Universiti Malaysia PAHANG

JABATAN PENYELIDIKAN & INOVASI
NO BORANG : UMP/JPI/REPORT
KUATKUASA: 01/06/2020

RESEARCH PROJECT REPORT FORM

Final [✓] Progress ☐ *Progress Period :* ____________ *√ Please tick*

PROJECT DETAILS *(Keterangan Projek)*

A	**Grant No**	RDU1703283
	Faculty/CoE	Faculty of Civil Engineering Technology
	Project Title	Optimization of active protein extraction from biomass waste for application in water treatment
	Project Leader	Mohd Nasrullah bin Zulkifli
	Project Member	1. PM. Dr. Che Ku Mohammad Faizal Bin Che Ku Yahya 2. Dr. Nurul Huda Binti Othman 3. PM. Dr. Mohamed Ariff Bin Ameedeen

PROJECT ACHIEVEMENT *(Pencapaian Projek)*

B

ACHIEVEMENT PERCENTAGE				
Project progress according to milestones achieved up to this period	**0 - 25%**	**26 - 50%**	**51 - 75%**	**76 - 100%**
Percentage (please state %)				/

EXPENDITURE *(Perbelanjaan)*

C

Budget Approved *Peruntukan diluluskan*	**Amount Spent** *Jumlah Perbelanjaan*	**Balance** *Baki*	**% of Amount Spent** *Peratusan Belanja*
RM 23,500.00	**RM 9,457.36**	**RM 14,042.64**	**40.24%**

RESEARCH OUTPUT *(Output Penyelidikan)*

D

NO OF PUBLICATION

KPI FOR NO OF PUBLICATION

	ISI	Scopus	Index Proceedings	Others
KPI	1	~~3~~ 1	1	
Achievement	2	1		

The contribution of funder (UMP, MOHE, MOSTI, Industry etc.) as the fund provider must be acknowledged at all times in all forms of publications. Please state the grant number (RDU/UIC) and grant name.

	ISI	Scopus
Number of articles/ manuscripts/books *(Please attach the **full paper** of Publication)*	1. Co-digestion of palm oil mill effluent for enhanced biogas production in a solar assisted bioreactor: supplementation with ammonium bicarbonate	1. Enhanced bioenergy production from palm oil mill effluent by co-digestion in solar assisted bioreactor: effects of hydrogen peroxide pretreatment 2. Enhanced coagulant extraction from jatropha curcas in aqueous solutions and its application in turbidity removal
	International	**National**
Conference Proceeding *(Please attach the **full paper** of Publication)*	1. 2.	1. 2.

HUMAN CAPITAL DEVELOPMENT

KPI FOR HUMAN CAPITAL DEVELOPMENT

	PhD Student	Master Student
KPI		1
Achievement		1

Human Capital Development	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
PhD Student					
Masters Student	/				
Undergraduate Student					
Total					

Name of Student: Zaied bin Khalid
ID Matric No: MKE18001
Faculty: FTKA
Thesis title: Enhanced biogas production from anaerobic co-digestion of palm oil mill effluent using solar-assisted bioreactor
Graduation Year: Waiting for senate later (2020)
*** enter for more space*

INTELECTUAL PROPERTIES

KPI FOR INTELECTUAL PROPERTIES

Patent, Copyright, Trademark,Industrial Design: ____________

Patent, Copyright, Trademark, Industrial Design ect	

OTHERS

KPI FOR OTHERS

Prototype, Technology,Collaborations etc: ____________

Prototype, Technology, Collaborations etc	

ASSET *(Aset)*

E

Bil	**Peralatan** *(Equipment)*	**Model**	**No Daftar Aset** *(Asset Tagging No)*	**Amount** (RM)	**Lokasi** *(Location)*

PRODUCT DESCRIPTION FOR UMP R&D DIRECTORY (SHORT & BRIEF) *Only for Final Report*

F

The protein extraction from biomass waste (Jatropha curcas seed cake and palm oil mill sludge) coagulator and biogass enhancer products.

PRODUCT PICTURE FOR UMP R&D DIRECTORY *Only for Final Report*

G

SUMMARY OF RESEARCH FINDINGS (*Ringkasan Penemuan Projek Penyelidikan*)

H	The protein extraction from biomass waste (Jatropha curcas seed cake and palm oil mill sludge) can be used for treatment of wastewater and biogas production.

PROBLEMS / CONSTRAINTS IF ANY (*Masalah/ Kekangan sekiranya ada*)

I	N/A

Date : 16/7/2020
Tarikh

Project Leader's Signature
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J

Recommend / N~~ot Recommend / KIV / Need Ammendment~~

....

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Nama:

Signature:
Tandatangan:

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Tarikh:

**** Dean/TDR/Director/Deputy Director**

COMMENTS, IF ANY/ ENDORSEMENT BY RMC PNI (*Komen, sekiranya ada / Pengesahan oleh RMC PNI*)

K	**Recommend / Not Recommend / KIV / Need Ammendment** .... **Name:** *Nama:* **Signature:** *Tandatangan:* **Date:** *Tarikh:*

RDU1703283

OPTIMIZATION OF ACTIVE PROTEIN EXTRACTION FROM BIOMASS WASTE FOR APPLICATION IN WATER TREATMENT

MOHD NASRULLAH BIN ZULKIFLI

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RESEARCH VOTE NO:

RDU1703283

Faculty of Civil Engineering Technology

Universiti Malaysia Pahang

2017

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My greatest wishes and thank to Allah, The Great Almighty God who gave my project thesis appropriateness. I am grateful and would like to express my sincere gratitude to all the members of this project which are Assoc. Prof Dr. Che Ku Mohammad Faizal bin Che Ku Yahya, Assoc. Prof Dr. Mohamed Ariff bin Ameedeen and Dr. Nurul Huda binti Othman for their effort in making this project a success.

ABSTRACT

In this study, the effect of the extraction medium on the properties and efficiency of bio-coagulant, extracted of *Jatropha curcas* (Jc), in turbidity removal from aqueous solutions has been investigated. The optimised values of NaCl concentration (*i.e.* NaCl), solution pH and solution temperature were identified to improve the extraction of the coagulant. The optimised conditions were associated with an optimum coagulant dosage and a maximum turbidity removal from the synthetic aqueous solutions. The highest turbidity reduction was achieved with the coagulant extracted at a solution pH of 10 and an extraction temperature of 60 °C (pH10/60°C-JcPc). Under these conditions, the coagulant dosage required was reduced by 80-90%, depending on the coagulation pH. At the coagulation pH=6, the pH10/60°C-JcPc well reduced the turbidity by 85%. However, the distilled water-based extract failed to lower the turbidity. Several analytical techniques were employed to characterize the nature of the active components derived from Jc. SDS-PAGE electrophoresis showed that Jc extract was mainly made up of proteins with molecular weights between 20 and 35 kDa. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. This study demonstrates the potential employability of these enhanced bio-coagulants. This can be a step ahead in helping with the development of sustainable processes in (waste)water treatment, particularly in tropical regions *e.g.* Malaysia with an abundant access to Jc.

ABSTRACT

Dalam kajian ini, pengaruh medium pengekstrakan terhadap sifat dan kecekapan bio-koagulan, diekstraksi Jatropha curcas (Jc), dalam penyingkiran kekeruhan dari larutan berair telah diselidiki. Nilai kepekatan NaCl yang dioptimumkan (iaitu NaCl), pH larutan dan suhu larutan dikenal pasti untuk meningkatkan pengekstrakan koagulan. Keadaan yang dioptimumkan dikaitkan dengan dos koagulan optimum dan penyingkiran kekeruhan maksimum dari larutan berair sintetik. Pengurangan kekeruhan tertinggi dicapai dengan koagulan yang diekstrak pada pH larutan 10 dan suhu pengekstrakan 60 ° C (pH10 / 60 ° C-JcPc). Dalam keadaan ini, dos koagulan yang diperlukan dikurangkan sebanyak 80-90%, bergantung pada pH pembekuan. Pada pembekuan pH = 6, pH 10/60 ° C-JcPc dengan baik menurunkan kekeruhan sebanyak 85%. Walau bagaimanapun, ekstrak berasaskan air suling gagal menurunkan kekeruhan. Beberapa teknik analisis digunakan untuk mencirikan sifat komponen aktif yang berasal dari Jc. Elektroforesis SDS-PAGE menunjukkan bahawa ekstrak Jc terutama terdiri dari protein dengan berat molekul antara 20 dan 35 kDa. Keadaan pengekstrakan yang dioptimumkan meningkatkan kecekapan koagulan bio-turunan yang menjanjikan ini dalam pengurangan kekeruhan. Kajian ini menunjukkan potensi penggunaan bio-koagulan yang dipertingkatkan. Ini dapat menjadi langkah maju dalam membantu pengembangan proses berkelanjutan dalam (air) rawatan air, terutama di kawasan tropika mis. Malaysia dengan akses yang banyak ke Jc.

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jc	*Jatropha curcas*

CHAPTER 1

INTRODUCTION

Access to safe drinking water is one of major global concern of humankind over the last decades. In developing countries, the cost of imported chemicals, such as aluminum salts and poly aluminum chloride, for conventional water purification is exorbitantly expensive. Disposal difficulties of the high volume sludge because of its aluminum content, residual aluminum concentration in the treated water, reaction of aluminum with alkalinity present in the water leading to the big drop in the pH of water and its low coagulating efficiency in cold water are the main problems which water treatment plants face with (Chatterjee T. et. al, 2009 and; Sanghi Rashmi and Singh Ajay, 2001). Additionally, recent studies have pointed out possible negative effect on consumers' health associated with using aluminum-based coagulants (Grahama et. al, 2008 and; Katayon et. al, 2006) McLachlan et. al, reported that extensive intake of alum may cause Alzheimer's disease (McLachlan, 1995).

Thus, in recent years, there has been an important challenge to develop sustainable technologies to treat turbid water with a better alternative for conventional coagulants, preferably from natural and locally grown or harvested. Aqueous extracts of several dry seeds have been recommended for their performance in water treatment applications in developing communities (Abidin et. al, 2011; Sanghi et. al, 2001; Ndbigengesere et. al, 1995 and; Pritchard et. al, 2009). Natural polyelectrolytes, which can be derived from plants and animal life such as Vegetable tannins and cactus mucilage (Miller et. al, 2008; Sanchez-Martin et. al, 2011 and; Zhang et. al, 2006) and chitosan (Chatterjee et. al, 2009) are alternatives to synthetic polymers or alum. A large number of bio materials in which coagulating properties have been observed, are available long time ago, but applying them in cheaper way and sustainable is still a serious challenge.

Present study is aimed to test the natural source materials that are indigenous and abundantly available in Southeast Asia region, particularly Malaysia, as renewable sources of natural coagulants. Four natural materials, which have properties similar to those previously described for natural coagulants, were chosen to examine the quality of the surface water treated by them and to compare them with that of the water treated with alum. Two seed plants, Moringa oleifera and Jatropha curcas, one animal-based polymer, chitosan, one plant mucilage, dragon fruit (Hylocereus costaricensis) foliage, have been selected to examine their applicability in surface water treatment.

The main objective is to conduct an investigation to improve the extract method of active agent from Jc seeds for water treatment. The specific objectives of this research are as follows:

1- To model and optimize the extraction of active agent from Jc press cake based on its coagulation activity using Response Surface Methodology.
2- To characterize the isolated protein from extraction coagulant.

