mg/l)	NH ₄ -N (mg/l)	T (°C)
7.58	0.78	46	4800	19306	2750	3282	25

The proteins extracted from the plant seeds have different turbidity removal efficiency (Fig. 4) and determine their capacity. The pumpkin seed's highest turbidity removal is 76.48%, the Cucumeropsis Mannii has 93.88%, the Luffa acutangula has 95.63%, and the Moringa oleifera has 96.88%. Their turbidity capacity is still limited because of the organic matter released in the water (Ghebremichael, Gunaratna et al. 2006).

The COD removal has different results (Fig. 5), with pumpkin seed's highest turbidity removal of 71.20%, The Cucumeropsis Mannii has 78.52%, the Luffa acutangula has 82.26%, and the Moringa oleifera has 88.76%. The purified proteins have high COD removal because the treatment process allowed for isolating and using the suitable proteins (Dezfooli, Uversky et al. 2016).

The ammonia nitrogen removal has been analyzed (Fig. 6). The plant seed-based bio-coagulant has high removal NH₃-N because the purification process has increased efficiency.

The sludge volume or concentration have observed and measured at the bottom of all beakers. The sludge volume obtained with the purified proteins has a high sludge concentration. Because the purified protein solution is more effective and generated large floc with a higher flocculate rate (1–2 min) in slow mixing for flocculation led to increased organic matter concentration than those produced by unpurified protein solution.

The result of the sludge volume has shown the efficiency of Moringa oleifera proteins over other proteins selected in removing turbidity, which is the primary process of clarifying water, the result can be observed by the sludge volume and the supernatant watercolor (Fig. 7).

Contrary to the sludge volume, the total solid result presents an interesting concentration for the novel plant seed-based bio-coagulant with Luffa acutangula result superior to the Moringa oleifera, and Cucumeropsis mannii result is equal to the moringa oleifera.

The TS is an essential parameter in this research to increase the biogas yield. The result shows that applying the plant seed-based bio-coagulant increases the TS > 2%. Therefore, this result is a significant change that can triple biogas production.

5.4 Biomethane potential test result

4.4.1- Methane yield

BMP tests were carried out at neutral pH (7.17 to 7.28), reducing the possibility of creating toxic conditions. The methane volume per gram of COD removed for all samples was lower than the theoretical value (350 NmL CH₄/gr COD removed). This difference shows loss of COD, probably, due to the presence of SO₄²⁻ and NO₃⁻ in the sludge (which may lead to the substrate competition of sulfate-reducing bacteria, nitrate-reducing bacteria, and acid-fermented microorganisms) by conversion into volatile fatty acids that have not yet finished the decomposition process. All methane yields have ranged from 60 ÷ 80%.

Results of BMP show FS + plant seed bio-coagulant in anaerobic digestion can produce more methane than FS without pre-treatment. Changes in specific CH₄ yield and CH₄ content consistently complied with the C/N ratio. A high C/N ratio indicates rapid nitrogen consumption by methanogens and lower biogas production. On the other hand, a lower C/N ratio causes ammonia accumulation which is toxic to methanogenic bacteria.

Table 13. Biomethane potential test result

Sample	Input values			Output values			Total CH ₄ Production (NmL)	Specific methane yield (NmL/gVS _{removed})	Total CH ₄ Prod from substrate (NmL)	Specific methane yield (NmL/gVS _{substrate})
	pH	VS (g/l)	COD (mg/l)	pH	VS (g/l)	COD (g/l)				
Raw FS + Inoculum	7.33	6.6±0.6	19,679.4	7.57	5.90±0.7	13231.2	82.8			
Inoculum + DW	7.48	6.8±0.1	17,638.8	7.84	5.61±0.7	12080.7	192.8±3.1			
FS+Luffa+ inoculum	7.27	10.02±0.6	19,306.8	7.94	8.39±	11.260	475.3±6.6	435.9±7.0	297.5±3.5	350.5±3.8
FS+Mannii + inoculum	7.33	9.8±0.2	16,414.6	7.83	8.54±0.1	10,866.4	459.8±7.4	421.4±5.9	287.0±4.2	296.0±4.6
FS+ Moringa + inoculum	7.38	9.7±0.3	18,895.7	7.73	8.62±0.6	9,912.2	448.8±11.6	409.8±10.8	275.9±8.6	285.3±9.2
FS+Pumkin+ inoculum	7.41	9.8±0.2	18,405.5	7.91	8.50±0.3	7,806.4	389.8±10.2	391.7±10.0	234.9±7.2	248.2±7.6

Discussion

This study presents more straightforward and more practical methods for obtaining effective and less expensive biocoagulants. The advantage of these methods is that they can be applied in developing and developed countries because the treatment conditions can be done in laboratories or at home. In addition, they present some advantages in the production methods and costs that facilitate the treatment of water and reduce environmental and human being health impact. The shortcomings of these bio-coagulant production methods are that they do not treat wastewater 100% in turbidity or microorganisms removal. However, this issue can be solved if the process is followed by an advanced treatment method.

The fecal sludge concentrates are highly biodegradable under suitable anaerobic digestion conditions. However, concentrates from the novel plant seed-based bio-coagulant Luffa acutangula had the highest methane production rate. Moringa oleifera and Cucumeropsis mannii have a similar result that is more than generated by pumpkin seed. The gas production increased gradually from the startup day to a stable state on the 4th day; the methane production per gram COD during the regular period was 350 ml CH₄/gCOD, while the production from FS without pretreatment with bio-coagulant was stable on day 10. Still, the methane production was less than that, with the production at a steady state within 172.8 mlCH₄ /gCOD. The period without discharge had the lowest biogas production. It was difficult for the highly concentrated sewage to degrade and produce biogas. However, the suitable concentrate for the BMP test was from Luffa acutangula, Moringa oleifera, and Cucumeropsi mannii.

Conclusion

This study considered and highlighted the simplified of plant seed proteins extraction and application as bio-coagulant for fecal sludge concentration to increase the biogas yield. Applying these plant seed-based bio-coagulants is a suitable method for FS pretreatment that increase the organic matter concentration for biogas production and removed the turbidity, COD, and Ammonia nitrogen in supernatant water that guaranteeing the water is reused. BMP batch tests have shown that the FS pretreated with plant seed-based bio-coagulant was a potential substrate for anaerobic digestion and methane production with the optimization of the biogas yield with or without a higher COD concentration. The BMP test results presented the suitability and importance of FS pretreated with bio-coagulant, which can produce the highest methane potential than the FS without pretreatment. Overall, the various methane potential test shows that the bio-coagulation treatment has a significant advantage in FS concentration and efficiency for the anaerobic digestion process.

Declarations

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Ethical Statement

Hereby, I Dima Francis Auguste Fleury Junior, consciously assure that for the manuscript: Plant Seed-Based Bio-coagulant Development and Application for Fecal Sludge treatment and Biogas Production Improvement, the following is fulfilled:

- 1) This paper is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.
- 6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- 7) All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

To verify originality, your article may be checked by the originality detection software iThenticate. See also <http://www.elsevier.com/editors/plagdetect>.

I agree with the above statements and declare that this submission follows the policies of Environmental Science and Pollution Research as outlined in the Guide for Authors and in the Ethical Statement.

Date:2022/05/15

Corresponding author's signature: Dima Francis Auguste Fleury Junior

Consent to Participate

All authors whose names appear on the submission

1. **Dima Francis Auguste Fleury Junior**
2. **Prof. Dr. Zifu Li**
3. **Prof. Zhou Xiaoqin**
4. **Lixin Zhu.**

- 1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work.
- 2) drafted the work or revised it critically for important intellectual content.
- 3) approved the version to be published; and
- 4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Credit Author Statement

Title: Plant Seed-Based Bio-coagulant Development and Application for Fecal Sludge treatment and Biogas Production Improvement

1. ***Dima Francis Auguste Fleury Junior:** Conceptualization, Methodology, Data curation, Writing- Original draft preparation.
2. **Prof. Dr. Zifu Li:** Visualization, Investigation, Supervision, Validation
3. **Zhou Xiaoqin :** Writing-Reviewing and Editing.
4. **Lixin Zhu: Senior engineer:** Supervision, Resources.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