CHAPTER 2

LITERATURE RIVIEW

Today, one of the key changes in the process industries is waste minimisation in the conversion of waste to value-added end products. Furthermore, the continual depletion of available resources has led to a rising growth in research opportunities *e.g.* the use of waste biomass in energy production and water/wastewater treatments. Natural materials are known to be effective coagulants. They are of great importance especially in countries where access to conventional chemicals has proven to be difficult or not economical (Megersa et. al, 2017 and; Saranya et. al, 2014). These easily-biodegradable biomaterials are readily found in abundance as waste by-products with practically little or no secondary large-scale application (Ozacar et. al, 2002 and; Hu et. al, 2013).

Active components derived from natural coagulant can be produced or extracted from microorganisms, animals (Rizzo et. al, 2008) and/or plants (Yin et. al, 2010). Plant-based coagulants are categorised into three main groups depending on the nature of their active components. The first group comprises oilseeds of which press-cake has shown a good coagulation activity with protein as their active component. The next group is mucilage of the cactus family plants with a mixture of polysaccharides, capable of treating water *via* floc forming. And finally, tannin, a general name given to large polyphenolic compounds derived from natural materials (Yin et. al, 2010).

Among plant-based coagulants, the active component in *Moringa oleifera* (*M. oleifera*) seeds is a promising alternative for the conventional coagulants. The corresponding coagulation mechanism has been thoroughly investigated in the literature (Katayon et. al, 2006 and; Yarahmadi et. al, 2009). It was shown that the active components in the water extract are the dimeric cationic polypeptides (6–17 kDa) with 10–11 isoelectric points (Fatombi et. al, 2013). Another potential source of bio-coagulant is *Jatropha curcas* (Jc), sometimes referred to as the "physic nut". The seeds are made up of about 30–35% oil. Jc has been widely grown for oil extraction from its seeds (Fatombi et. al, 2013; Lestari et. al, 2011 and; Makkar et. al, 2008). A solid waste residue, called press cake (Pc), with a good protein content is generated after the oil extraction process (Lestari et. al, 2011; Da Luz et. al, 2014 and; Makkar et. al, 2008).

In comparison to *M. oleifera*, it has been reported (Khodapanah et. al, 2013) that the aqueous extracts of Jc kernel demonstrate an acceptable coagulation activity when tested on river water samples. The active coagulant component in the Jc is believed to be a soluble protein (Jc is an oilseed with a high protein content). It has also been reported that the key factors in increasing the solubility of protein in aqueous solutions are the ionic strength, solution pH and temperature (Okuda et. al, 2001; Álvarez et. al, 2018 and; Saetae et. al, 2011).

The first objective of the present work is to investigate and optimize the extraction conditions in order to reduce turbidity in aqueous solutions with a minimum dosage of bio-coagulant. This study also seeks to specify and characterize the produced extract. To the best of our knowledge, the composition, nature and properties of Jc extract has not been reported elsewhere. Elemental analysis, FTIR analysis, enzymatic hydrolysis and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) measurements were employed in order to characterize the active components.

Coagulation activity of each isolated coagulant is evaluated in Jar tester. The study involved steps such as rapid mixing, slow mixing and sedimentation in a batch process. Samples of synthetic turbid water were prepared by adding a stock kaolin suspension to tap water for all coagulation experiments. pH of synthetic turbid water is adjusted to desired pH before each run. During a rapid mixing, the different dosage of isolated coagulant sample is added to each beaker of turbid water sample. One of the jars receiving no treatment (i.e. dosage of 0 mg/L of coagulant), serving as a blank against in which turbidity removal can be compared with. Residual (final) turbidity of treated water mples is measured after sedimentation period by turbiditimeter.

In order to extract the active agent, Jc press cake is mixed with the extraction solution in different conditions. Then the extracted proteins are isolated and characterized using a range of techniques including isoelectric precipitation, dialysis and electrophoresis. To isolate the active protein from extracted solution, the pH of the coagulant solution was adjusted to isoelectric point of Jc proteins. The generated suspension is centrifuged to seperate from supernatant then dissolved in distilled water and continually stirred for several hours by magnetic stirrer. Subsequently, the non-soluble part is filtered. The supernatant was dialyzed overnight at 4 °C against deionized water in a dialysis bag.

As a first optimization step, the critical variables of the extraction along with their variation ranges are estimated based on a classical "one-variable-a-time" (OVAT) procedure. The effective variables (results from OVAT) are then used as input variables in a full factorial analysis, with three (or five) levels, and with 3 replicates. The input levels of different factors, including center points, for the full factorial procedure are: temperature (estimated 25 - 75 °C), NaCl concentration (estimated 0 – 0.5 M) and pH (estimated 8 - 12).

The responses are coagulation activity, optimum dose and protein content of isolated protein from Jc.

Various tests are performed on isolated protein from Jc to characterise the active agent. Total protein/carbohydrate content, FTIR spectroscopy, HPLC, and the molecular size of active protein by SDS-PAGE electro-phoresis are several analytical techniques to characterize the isolated active agent.

CHAPTER 3

METHODOLOGY

3.1 Materials

The Jc seeds were supplied by the Malaysian Agricultural Research and Development Institute (MARDI). The raw plant was freshly collected from Universiti Putra Malaysia's farm. Kaolin, sodium hydroxide pellets and sodium chloride were purchased from R&M Chemicals, Essex, UK. Hexane from Systerm Co., Shah Alam, Malaysia, Alcalase from Novozymes A/S, Baegsvard, Denmark and Hydrochloric acid (HCl, 36%) from Fisher Scientific, UK were the other chemicals used in the present work.

3.2 Characterisation of Jc

A hexane-based solid–liquid extraction process was employed in order to prepare the Jc press cake (Sayyar et. al, 2009). The results from the proximate and elemental analyses (Truspec CHNS/CHNSO elemental analyzer (LECO, USA)) of the shelled Jc seeds as well as the press cake are shown in Table 1. All chemicals used in the experiments were of analytical grade and were used as received without any further purification. In addition, the functional groups in Jc and JcPc were detected on a Spectrum 100 FTIR spectrometer (Thermo Nicolet) within a spectral range of 4000-400 cm^{-1}. The spectra of the kernel and the press cake were similarly recorded.

Table 1. Elemental and proximate analyses of shelled Jc seed.

	Elemental analysis (dry wt%)				
	Nitrogen	Hydrogen	Carbon	Sulfur	Oxygen
Kernel	4.98±0.03	8.87±0.17	57.05±0.24	2.20±0.06	22.56±0.16
Press cake	8.38±0.30	6.80±0.12	47.69±0.15	0.18±0.02	33.11±0.23

	Proximate analysis (raw wt%)					
	Crude protein	Crude Lipids	Ash Content	Moisture Content	Crude Fiber	Total Carbohydrates*
Kernel	31.52±0.35	51.79±0.09	3.30±0.14	3.95±0.04	6.56±0.27	2.88±0.06
Press cake	54.04±0.43	16.19±0.02	6.03±0.03	5.20±0.02	7.64±0.13	10.90±0.12

* Calculated from difference

3.3 Extraction of the coagulant's active component

In order to extract the active component, two grams of the sieved JcPc (100-550 μm) was mixed with 100 ml of the extraction solution. In order to determine the optimum extraction conditions, the effects of three experimental parameters *i.e.* solution temperature, NaCl concentration and solution pH (*i.e.* the extraction pH), were studied. To achieve this, different aqueous media were used: distilled water (Favorit Water Still W4L) at neutral pH (*i.e.* pH 7) as well as at pH values of 9 and 10 (adjusted by using 0.1 and 1 M NaOH) and NaCl solutions (0.1, 0.2, 0.3, 0.5, 1.0, 2.0 M) (Lestari et. al, 2011 and 2010). The extraction temperature was studied at three levels: the room temperature (RT), 40 °C and 60 °C (Makkar et. al, 2008). All mixtures were continuously stirred for five minutes on a heated hotplate, using a magnetic stirrer (Fisher Scientific Isotemp). The suspension was next filtered through a muslin cloth and cooled down to room temperature. The supernatant was then centrifuged (High Speed Refrigerated Centrifuge CUBOTA 6500) at 3000 rpm for 15 minutes. These coagulant solutions, extracted in distilled water (DW) and NaCl solutions, were referred to as DWE-JcPc and SCE-JcPc (*i.e.* Distilled Water Extract/Sodium Chloride

Extract of JcPc), respectively. A fresh solution was prepared for each sequence of experiments to prevent any change in coagulation activity due to microbial decomposition of the organic compounds during storage. The coagulant extract from JcPc at optimum extraction condition was labelled as pH10/60°C-JcPc indicating that the extraction solution pH and solution temperature were adjusted at 10 and 60 °C, respectively.

3.4 Isolation, partial purification and characterization of the active component

In order to identify the nature of the active components in Jc, enzymatic hydrolysis was performed by adding Alcalase to pH10/60°C-JcPc. The solution pH of the pH10/60°C-JcPc was adjusted at 8 using HCl (0.1 M and 1 M) prior to the addition of the enzyme (pre-adjusted medium pH). The mixture was then continually stirred inside a water bath regulated at 50 °C for 6 hr (Li et. al, 2010 and; Espejo-Carpio et. al, 2015) During this process, the entire peptide bonds were broken down (Marrufo-Estrada et. al, 2013). This would then allow a coagulation test to potentially prove the quality of the extracted active component in the solution (Li et. al, 2010).

In order to study the nature of the active component, three samples from the original pH10/60°C-JcPc solution (30 ml, pH=8) were prepared in three volumetric flasks (100 ml). Alcalase (500 µl) was next added to one of the three samples. The second sample was used as the control experiment with no added Alcalase. Finally, the third sample (Alcalase free) was placed and kept inside the refrigerator (4-7 °C) in order to prevent any microbial decomposition of the organic compounds. An enzyme control test was also employed by adding of Alcalase (500 µl) to distilled water (30 ml, pH = 8). After the completion of the hydrolysis reactions, the coagulation activities of all four samples were tested *via* the jar test. Active proteins were isolated and characterized using a range of techniques including isoelectric precipitation, dialysis and electrophoresis. A modified version of an existing method (Makkar et. al, 2008) was employed in order to precipitate the active proteins from the JcPc solutions. In order to isolate the protein from the pH10/60°C-JcPc, the pH of the coagulant solution was adjusted to 5 using HCl (Fisher Scientific 36%) before being centrifuged at 3000 rpm for 20 min at room temperature (High Speed Refrigerated Centrifuge CUBOTA 6500). The generated white suspension was then dissolved in distilled water and was continually stirred for four hours on a magnetic hotplate (Fisher Scientific Isotemp). Finally, the non-soluble residue was filtered firstly through a Whatman No. 1 filter paper and then through a 0.45 and 0.22 µm nylon membrane. The supernatant was dialyzed overnight at 4 °C against deionized water in a dialysis bag (UC36-32-100, Viskase Sales Corp) with a molecular weight cut-off of 12-14 kDa.

The protein's Molecular Weight Distribution of the distilled-water extract and the purified pH10/60°C-JcPc samples were monitored by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) on 12.5 % (w/v) polyacrilamid gel. Protein bands were visualized *via* Coomassie brilliant blue R-250 staining (Hamarneh et. al, 2010).

The total sugar and protein contents of the natural coagulant solutions were measured *via* the phenol-sulfuric acid method with glucose as the standard (Zheng et. al, 2008) and the Bradford method with bovine serum albumin as the standard, respectively (Da Luz et. al,2014).

3.5 Turbid water samples

In order to identify the optimised experimental condition for the extraction of the active component of Jc, synthetic kaolin turbid water was prepared and used in the subsequent turbidity removal experiments. Kaolin was used to establish a desired level of turbidity. By adding 7.5 ml of kaolin stock solution (Sciban et. al, 2009) to 500 ml of tap water and storing the solution over night to maintain a constant temperature and remove any residual chlorine, a 200-NTU turbid water was successfully synthesized. The turbidity was determined using a HACH Turbidimeter (Model 2100 N). The pH of the turbid water samples was 7.41 ± 0.07 and where indicated, the desired pH (*i.e.* coagulation pH) was adjusted by adding hydrochloric acid (0.1 M and 1 M) and sodium hydroxide (0.1 M and 1 M).

3.6 Coagulation experiments

Jar floc test, comprising a series of batch experiments, was employed in order to study the coagulation process. All experimental parameters were kept constant during the tests except for the target variable. The coagulation activities of the different extracts were evaluated in a jar tester (VELP, model JLT6). Samples were added to beakers filled with turbid water in different dosages (10-400 mgL^{-1}). The solutions were then constantly stirred (100 rpm) for 4 min, followed by a slow mixing phase (40 rpm) for 25 min. After about 30 minutes of sedimentation, an aliquot of the clarified sample (20 mL) was collected from the top of the beaker and the residual turbidity was measured. The final (residual) turbidity of the treated water samples was measured and the turbidity removal percentage was calculated by the difference between the initial and the final turbidity values divided by the initial turbidity, multiplied by 100. All experiments were run at room temperature (25 ± 1 °C).

In order to investigate the reproducibility of the results, the extraction experiments in pH 10 and temperature 60 °C were repeated in quintuples and in irregular intervals for two months followed by the subsequent analyses of the efficiency of the pH10/60°C-JcPc in turbidity removal at coagulation pH of 4. Each experiment was run in triplicates. The residual turbidity of these runs *i.e.* 4.22±0.43, 4.02±0.20, 4.63±0.34, 5.48±0.45, 4.65±0.31 NTU, indicates that the extracted natural coagulant could successfully reduce the turbidity of the synthetic turbid water with a high degree of reproducibility.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Optimisation of the extraction conditions

The coagulation activity of the DWE-JcPc was investigated in a synthetic aqueous kaolin solution. Through a series of initial tests, it was concluded that acidic pH facilitates turbidity reduction from synthetic water. Fig. 1 presents the effect of DWE-JcPc dosage on residual turbidity of synthetic aqueous kaolin solution at coagulation pH values of 4, 5 and 6. The residual turbidities of water treated with the optimum dose of the coagulating solution - corresponding to the highest turbidity reduction - were found to be 5 and 33 NTU at pH 4 and 5, respectively. The optimum doses of coagulant at pH 4 and 5 were 100 and 200 mgL^{-1}, respectively. However, at a coagulation pH of 6, DWE-JcPc could not reduce the turbidity to an acceptable level (*i.e.* >30%, Miller et. al, 2008) and therefore, distilled-water extraction proved not to be suitable for successful coagulation at coagulation pH of 6.

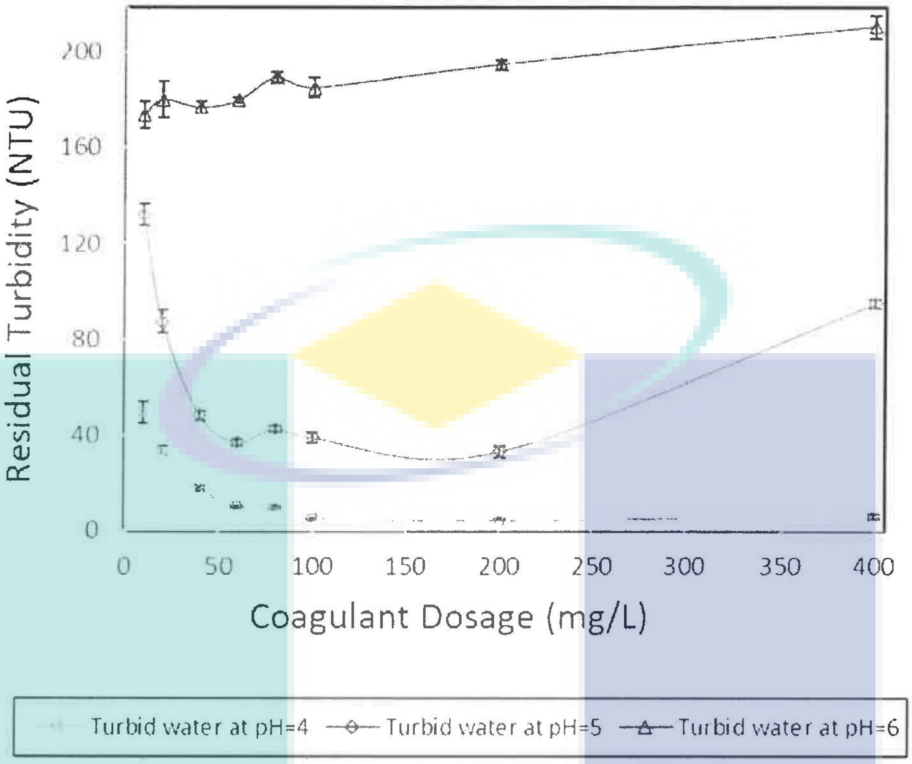


Fig. 1. Effect of water-extracted coagulant (DWE-JcPc) dosage on turbidity reduction of synthetic kaolin/water at coagulation pH values of 4, 5 and 6.

The elemental analysis on the JcPc (Table 1) showed an enrichment of protein in the sample: 8.38 wt% nitrogen (7.69 wt% carbon) as compared to the kernel, which contained only 4.98 wt% nitrogen (57.05 wt% carbon). The crude protein contents of Jc's kernel and JcPc are 31.52% and 54.04%, respectively (Table 1). Both kernel and press cake were further analysed *via* FTIR to confirm the presence of the protein. As illustrated in Fig. 2, FTIR analysis for Jc kernel and press cake showed two strong absorption bands at 1635 cm^{-1} and another at 1535 cm^{-1}. Another band was also recorded in the range of 1240-1246 cm^{-1}. These peaks are characteristic of carbonyl C=O stretching vibrations in primary, secondary, and tertiary amides, respectively (Prasuna et. al, 2009 and; Kwaambwa et. al, 2008). This suggests that the Jc seeds hold up a high percentage of protein within them. The comparison between spectra of the press cake and the kernel demonstrated that the amide infrared absorbance has not been remarkably altered in the press cake due to the structural changes in protein after solvent extraction of lipids.

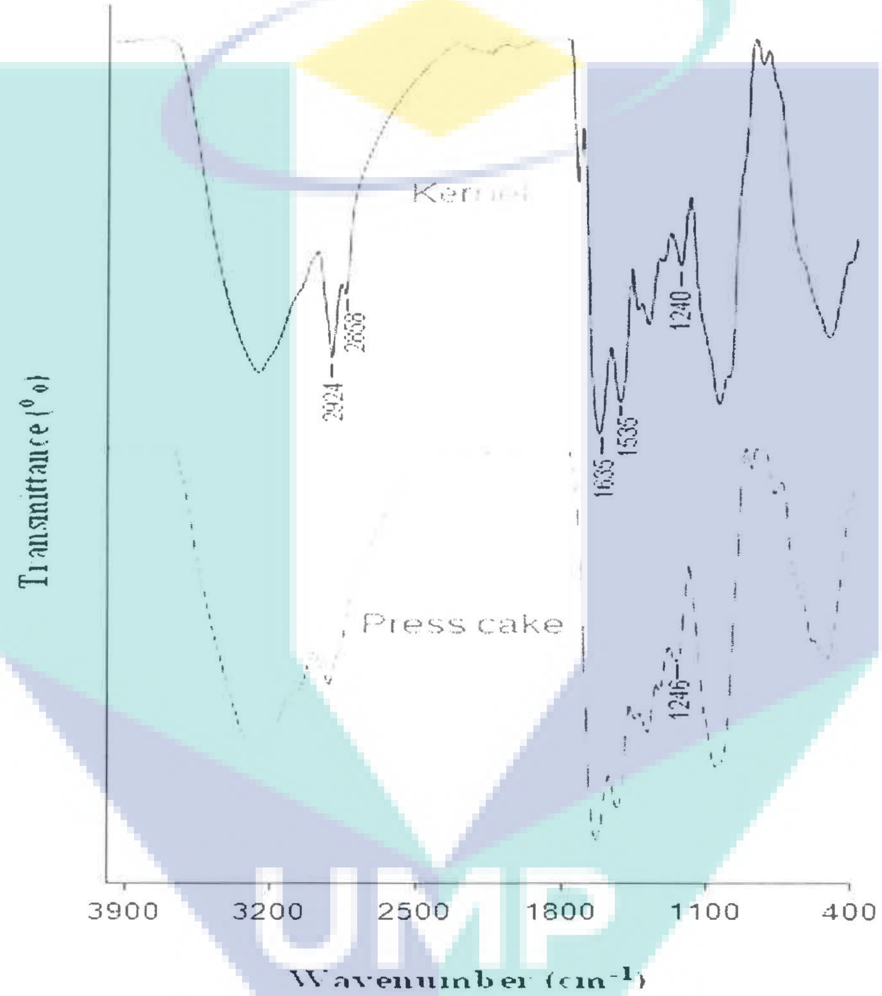


Fig. 2. FTIR spectra of Jc kernel and press cake.

Therefore, the active component of Jc press cake as a coagulant might be protein molecules. In both laboratory and commercial-scale extraction processes, water with sodium or potassium hydroxide are used to modify the pH and to ultimately extract protein from press cake (Makkar et. al, 2008 and; Pelegrine et. al, 2005). On this ground, the same approach was adapted throughout the JcPc active component extraction process.

With NaCl solution, it was observed that the optimum dosage of JcPc extract was halved compared to when water was used as the extraction medium at coagulation pH 4. The corresponding amount of SCE-JcPc was 40 mgL^{-1}. On the other hand, the lowest concentration of NaCl showing the same degree of turbidity reduction was 0.3 M. Additionally, higher concentrations of up to 0.5 M did not show any significant difference while elevated salt concentrations (1.0 and 2.0 M NaCl) had a negative impact on turbidity reduction. The decrease in coagulation performance at 1.0 and 2.0 M could be due to the "salting-out" phenomena (Makkar et. al, 2008).

In the next phase of the experiments, the effects of temperature and pH on extraction were investigated. Although the temperature of the extraction solution was expected to improve the extraction yield (increasing temperatures facilitated the molecular dissolution of solutes in the solvents), the results indicated that solely preheating the coagulant solution would not have a significant effect on the coagulating efficiency. It has been observed that in some cases, an increase in temperature could decrease the protein solubility at isoelectric point (pI); however, at other pH values (below and above pI), the effect of temperature on solubility of protein was strongly affected by solution pH (Pelegrine et. al, 2005). On the other hand, Saetae et al. 2011, reported that Jc protein solubility increases with temperatures within the range of 30 to 50 °C at an extraction pH of 12. In general, it is understood that the effect of temperature on solubility is strongly dependent on the pH of the extracting solution and therefore, there exists an interaction between these two experimental factors. Hence, in the subsequent experiments, the effects of temperature and pH of the media were studied together.