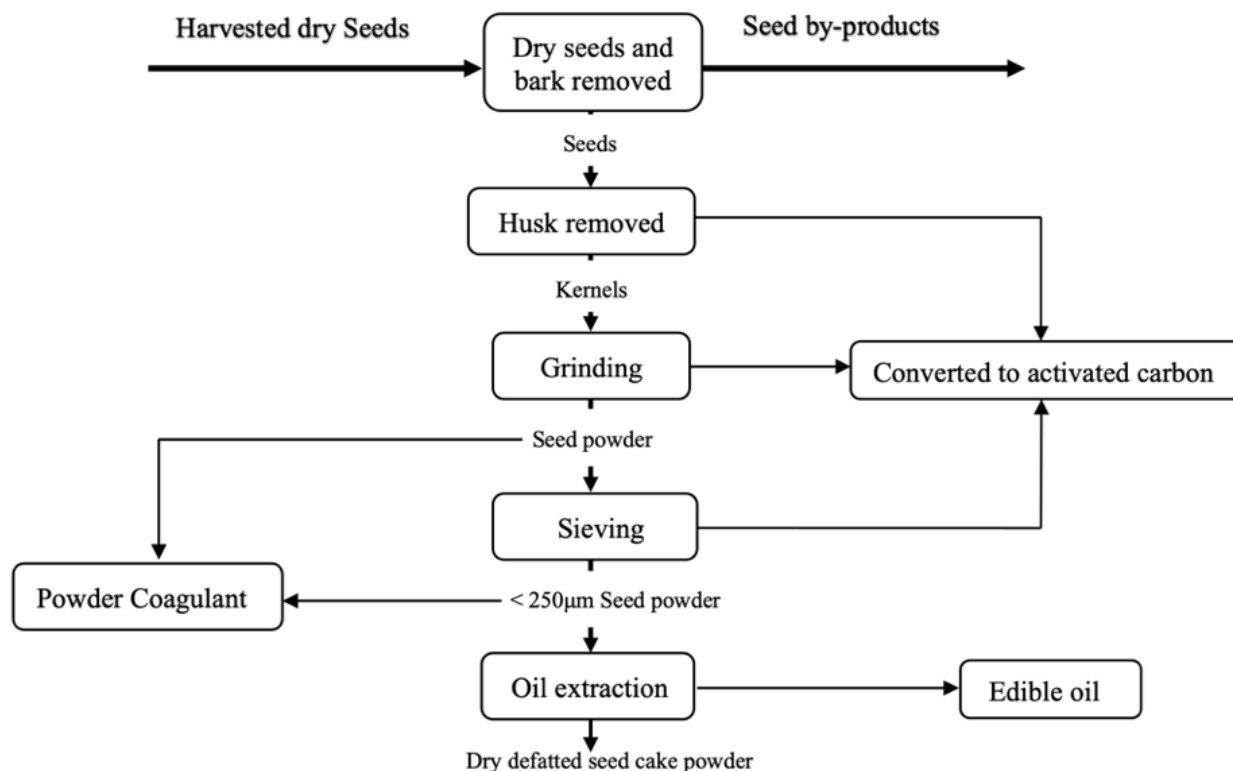


Figure 1

Cucumeropsis mannii seed oil extraction process.

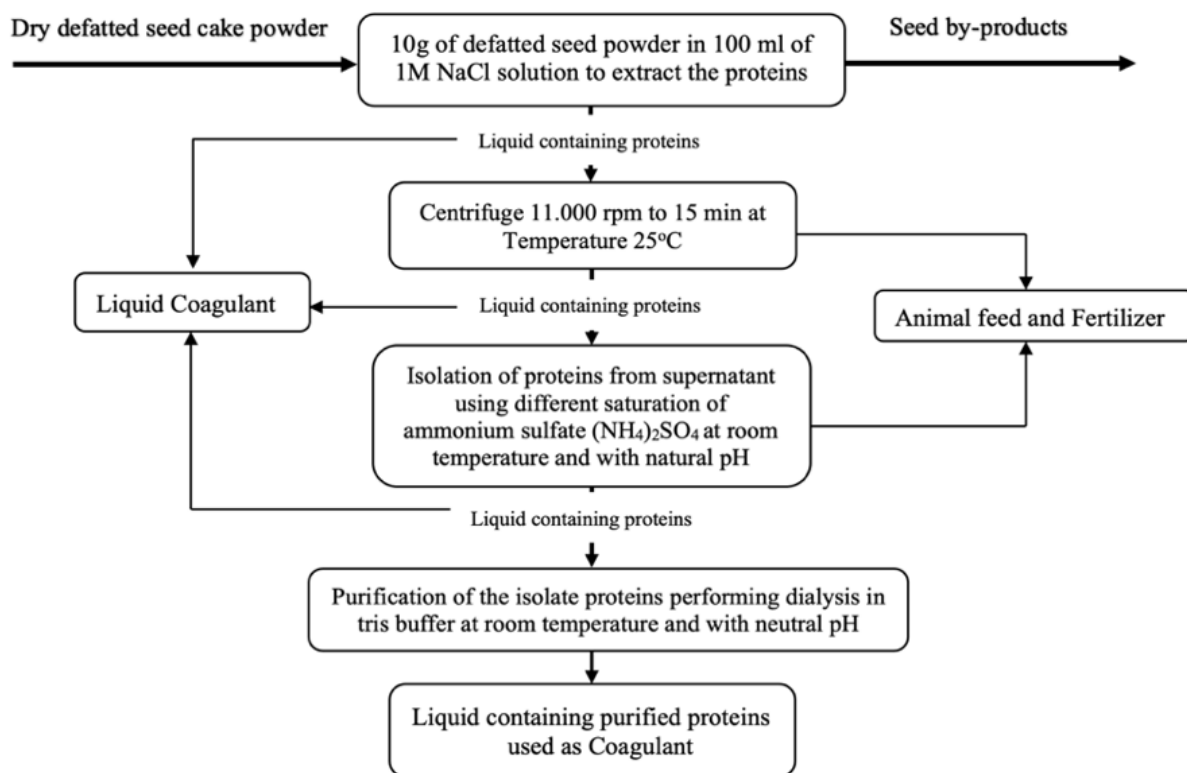


Figure 2

Cucumeropsis mannii seed functional proteins extraction process.