Fig. 3 demonstrates the effects of ionic strength, pH and temperature of the extraction solution on the optimum coagulant dosage to achieve a residual turbidity of 5 NTU ≤ in synthetic water at a coagulation pH of 4.

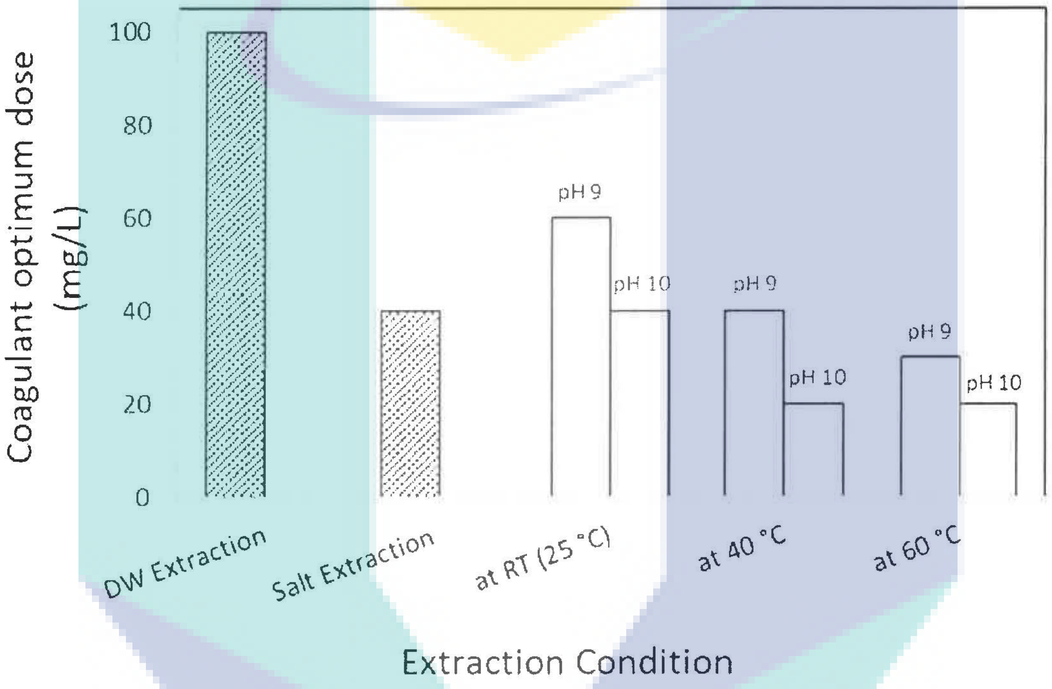


Fig. 3. Optimum dose corresponding to a residual turbidity of 5 NTU ≤ of water at coagulation pH 4 using various extracting solutions.

The optimum dosage was observed to be 100, 40 and 20 mgL^{-1} for DWE-JcPc, SCE-JcPc and pH10/60°C-JcPc at a coagulation pH of 4, respectively. A notable achievement was the improvement in coagulation activity of JcPc extract at coagulation pH values of 5 and 6 by changing the extraction pH and the temperature of the extraction media. Fig. 4 draws a comparison among various extracting solutions with respect to turbidity removal at coagulation pH values of 5 and 6. The results show that the extraction of active components at an extraction pH of 10 and higher temperature substantially improved the performance of coagulant at coagulation pH values greater than 4.

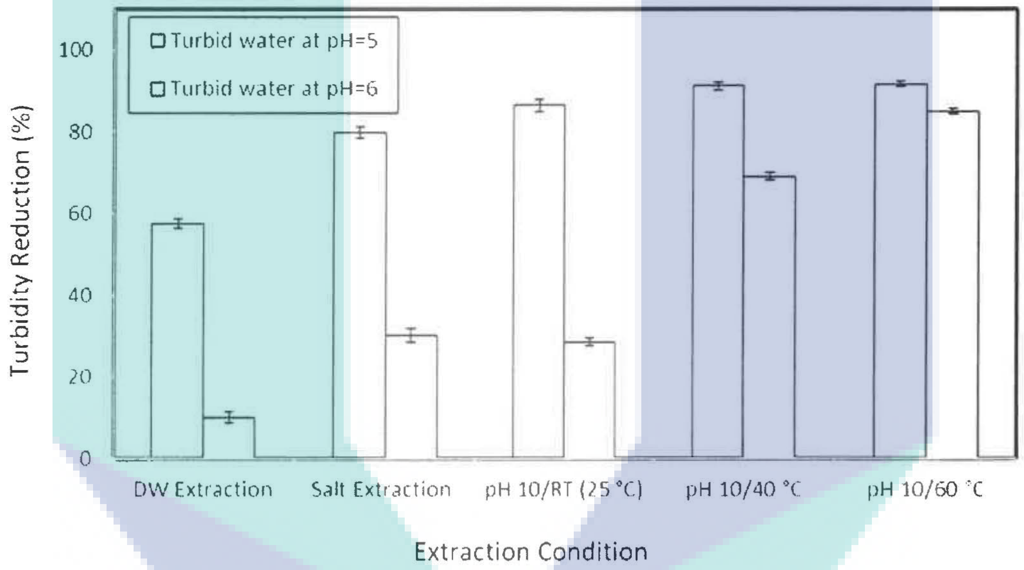


Fig. 4. Turbidity reduction of synthetic water at two coagulation pH values of 5 and 6 with various extracting solutions with a constant coagulant solution dosage of 20 mgL^{-1}.

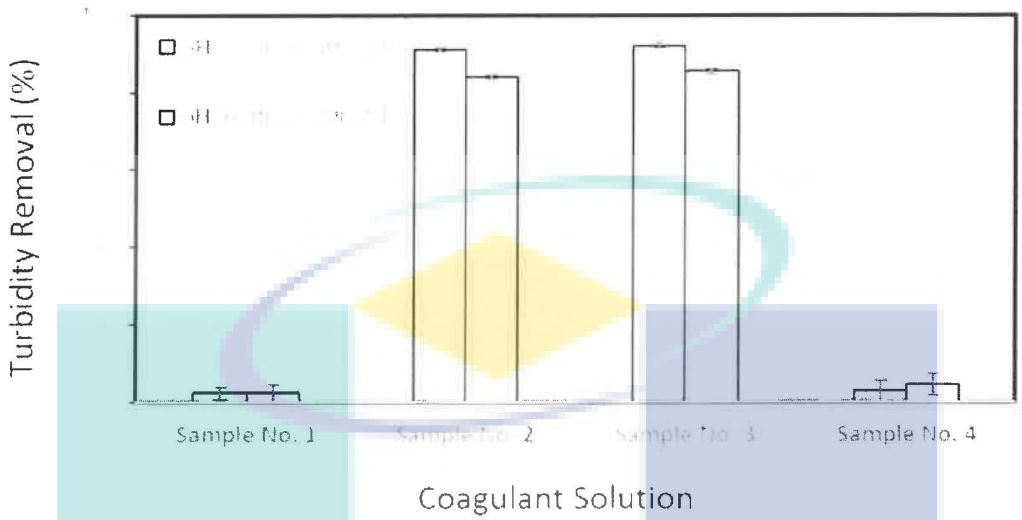
The efficiency of different extracting solutions at coagulation pH=4 was understood to be the same in terms of the observed residual turbidity. They all showed residual turbidity of ≤ 5 NTU. However, the optimum coagulant dosages dropped by 80% and 90% for coagulation pH 4 and 5, respectively. Additionally, a higher coagulation activity (by 91.6%) with the pH10/60°C-JcPc was observed at coagulation pH=5 comparing with 83% when using DWE-JcPc. Therefore, regarding the optimum dosage improvemnt, the highest achieved turbidity removal with pH10/60°C-JcPc at coagulation pH=5 was almost 11 times higher than that of distilled water extract. Interestingly, at the coagulation pH=6, and with the use of an alkaline-medium extract, the coagulation activity increased by 85% despite the absence of coagulation activity with distilled water extract. Therefore, it is concluded that extraction at pH 10 and at 60 °C presents two major advantages: a reduction in the optimum dosage to achieve the maximum turbidity removal and the improvement of coagulation activity at coagulation pH=5 & 6. Although salt extraction solution showed its capability in reducing the optimum dosage at coagulation pH=4 and it also indicated an improvement in coagulation activity at coagulation pH=5 compared to DWE-JcPc, it was still not as efficient as the solution extracted at pH 10 and at 60 °C.

Since the active coagulant component was now believed to be protein, the protein and carbohydrate content of the extractant were subsequently analysed. The results indicate a significant increase in the yield of protein extraction from JcPc through alkaline extraction. Extraction at 60 °C and a pH of 10 corresponded to a higher protein content (*i.e.* 6.65 ± 0.20 gL^{-1}) than extraction in a water-only medium (*i.e.* 0.81 ± 0.06 gL^{-1}). Furthermore, the total amount of carbohydrate in DWE-JcPc and pH10/60°C-JcPc were 1.35 ± 0.17 gL^{-1} and 1.63 ± 0.02 gL^{-1}, respectively. Similar findings by other researchers (Madrona et. al, 2011 and; Makkar et. al, 2008) have also been reported in the literature for optimising the extraction of protein from oil seed biomass.

4.2 Isolation and identification of pH10/60°C-JcPc active component

In order to characterize the extracts, several characterization techniques were employed. Protein hydrolysis and SDS-PAGE electrophoresis were used to characterize the coagulation active components in Jc.

The hydrolysis process is a reaction in which a substance is degraded. Enzymatic hydrolysis involves minimum side reactions and leads to a limited hydrolysis of selected bonds. Proteases hydrolysis breaks down proteins into various peptides which are further reduced into amino acids. This leads to a complete degradation of protein structure [19]. The results could support our hypothesis that the active component of Jc-derived coagulant is a protein if the hydrolysed sample does not show a significant coagulation activity. Fig. 5 compares the performance of the pH10/60°C-JcPc and the hydrolysed pH10/60°C-JcPc solutions for their turbidity removal.

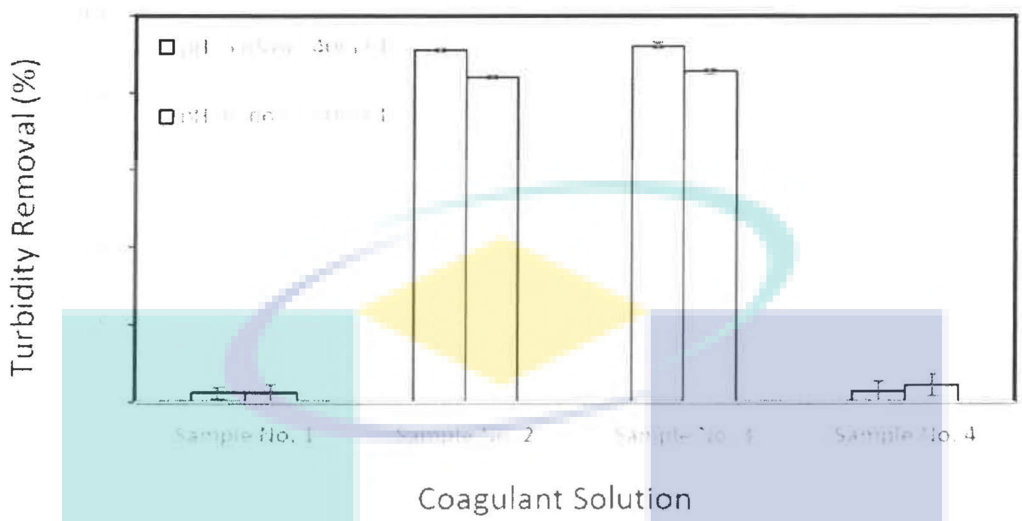


Sample:
No. 1: pH10/60°C-JcPc+Enzyme
No. 2: pH10/60°C-JcPc inside water bath
No. 3: pH10/60°C-JcPc inside refrigerator
No. 4: Distilled water +Enzyme

Fig. 5. Residual turbidity of kaolin water at two-coagulation pH values of 5 and 6 by using pH10/60°C-JcPc solution under protein hydrolysis reaction.