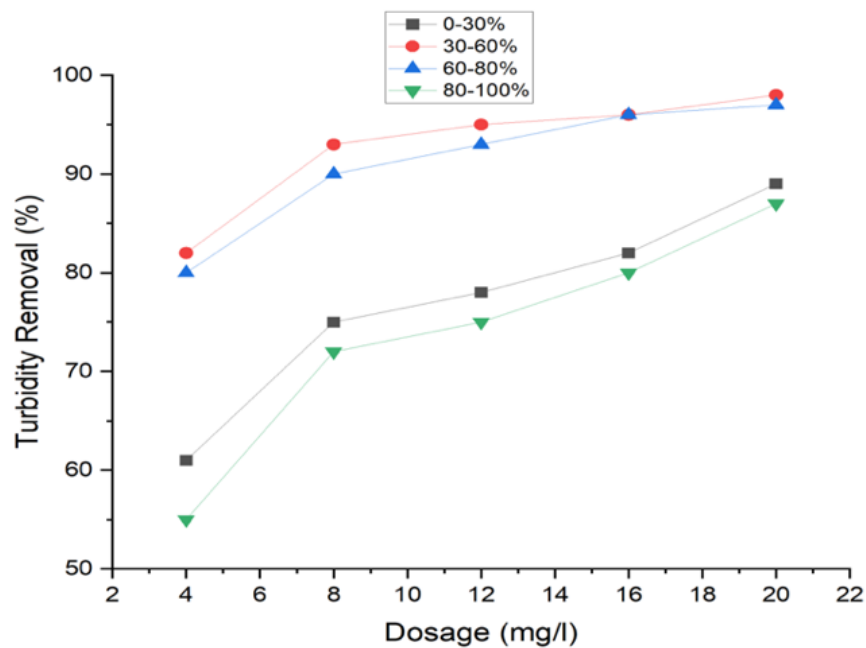


Figure 3

Bio-coagulant test

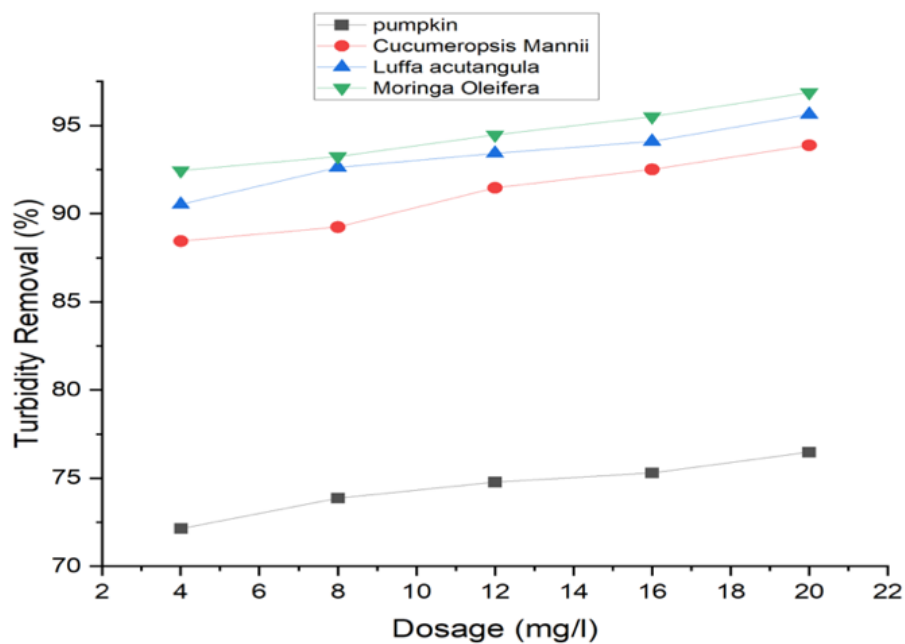


Figure 4

Turbidity removal by different steps of treatment.

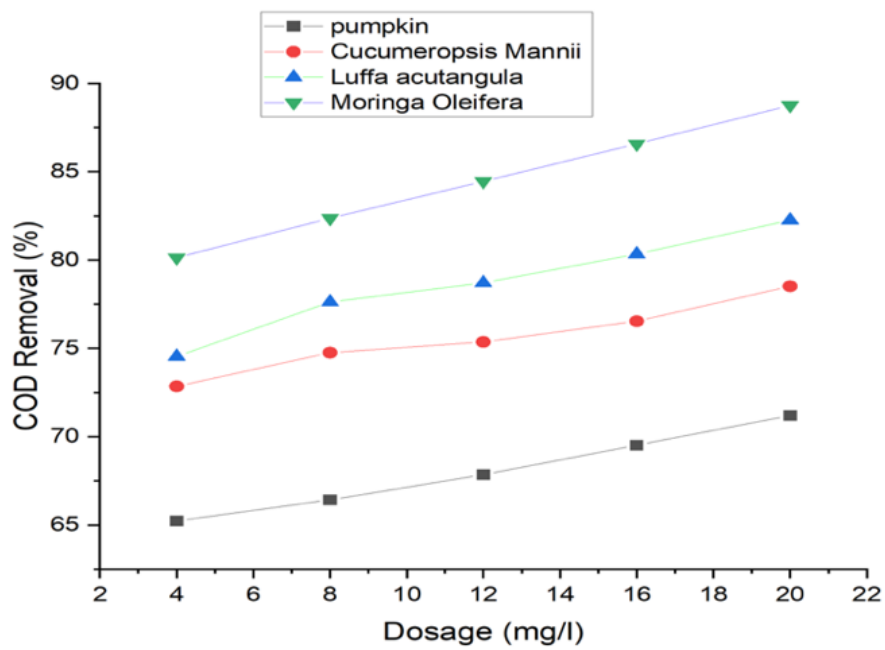


Figure 5

COD removal by different processes of treatment.