Sample no. 1, 2 and 4 were maintained inside a water bath under identical Alcalase hydrolysis conditions. The hydrolysed pH10/60°C-JcPc solution did not show any coagulation activity. The turbidity removal of the treated water using the hydrolysed pH10/60°C-JcPc was measured to be 2-5% compared to the turbid water treated with sample no. 2 and 3 (*i.e.* 85-92%). This indicated that sample no. 1 completely failed to reduce the turbidity and therefore, no coagulation activity was observed. Alcalase just reacts with protein components and break downs peptide bonds, resulting in new components such as amino acids with completely different properties from proteins. In addition, the results of sample no. 4 confirmed that Alcalase did not demonstrate any coagulation activity.

The SDS-PAGE was used to determine the molecular weight profile for the isolated proteins. The SDS-PAGE patterns of pH10/60°C-JcPc (Fig. 6 (a)) contain three major bands indicating the presence of various types of proteins with different molecular weights. The highest protein intensities of pH10/60°C-JcPc are grouped into two classes of molecular weights: <25 kDa and >25-35 kDa. In general, a minimum number of 25 protein bands are present in the Jc seed protein (Lestari et. al, 2010). The number of proteins extracted in distilled water was much larger than the optimum extraction conditions while the concentration of the target protein was significantly higher in the pH10/60°C-JcPc solution. These results were consistent with the findings by (Hamarneh et al. 2010). They stated that the molecular weight of the protein in the Jc extract (under isoelectric conditions) contains three major bands between 20 and 45 kDa (Fig. 6 (b)). In our study, this was between 20 and 35 kDa.



Sample:
No. 1: pH10/60°C-JcPc+Enzyme
No. 2: pH10/60°C-JcPc inside water bath
No. 3: pH10/60°C-JcPc inside refrigerator
No. 4: Distilled water +Enzyme

Fig. 6. Protein molecular weight distributions of JcPc extracts: (a) Isolated protein from pH10/60°C-JcPc by isoelectric precipitation & dialysis (2), DWE-JcPc (3), and protein marker (1) and (b) Jc proteins extracted by isoelectric precipitation.

The total protein content in pH10/60°C-JcPc after isoelectric precipitation was measured to be 1.5 ± 0.06 gL^{-1}. However, the total amount of carbohydrate was observed to be negligible. This also confirmed that the protein isolation procedure was successful. The protein content of the pH10/60°C-JcPc after isoelectric precipitation was 77% less than of the pH10/60°C-JcPc, owing to the loss of proteins during the precipitation and dissolution stages.

CHAPTER 5

CONCLUSION

The purpose of this study was to optimise the extraction method of the active coagulation component from Jc seeds. In order to identify the extracted species, a range of characterisation techniques were employed. The key findings in this study are summarised as follows:

1- Among various extraction media employed to extract the active components, an extraction medium with a pH of 10 and a temperature of 60 °C was found to be the optimised condition for both maximum turbidity removal and optimum coagulant dosage required. In the removal of kaolinite turbidity at the coagulation pH 4 and 5, JcPc extracted in an alkaline media demonstrated an improved coagulation activity with a five- and tenfold reductions in the required coagulant dosage compared to JcPc extracted in distilled water, respectively. The experimental results indicate the efficiency of the optimized extraction conditions in the active component extraction from JcPc. The protein content of pH10/60°C-JcPc was 8.2 times higher than that of the DWE-JcPc. The pH10/60°C-JcPc could efficiently coagulate more than 91% of the initial 200-NTU kaolin turbidity using only a dosage of 20 mgL^{-1} at a coagulation pH of 5. However, 200 mgL^{-1} of the DWE-JcPc was required to remove only 80% of the same kaolin water. With an alkaline solution as the extractant, a coagulation activity at pH 6 corresponding to more than 85% turbidity removal was observed. However, distilled water extract did not show any coagulation activity at this pH.

2- As confirmed *via* the elemental/proximate analyses as well as the FTIR spectra, the effective coagulant components were protein molecules. This was further confirmed through enzymatic hydrolysis. The water-extract solution demonstrated at least 20 protein bands in SDS-PAGE gel. Comparing the two SDS-PAGE gels, it was realised that the active proteins had a molecular weight between 20 and 35 kDa.

3- This study has showed that the pH10/60°C-JcPc can be a promising coagulant to be used in water treatment plants, especially in tropical countries such as Malaysia with an easy access to Jc. It was realized that the Jc seeds could be used as an efficient coagulant in water and wastewater treatment upon an initial purification of the active component. It is mentionable that the ultimate application of this coagulant in pilot and full-scale plants requires appropriate cost-benefit and a full techno-economic analyses for a viable commercial application of pH10/60°C-JcPc. Therefore, the authors would see it critical and with key benefits to perform such calculations in future works.

References

Grahama N., Gang F., Fowler G. and Watts M., Characterisation and Coagulation Performance of a Tannin-Based Cationic Polymer: A Preliminary Assessment, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 327, 9 (2008)

Hamarneh A. I., H.J Heeres, A.A Broekhuis, F. Picchioni, Extraction of Jatropha curcas proteins and application in polyketone-based wood adhesives, Int. J. Adhes. Adhes., 30 (7) (2010) 615-625.

Hu C. Y., S.L. Lo, C.L. Chang, F.L. Chen, Y.D. Wu, J.L. Ma, Treatment of highly turbid water using chitosan and aluminum salts, Sep. Purif. Technol., 104 (2013) 322-326.

Huang Ch., Chen Sh. and Pan J. R., Optimal Condition for Modification of Chitosan: a Biopolymer for Coagulation of Colloidal Particles, Wat. Res., 34, 1057 (2000)

Idris J., Sam A. Md., Musa M., Ku Hamid K. H., Husen R. and Muhd Rodhi M. N., Dragon Fruit Foliage Plant-Based Coagulant for Treatment of Concentrated Latex Effluent: Comparison of Treatment with Ferric Sulfate, Journal of Chemistry, 2013, Article ID 230860, 7 (2013)

Katayon S., Megat Mohd Noor M. J., Asma M., Abdul Ghani L. A., Thamer A. M., Azni I., Ahmad J., Khor B. C. and Suleyman A. M., Effects of Storage Conditions of Moringa oleifera Seeds on its Performance in Coagulation, Bioresource Technology, 97, 1455 (2006)

Katayon S., S.C. Ng, M.M.N. Megat Johari, L.A. Abdul Ghani, Preservation of coagulation efficiency of Moringa oleifera, a natural coagulant, Biotechnol. Bioprocess Eng., 11 (6) (2006) 489-495.

Khodapanah N., I.S. Ahamad, A. Idris, Potential of using bio-coagulants indigenous to Malaysia for surface water clarification, Res. J. Chem. Environ., 17 (9) (2013) 70-75.

Kwaambwa H. M., R. Maikokera, Infrared and circular dichroism spectroscopic characterisation of secondary structure components of a water treatment coagulant protein extracted from Moringa oleifera seeds, Colloids Surf. B., 64 (1) (2008) 118-125.

Lestari D., W. Mulder, J. Sanders, Improving Jatropha curcas seed protein recovery by using counter current multistage extraction, Biochem. Eng. J., 50 (1-2) (2010) 16-23.

Lestari D., W.J. Mulder, J.P.M. Sanders, Jatropha seed protein functional properties for technical application, Biochem. Eng. J., 53 (3) (2011) 297-304.

Li Z. Y., W. Youravong, A.H. Kittikun, Protein hydrolysis by protease isolated from tuna spleen by membrane filtration: A comparative study with commercial proteases, LWT-Food Sci. Technol., 43 (1) (2010) 166-172.

Madrona G. S., R. Bergamasco, V.J. Seolin, M.R. Fagundes Klen, The potential of different saline solution on the extraction of the Moringa oleifera seed's active component for water treatment, Int. J. Chem. React. Eng., 9 (1) (2011).

Makkar H. P. S, G. Francis, K. Becker, Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate, J. Sci. Food Agric., 88 (9) (2008) 1542-1548.

Marrufo-Estrada D. M., M.R. Segura-Campos, L.A. Chel-Guerrero, D.A. Betancur-Ancona, Defatted Jatropha curcas flour and protein isolate as materials for protein hydrolysates with biological activity, Food chem., 138 (1) (2013) 77-83.

McLachlan D. R. C., Aluminum and the Risk for Alzheimer's disease, Environmetrics, 6, 233 (1995)

Megersa M., A. Beyene, A. Ambelu, L. Triest, Extraction of natural coagulants from Maerua subcordata and Moringa stenopetala for use in turbid water treatment. Desalination Water Treat., 59 (2017) 127-134.

Miller S. M., E.J. Fugate, V.O. Craver, J.A. Smith, J.B. Zimmerman, Toward understanding the efficacy and mechanism of Opuntia spp. as a natural coagulant for potential application in water treatment. Environ. Sci. Technol., 42 (12) (2008) 4274-4279.

Miller S. M., Fugate E. J., Craver V. O., Smith J. A. and Zimmerman J. B., Toward Understanding the Efficacy and Mechanism of Opuntia spp. as a Natural Coagulant for Potential Application in Water Treatment, Environ. Sci. Technol., 42, 4274 (2008)

Ndbigengesere A., Narasiah K.S. and Talbot B.G., Active Agent and Mechanism of Coagulation of Turbid Waters Using Moringa oleifera, Wat. Res., 29, 703 (1995)

Nkurunziza T., Nduwayezu J. B., Banadda E. N. and Nhapi I., The Effect of Turbidity Levels and Moringa oleifera Concentration on the Effectiveness of Coagulation in Water Treatment, Water Science and Technology, 59, 1551 (2009)

Okuda T., A.U. Baes, W. Nishijima, M. Okada, Isolation and characterization of coagulant extracted from Moringa oleifera seed by salt solution, Water Res., 35 (2) (2001) 405-410.

Ozacar M., I.A. Sengil, The use of tannins from Turkish acorns (valonia) in water treatment as a coagulant and coagulant aid, Turkish J. Eng. Env. Sci., 26 (2002) 255-263.

Pelegrine D. H. G., C.A. Gasparetto, Whey proteins solubility as function of temperature and pH, LWT-Food Sci. Technol., 38 (1) (2005) 77-80.

Prasuna C. P. L., R.P.S. Chakradhar, J.L. Rao, N.O. Gopal, EPR and IR spectral investigations on some leafy vegetables of Indian origin, Spectrochim. Acta Mol. Biomol. Spectrosc., 74 (1) (2009) 140-147.

Pritchard M., Mkandawire T., Edmondson A., O'Neill J. G. and Kululanga G., Potential of Using Plant Extracts for Purification of Shallow Well Water in Malawi, Physics and Chemistry of the Earth, 34, 799 (2009)

Rizzo L., A Di Gennaro, M. Gallo, V. Belgiorno, Coagulation/chlorination of surface water: A comparison between chitosan and metal salts, Sep. Purif. Technol., 62 (1) (2008) 79-85.

Rizzo L., Lofrano G., Grassi M. and Belgiorno V., PreTreatment of Olive Mill Wastewater by Chitosan Coagulation and Advanced Oxidation Processes, Separation and Purification Technology, 63, 648 (2008)

Saetae D., T. Kleekayai, V. Jayasena, W. Suntornsuk, Functional properties of protein isolate obtained from physic nut (Jatropha curcas L.) seed cake, Food Sci. Biotechnol., 20 (1) (2011) 29-37.

Sanchez-Martin J., Beltran-Heredia J. and Gibello-Pérez P., Adsorbent Biopolymers from Tannin Extracts for Water Treatment, Chemical Engineering Journal, 168, 1241 (2011)

Sanghi Rashmi and Singh Ajay, Comparative Decolorisation Study of Malachite Green Dye Solutions using Synthetic and Natural Coagulants, Res. J. Chem. Environ., 5(2), 35-37 (2001)

Saranya P., S.T. Ramesh, R. Gandhimathi, Effectiveness of natural coagulants from non-plant-based sources for water and wastewater treatment—A review. Desalination Water Treat., 52 (31-33) (2014) 6030-6039.

Sayyar S., Z.Z. Abidin, R. Yunus, A. Muhammad, Extraction of oil from Jatropha seeds-optimization and kinetics, Am. J. Appl. Sci., 6 (7) (2009) 1390-1395.

Sciban M., M. Klasnja, M. Antov, B. Skrbic, Removal of water turbidity by natural coagulants obtained from chestnut and acorn, Bioresour. technol., 100 (24) (2009) 6639-6643.

WHO (World Health Organisation), Guidelines for DrinkingWater Quality, Fourth Edition, (2011) Yarahmadi M., M. Hossieni, B. Bina, M.H. Mahmoudian, A. Naimabadie, A. Shahsavani, Application of Moringa oleifera seed extract and polyaluminum chloride in water treatment, World Appl. Sci. J., 7 (8) (2009) 962-967.

Yin C. Y., Emerging usage of plant-based coagulants for water and wastewater treatment, Process Biochem., 45 (9) (2010) 1437-1444.

Zhang J., Zhang F., Luo Y. and Yang H., A Preliminary Study on Cactus as Coagulant in Water Treatment, Process Biochemistry, 41, 730 (2006).

Zheng Y., Z.L. Ye, X.L. Fang, Y.H. Li, W.M. Cai, Production and characteristics of a bioflocculant produced by Bacillus sp. F19, Bioresour. Technol., 99 (16) (2008) 7686-7691.

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Enhanced coagulant extraction from *Jatropha curcas* in aqueous solutions and its application in turbidity removal

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ABSTRACT

In this study, the effect of the extraction medium on the properties and efficiency of bio-coagulant, extracted of *Jatropha curcas* (Jc), in turbidity removal from aqueous solutions has been investigated. The optimized values of NaCl concentration (i.e., NaCl), solution pH and solution temperature were identified to improve the extraction of the coagulant. The optimized conditions were associated with an optimum coagulant dosage and a maximum turbidity removal from the synthetic aqueous solutions. The highest turbidity reduction was achieved with the coagulant extracted at a solution pH of 10 and an extraction temperature of 60°C (pH10/60°C-Jc press cake). Under these conditions, the coagulant dosage required was reduced by 80%–90%, depending on the coagulation pH. At the coagulation pH = 6, the pH10/60°C-Jc press cake well reduced the turbidity by 85%. However, the distilled water-based extract failed to lower the turbidity. Several analytical techniques were employed to characterize the nature of the active components derived from Jc. SDS-PAGE electrophoresis showed that Jc extract was mainly made up of proteins with molecular weights between 20 and 35 kDa. The optimized extraction conditions significantly improved the efficiency of this promising bio-derived coagulant in turbidity reduction. This study demonstrates the potential employability of these enhanced bio-coagulants. This can be a step ahead in helping with the development of sustainable processes in (waste)water treatment, particularly in tropical regions, for example, Malaysia with an abundant access to Jc.

Keywords: Jatropha curcas; Natural coagulant; Water treatment; Biomass; Protein; Turbidity removal

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Enhanced bioenergy production from palm oil mill effluent by co-digestion in solar assisted bioreactor: Effects of hydrogen peroxide pretreatment

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ABSTRACT

Pretreatment is significant for the bioenergy yield enhancement by anaerobic co-digestion (ACoD) process. Oxidization by hydrogen peroxide (OHP) had substantial impacts on biological break down through pretreatment of substrate and bioenergy production by ACoD methods. It is considered as an environmentally friendly and economical pretreatment method of ACoD for different wastewater treatment. This work aims to study the potential effects of OHP pretreatment in treating palm oil mill effluent (POME) for greater bioenergy yield using a solar assisted bioreactor (SABr). In this study, the solar panel first converted solar radiation into electricity, which warmed up POME and cattle manure (CM) mixture to keep the reactor in mesophilic temperature. The operation was done semi-continuously, and the ACoD operation was analysed at a 50:50 mixing ratio for POME and CM. The Fenton oxidation effects of 1.00 % OHP doses with 1 mM Fe^{3+} on the POME at 30 min exposure for COD and TOC removal were 33.80 % and 28.31 %. The improvement of biodegradable dissolved organic carbon (BDOC) was 59 % more for POME at 1.00 % OHP doses, which were maximum for any other OHP percentage dose and thus BOD/COD was also enhanced up to 0.72 for POME. Biogas and methane production can be enhanced up to 46.00 % and 64.83 % if pretreated by 1.00 % OHP doses. The methane composition is also enhanced up to 72.4 % compared to non-pretreated which was 64.13 %. Kinetic study of potential methane production from POME was determined for measuring final methane production as well as kinetic constants. The consequences of OHP pretreatment for POME subsidize advantageous evidence for the effectiveness of the ACoD process for the treatment of POME.

1. Introduction

Anaerobic co-digestion (ACoD) is one of the prominent wastewater treatment methods that process multiple substrates to produce bioenergy, which consequences also in an enhancement of wastewater treatment efficiency [illegible]. It consequently regulates higher bioenergy production and improved ACoD system steadiness compared to mono anaerobic digestion (AD) of the substrate [illegible]. It is considered as one of the most energy-efficient and eco-friendly methods for bioenergy yield from sustainable energy sources [illegible]. However, palm oil mill effluent (POME) is like dark liquid which can easily dissolve and become suspended and produces bad odor after a chemical breakdown by bacteria. Its characteristics are acidic, and its degradation is quite tricky [illegible]. If discharged directly into the waterways, the environment can be polluted by POME because of its greater chemical oxygen demand (COD) and biochemical oxygen demand (BOD) concentration [illegible]. In contrast, there is a relatively new issue which is feedlot farming with slaughterhouse incorporation, and so it is necessary to administrate carefully for waste management. Though the cattle are kept only for three months sequence to make fatter before sending for slaughter, the cow dung and effluent produced from slaughterhouse per day are about 200 – 300 tonnes [illegible]. Conversely, the element analysis of cattle manure (CM) indicates a large proportion of oxygenated compounds, perhaps for the nature of the food which is consumed by cattle [illegible]. In the ACoD, the microbial growth of wastewater requires different cationic elements like sodium, potassium, and others; if these element exist in high

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absorptions, the microbial activity can be hindered [9]. It is positively affected by the presence of metal contents because of its low percentage. However, as an exceptional inoculum in the ACoD, CM contains prominent buffering capacity, anaerobic microorganisms abundance, and a wide variety of essential nutrients for optimum bacterial growing [10].

Pretreatment of POME is required for improving bioenergy yield through ACoD. To enhance the organic degradation efficiency of several stubborn materials, an effective pretreatment could be supportive. The pretreatment can change several stubborn organic materials to decomposable organics, which might be degraded easily later in the bioreactor [11]. Many pretreatment techniques are existing for POME including chemical, biological, and physical methods to increase the hydrolysis of organic materials [12–14]. But, keeping these chemical reactions, circumstances were taken as expensive and might produce toxicity, which can create serious ecological difficulties. Oxidation by hydrogen peroxide (OHP), a safe technology appeared to come across the gaps in between the substrates treatment techniques with average to rich stubborn organic content and dumpy digestibility [15]. Moreover, this method is appropriate to treat all types of carbon-based wastewater. Hydrogen peroxide is broadly used as an oxidizing reagent in wastewater treatment and can improve degradation at neutral pH because the quick disintegration of hydrogen peroxide at alkaline pH confines the oxidization. So, alkaline OHP pretreatment was considered as one of the most excellent processes to improve the hydrolysis [16–19]. However, OHP pretreatment of POME needs more considerable amounts of alkaline conditions; substrate needs to be detached and eroded before future usage. These aspects could intensify the pretreatment cost and wastewater management difficulties.

On the other hand, solar energy is used for less costly anaerobic reactors. The anaerobic solar heating system for the bioreactor is allied with greater bioenergy yield. Rather than using electric and diesel heating system for bioreactor, solar-powered reactor also produces a greater amount of bioenergy, and henceforth the cost of bioenergy generation could be lessened. The solar-assisted bioreactor (SABr) consists of a solar thermal energy accumulator, a temperature controller, a reactor, and a produced biogas collector [20]. Solar radioactivity coming by solar panel becomes electricity, which will provide the required temperature for biological waste degradation and keep balance the reactor temperature [21]. Then, the main reactor at the ideal reaction temperature produces biogas and methane that capitalize on the net energy productivity [22]. The previous study showed that mesophilic (308°K) and thermophilic (328°K) conditions are the stable functioning temperature for the bioenergy production process by the ACOD method. The bioreactor heating system can be easily achieved from a solar energy storage system and produce bioenergy throughout the year. In this research study, the mesophilic (308°K) temperature was maintained to warm the organic substances in anaerobic conditions.

The main aim of this research study was to examine the effects of hydrogen peroxide for the enhancement of bioenergy production at different OHP concentrations. This work developed a pretreatment technique for POME which is ecologically proficient as it enhanced bioenergy production which is environment friendly, cost-effective because it requires very small amount, and have greater effects on biological breakdown of POME so that it can enhance bioenergy production. There is still a gap of investigations and researches on the effects of OHP on POME degradation efficiency during chemical pretreatment at neutral pH and its influence on subsequent bioenergy production [23]. In this study, the first-order kinetic model was also assessed for the methane production in different OHP concentrations. The POME pretreatment by OHP used in this research was operated at room temperature, basic pH, and post pretreatment cleaning step was avoided.

Table 1

Compositions and Characteristics of Palm Oil Mill Effluent and Cattle Manure.

Parameter	Raw POME	Raw CM
pH	4.60 ± 0.40	5.40 ± 0.50
COD	28.34 ± 0.30	16.72 ± 0.20
BOD	15.28 ± 0.20	9.28 ± 0.10
TS	39.75 ± 0.30	2.38 ± 0.10
VS	32.56 ± 0.30	1.17 ± 0.10
VFA	3.20 ± 0.10	2.80 ± 0.05
TC	15.69 ± 0.20	2.62 ± 0.05
TN	0.73 ± 0.05	0.32 ± 0.05
TP	0.13 ± 0.02	0.03 ± 0.02
C/N Ratio	21.64	8.27

Note: All parameters are in g/L except pH.