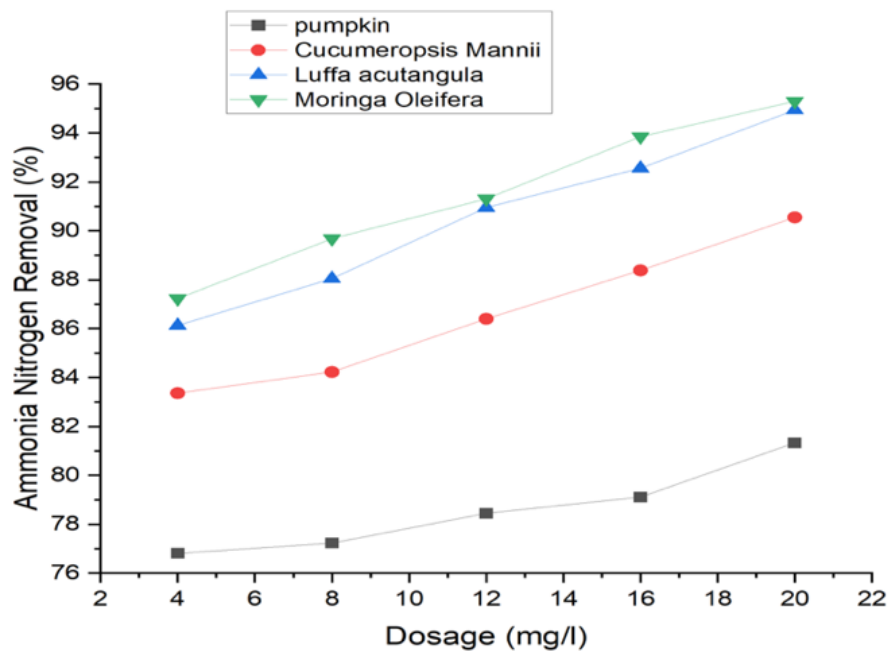


Figure 6

Ammonia nitrogen removal

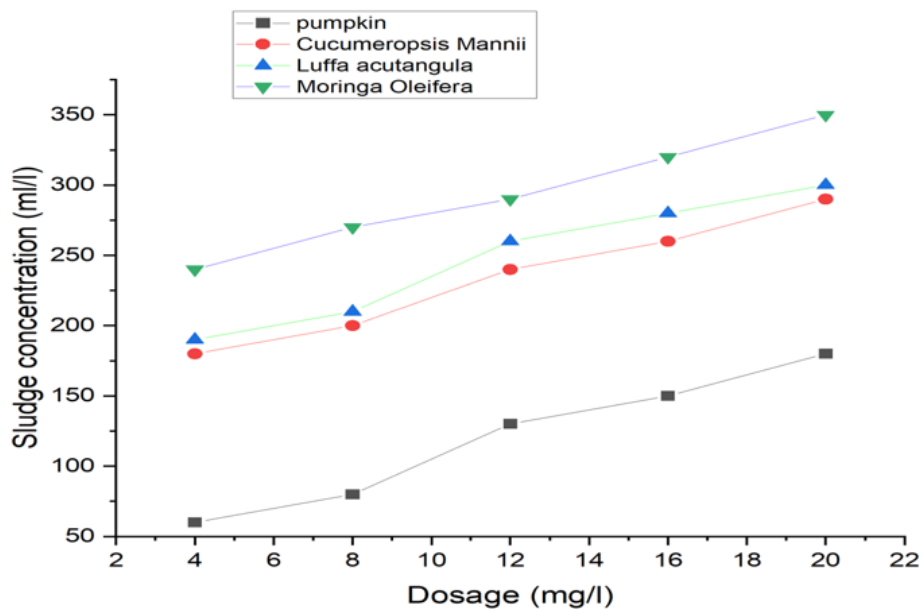


Figure 7

fecal sludge treatment final result