2. Materials and methods

2.1. *Feedstock collection, preparation, and characterization*

A 100.00 L sample of POME was accumulated in sample collection container from the anaerobic pond of the LKPP Corporation Sdn. Bhd., No.45/4, Jalan Teluk Sisek, 25,000 Kuantan, Pahang, Malaysia. Approximately, 100.00 kg partially digested CM was collected duly from the ejection of the average-sized farm in Gambang, Malaysia. POME sample was subjected to the simple screening to eliminate coarse materials. The dilution of CM in the water at a proportion of 1:25 was made and filtered through a sieve (20 μm) to remove debris. The physicochemical characterization of POME and CM before co-digestion is presented in Table 1. Total solids (TS) and volatile solids (VS) value of POME had of 39.75 g/L and 32.56 g/L, respectively. This large amount of presence indicates that microorganisms are readily-available in the substrate. Mostly, the POME holds cellulose, hemicellulose, sugars, carbohydrate, and lignin while CM holds prominent buffering capacity, plenty of anaerobic microbes and a wide variety of important nutrients for optimum bacteriological rising [10,24]. The pH of POME was found 4.60 while the pH of the co-substrate, i.e., CM was found 5.40. The COD value of the POME was found 28.34 g/L. The nitrogen content quantity was more in manure livestock than other surplus constituents. The ammonia from CM during the digestion process subsidized the advanced process steadiness. The carbon to nitrogen ratio (C:N) is another utmost important parameter for anaerobic digestion process [25]. To form new cells, carbon and nitrogen are vital nutrients.

2.2. *Pretreatment of palm oil mill effluent (oxidation by hydrogen peroxide)*

Five POME samples of the same volume, which is 150 mL were taken in airtight conical flasks. Then, each volume of POME was treated with 50 mL standard volume of 30 % concentrated hydrogen peroxide solution and 1 mM Fe^{3+} by Fenton oxidation process. In the process of Fenton reaction, hydroxyl radicals (• OH) are generated from the hydrogen peroxide reduction. The Fenton reaction make happens the oxidant dissociation and the creation of highly responsive hydroxyl radicals that attack the organic pollutants and finally destroy them [26]. To assess the optimum dose of hydrogen peroxide solution intended for improved organic degradation, percentage of hydrogen peroxide addition was gradually added like 0.25 %, 0.5 %, 0.75 %, 1 %, 1.25 % and then pH was changed to 7.0 ± 0.05 with adding 1 N NaOH solution. The liquid composition of the wastewater with hydrogen peroxide was done for 30 min agitation with a magnetic stirrer. The optimum hydrogen peroxide dosing was determined by obtaining high biodegradability from BOD/COD ratio. Then, all the samples were set aside about 2.5 h for oxidation by hydrogen peroxide at 298°K for better biodegradability enhancement, COD and TOC reduction.

The reaction between H_2O_2 and Fe^{3+} generates a non-selective resilient oxidant known as hydroxyl radical. The reaction pathway is expressed below:

$$H_2O_2 + FeCl_3 \rightarrow Fe^{3+} + \bullet OH + OH^- + 3Cl \quad (1)$$

The reactions creating oxidizing groups basically accountable for the absolute attack on organic carbon can be shown as follows:

$$RH + \bullet OH \rightarrow \bullet R + H_2O \quad (2)$$

$$\bullet R + O_2 \rightarrow \bullet ROO \quad (3)$$

$$\bullet ROO + RH \rightarrow ROOH + \bullet R \quad (4)$$

2.3. Reactor design, fabrication, and operation

The conventional solar reactor design is not appropriately proficient at maintaining the pH and temperature. So, pH and temperature controllers were installed to attain good control over the system. There was a two-unit battery cell that stored solar energy from the solar panel and converts to electrical power by a DC-AC converter. The total volume for the reactor was 5.00 L, where 3.50 L was working volume. The reactor was made of the cylindrical global configuration system. The main reactor was made of glass and stainless steel. It was tightly closed with a steel frame topping in combination with four nuts. The stirrer motor was fixed over the reactor. A speed control device was also installed for the stirrer which ranged from 0 − 450 rpm. There was also a water jacketing to provide the required temperature for bacteria degradation. There were also two feeding injectors to feed the sample into the reactor. Produced gas was collected through a gas collection bag. The following Fig. 1 is the schematic diagram of the solar-assisted bioreactor for the anaerobic co-digestion process.

The reactor was run at 308°K for 24 days while it was fed with 437.50 mL of the substrates of the same every three days until 3.50 L of its working volume was achieved for 24 days digestion. The volume mixing ratio was analyzed which is 50:50 of POME:CM [illegible]. Mixing was generated by direct motors powered from solar energy and joined to blades functioning at 60 rpm. Meanwhile, the anaerobic bacteria consume organic compounds in the sludge as a substrate and formed anaerobic environments suitable for the development of firm anaerobes. The properties of fed wastewater were analyzed in every three days, excluding pH that was examined daily. pH at 7.00 ± 0.10 was maintained using 1 N NaOH throughout the co-digestion time. The outcomes of the reactor operation were like the consequence of co-digestion on biodegradation, biogas production, and moreover system stability. A gas bag was also attached for the collection of the produced gas. The mixture of POME and CM was gradually increased because it took some time for the microbes to adapt to the new situation.

2.4. Biogas and methane composition analysis

Biogas production was determined by water displacement method at a fixed temperature and pressure condition [illegible]. The daily and cumulative biogas amount was established by the procedure of water displacement. Biogas production was represented as volume yield expressed in mL. As much as the water displaced, the more biogas was generated. Biogas yield and methane composition were analyzed with an Agilent's gas chromatography (GC) device. Helium was used as the carrier gas, which has a flow rate of about 30.00 ml/min. 343°K, 393°K, and 473°K temperature were kept for the oven, inlet and detector respectively.

2.5. Statistical analysis

The data were analyzed for three replicates by using Microsoft Excel 2016. All necessary statistical data were derived in this software. The mean, standard deviation, and standard error results were calculated from replicates by OriginPro 9.1 and applied to each figure and table values.

3. Results & discussions

3.1. Effects of pretreatment on palm oil mill effluent characteristics

Table 2 represents the biological fermentation of POME with different applied doses of OHP under semi batch-test operation. The implemented OHP doses varied between 0.25–1.25% and whereas the oxidation process continued until 30 min for maximum removal of pollutants. The pretreatment efficiency of OHP to promote the wastewater quality was actively subjected to the applied OHP dose. The effect of OHP dosing on the wastewater properties at 30 min of exposure was presented in Table 2. COD and TOC removal were 33.80 % and 28.31 % at 1.00 % OHP dose. Mater et al. reported that at 1.00 % OHP doses with 1 mM Fe^{3+}, 28 % TOC removal was done [illegible]. Results showed that OHP dose of 1.00 % was found to be optimal as higher OHP doses were not so effective in pollutant removal because it has a toxic effect on the survival of microorganisms. As seen, TN removal was very low and did not exceed 24.66 %. Allen et al. reported that the substantial rise in oxidation potential of ammonia concerning non-oxygenated controls during the treatment process is the reason for low TN removal [illegible]. VSS removal was significantly dependent upon OHP dose. However, OHP paths contain hydroxyl radicals and attack on the short fatty acid chains. The aromatic rings attached by the hydroxyl group that exists in the short fatty acid chains are ruptured by powerful OH

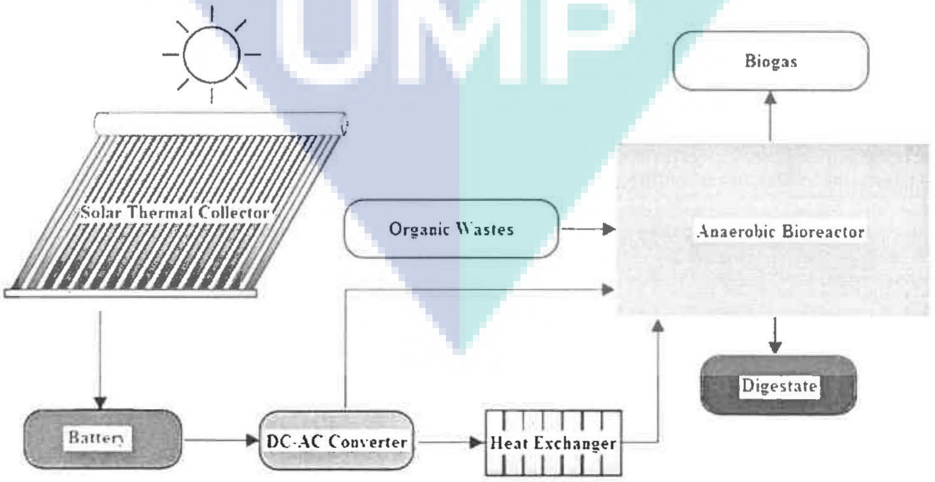

Fig. 1. Schematic Diagram of the Solar Assisted Bioreactor2.

Table 2
Physicochemical Characteristics Changes of POME with Different OHP Doses.

Parameter	Raw POME	0.25 %	0.5 %	0.75 %	1.00 %	1.25 %
pH	4.60 ± 0.40	7.0 ± 0.05	7.0 ± 0.05	7.0 ± 0.05	7.0 ± 0.05	7.0 ± 0.05
COD	28.34 ± 0.30	25.32 ± 0.30	22.89 ± 0.30	20.64 ± 0.30	18.76 ± 0.30	17.44 ± 0.30
BOD	15.28 ± 0.20	14.69 ± 0.20	14.42 ± 0.20	14.24 ± 0.20	13.52 ± 0.20	12.90 ± 0.20
TOC	9.68 ± 0.20	8.75 ± 0.20	7.83 ± 0.20	7.03 ± 0.20	6.64 ± 0.20	6.13 ± 0.20
TN	0.73 ± 0.05	0.68 ± 0.05	0.62 ± 0.05	0.59 ± 0.05	0.55 ± 0.05	0.52 ± 0.05
VSS	15.54 ± 0.30	12.92 ± 0.30	10.10 ± 0.30	9.80 ± 0.30	9.17 ± 0.30	8.32 ± 0.3
BOD/COD Ratio	0.54	0.58	0.63	0.69	0.72	0.74

Note: All parameters are in g/L except pH.

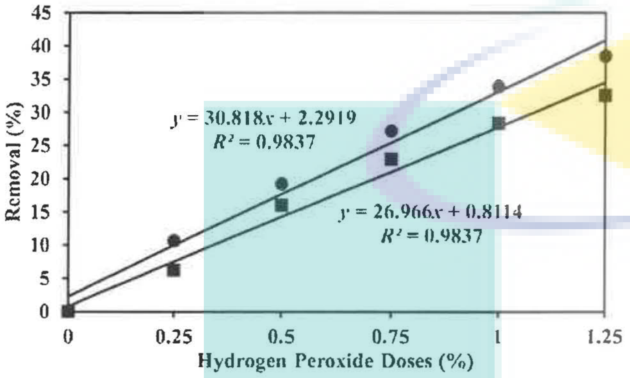

Fig. 2. Effects of Oxidation by Hydrogen Peroxide (OHP) on COD and TOC Removal in POME at Different Hydrogen Peroxide Doses (%) (● COD, ■ TOC)3.

radicals. This consequence in the generation of water-soluble compositions using hydrogen abstraction and oxygen atoms insertion with the existence of ferrous or ferric ions. The consequence of this reaction yields minor aliphatic compositions, consequent from the eradicating of the lengthy hydrocarbon chains (C–C) of short-chain fatty acids and eventually promote the mineralizing of the antecedent organics. Organic substances exist in POME are utilized by microbes as nutrients and are transformed into simple end products [[illegible], [illegible]].