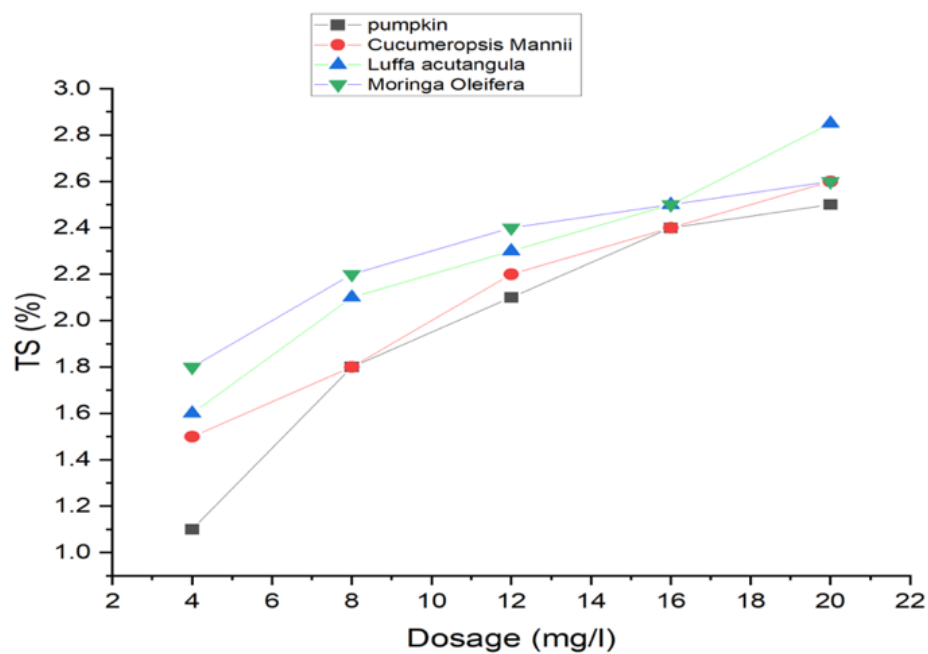


Figure 8

total solids

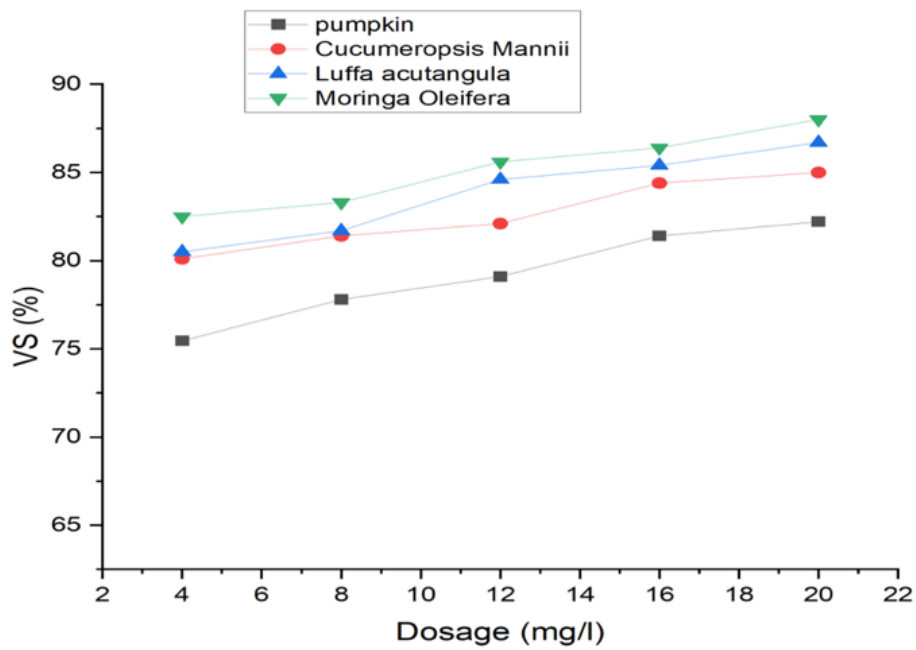


Figure 9

Volatile solids

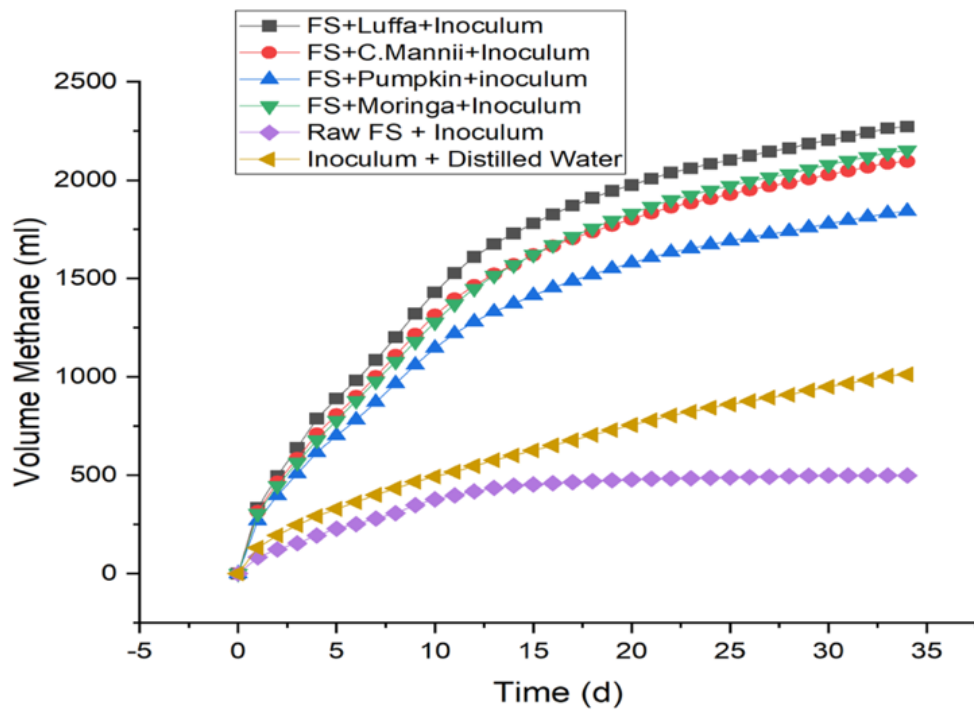


Figure 10

volume methane

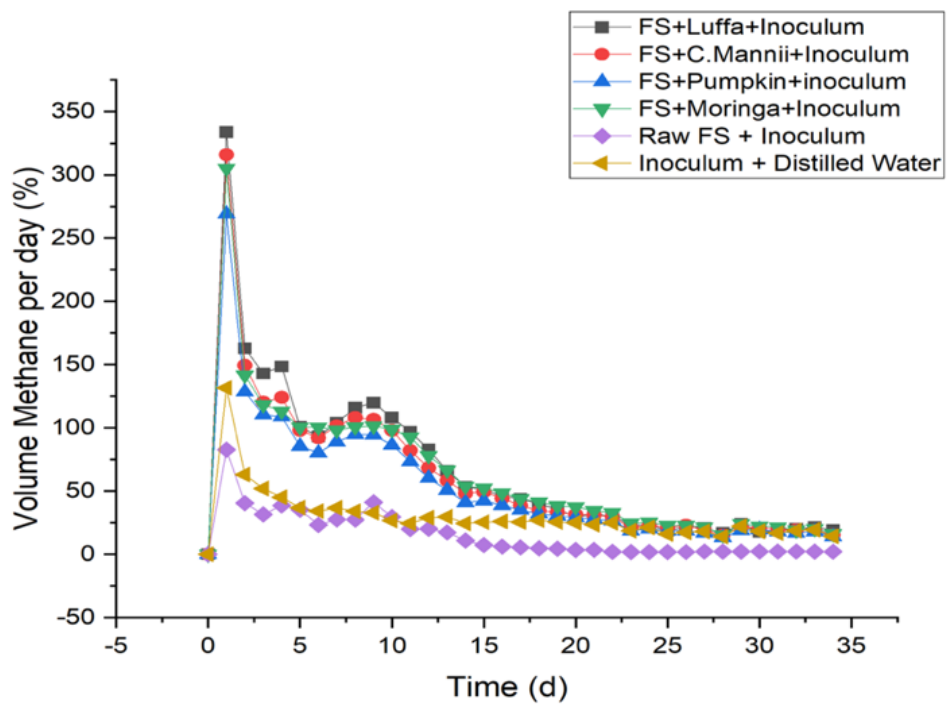


Figure 11

Volume methane produced/per day