The biodegradability index (BOD/COD) in wastewater ought to be in the range of 0.40 to 0.80 for the treatment by the biological procedure as reported by many authors [[illegible]]. [illegible] describes the initial value of the BOD/COD ratio was 0.54 in POME, and hence pretreatment was planned to be done with chemical oxidation by hydrogen peroxide. At the end of 30 min of chemical oxidation in different dosing, BOD/COD was enhanced up to 0.72 for POME with 1.00 % OHP doses. Oxidation with Fenton reagent based on ferrous ions may possess the capability to attack the short-chain fatty acids generating most effective hydroxyl radicals [[illegible], [illegible]]. Fenton oxidization handling of POME changes high atomic fatty acids to small atomic fatty acids like acetic acids and formic acids. Mae et al. reported that eliminating oxidizing organics present in POME, the OHP removes fatty acid [[illegible]].

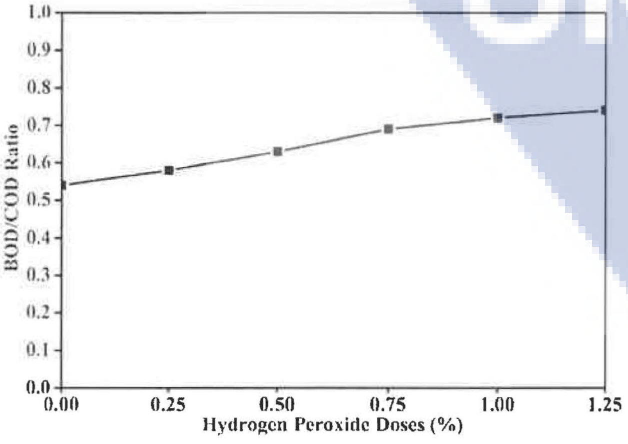

Fig. 3. Increase in Biodegradability Index at Different OHP Doses4.

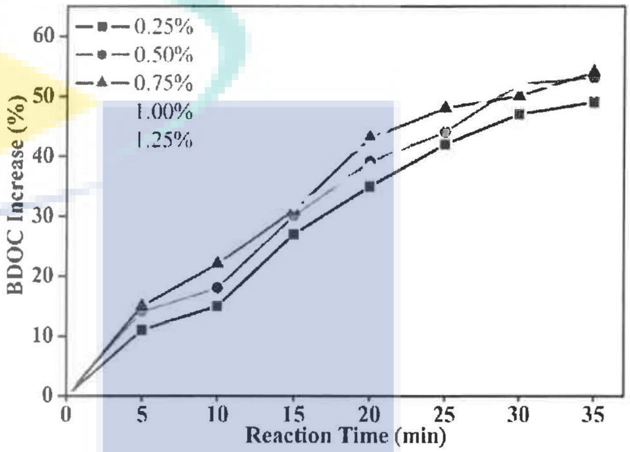

Fig. 4. BDOC Increase % with Various Reaction Time for Different OHP Doses5.

The effect of OHP on biodegradable dissolved organic carbon (BDOC) was shown in [illegible]. BDOC is the indicator of bacterial regrowth potential in terms of the existence of biodegradable organic contents in the wastewater [[illegible]]. The BDOC section contains organic particles, and the heterotrophic microbes can be used as a potential source of carbon to produce bioenergy. At an applied dose of OHP for 30 min contact time, BDOC improvement was 59 % more for POME, which was maximum for any other OHP doses. The relation between hydrogen peroxide aromatics and unsaturated organic compounds in the formation of saturated polycarbonaceous contents; moreover, it also works on aliphatic acids generating OH^- radicals and thus BDOC increases [[illegible]]. The reason is due to split and cyclic soaked hydrocarbons highly and aromatic contents are more tough to biodegradation. The result also indicates that OHP can expressively enhance the biodegradability of the POME; particularly, the biological treatment competence might be enhanced by oxidation.

3.2. Effects on biogas production

Pretreated and nonpretreated POME with CM having 50:50 mixing ratios was subjected to ACoD operation in SABr. The cumulative biogas production is shown in [illegible]. The maximum cumulative biogas production was attained from 1.00 % OHP doses. The biogas yields of 0.25 %, 0.50 %, 0.75 %, 1.00 % and 1.25 % OHP doses were 13.17 %, 22.04 %, 29.32 %, 46.00 % and 24.94 % more than non-pretreated sample respectively. So, cumulative biogas productions were enhanced after pretreatment of POME by OHP as it enhanced the substrate hydrolysis and promotes a substantial amount of biogas production. It is essential to find out maximum OHP concentration at basic pH for greater biogas production. In conventional pretreatment by OHP, the pretreated debris

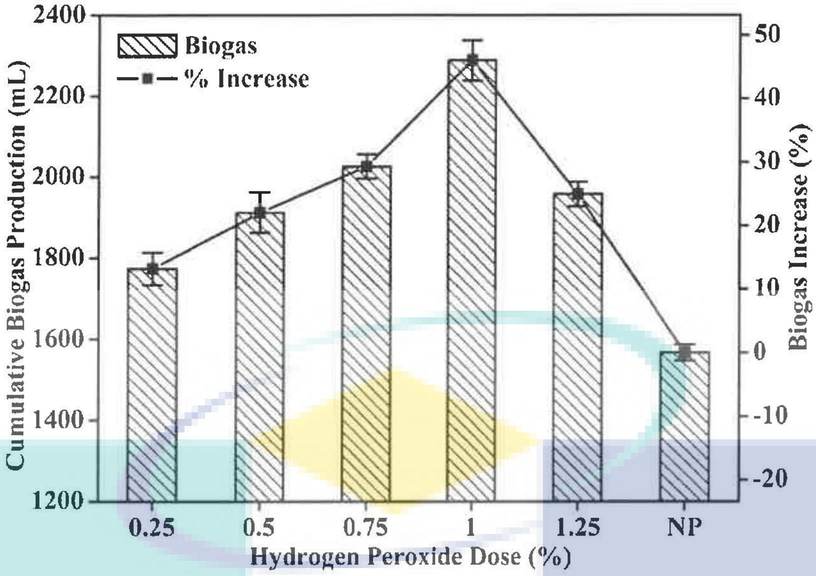

Fig. 5. Cumulative Biogas Production and Biogas Increase (%) for OHP Pretreated and Nonpretreated (NP) during 24 Days of Anaerobic Co-digestion (Error Bar is Standard Deviation)6.

need to be detached to avoid liquid toxicity (lignin break down end products and sodium ions pressure) on supplementary digestion [[illegible], [illegible]]. It caused not only the loss of a substantial percentage of hemicellulose but also produced numerous liquid wastes. Hereafter, the pretreatment methods utilized in this research was prominent due to enhanced production of bioenergy and more cost-effective regarding alkali consumption.

From [illegible], the gradual increase of OHP pretreatment increases from 0.25 % to 1.00 % as the co-digestion duration prolonged, and so the biogas production has improved. Again, the OHP doses increasing more than 1.00 %; the cumulative biogas production deteriorated concerning 1.00 % OHP doses. With pretreatment of POME by OHP, the maximum biogas production was found 2287.82 mL in the case of 1.0 % OHP, which is 46.00 % more production concerning OHP untreated co-digestion. This effect is quite similar to other research that proved that the maximum biogas production was found in 6.8 % OHP pretreated Sorghum bicolor, which is 65.00 % higher than the untreated samples [[illegible]]. The OHP was insufficient in lower concentration doses, and even high concentration was not also appropriate. When the OHP composition is too high, the generation of OH radicals may help in nutrients precipitation, their gathering onto bioreactor partitions and action can decrease methanogenic archaea. This phenomenon further consequences in the nutrition loss and lowering buffering capacity in the co-digestion system. Zhu et al. commented that the production of biogas from corn stover by alkaline pretreatment decreased when the concentration of NaOH improved from 5.00%–7.50 % and induces an additional short-chain fatty acid and methanogenesis inhibition [[illegible]]. As well, these aspects could be caused by the increased break down of cellulose and hemicellulose for the high percentages of OHP doses [[illegible]].

3.3. *Effects on methane production*

Methane composition and cumulative methane production (at 24 days of co-digestion) from different POME pretreatment by OHP doses are presented in [illegible]. Comparatively low methane production from nonpretreated POME is because of the density of the plant surface to ACoD and high lignin composition. The structure between cellulose, hemicellulose, and lignin restricted the hydrolysis and enzyme process, therefore, lignocellulosic digestion is hindered [[illegible]]. The methane production is similar to a series of current research with POME as ACoD substrates. As shown in [illegible], different OHP pretreatment dosing has a greater amount of methane production than the nonpretreated co-digestion. Methane composition rises with the rise of OHP dosing from 0.25 % to 1.00 % and decreases after 1.00 %. The results indicate that OHP pretreatment enhances methane production. With the appropriate percentages of OHP dosing, the chemical bond of cellulose, hemicellulose, and lignin were demolished by alkali, preparing POME more biodegradable by the microbes. By pretreating with different OHP dosing, the maximum methane production was found 1656.38 mL, which was 64.83 % more than the nonpretreated co-digestion, which was observed at 1.00 % OHP pretreatment of POME. This methane composition was 72.40 % in terms of biogas production. In a similar study, pretreatment by OHP was also used to improve the ACoD efficiency for Miscanthus floridulus [[illegible]]. Their outcomes displayed that the highest methane production was found 278.7 mL/g VS at 0.8 % OHP doses maintaining neutral pH and improved by 49 % concerning nonpretreated samples.

Actually, pretreatment plays important role for the enhancement of bioenergy yield from biomass through ACoD process, particularly for lignocellulosic substrates. Pretreatment by hydrogen peroxide was used to increase the anaerobic digestion of Miscanthus floridulus [[illegible]]. The outcomes of this study showed that the highest methane production was yield 278.7 mL/g VS at 0.8 % H_2O_2 concentration having neutral pH which is an increment of 49 % with respect to the non-pretreated sample. In another study, the effect of hydrothermal and $Ca(OH)_2$ pretreatment on the biogas production by the AD of sugarcane bagasse (SCB) was examined. Hydrothermal, $Ca(OH)_2$ and their combination of pretreatment had substantial effects on hemicellulose and lignin breakdown by pretreatment and methane production through anaerobic digester [[illegible]]. The maximum production of biogas was observed with combined pretreatment effect was reached 318 mL/g VS, that was 47 % more than the untreated sugarcane bagasse where the maximum methane content was 69 % and maximum lignin degradation was 44 %. So, it is crystal clear that pretreatment is very effective in enhancing bioenergy production.

Again, the impact of alkaline pretreatment by H_2O_2 on the biodegradation and the methane yield of greenhouse crop waste was explored [[illegible]]. In this approach, H_2O_2 concentration (1–3 %) was maintained in different groupings to fix the effects of alkaline pretreatment of H_2O_2. The consequences shown that the alkaline pretreatment H_2O_2 made a substantial rise in the range of 200–800 % in soluble COD leakage and enhanced the methane production from 174 mL CH_4/g VS

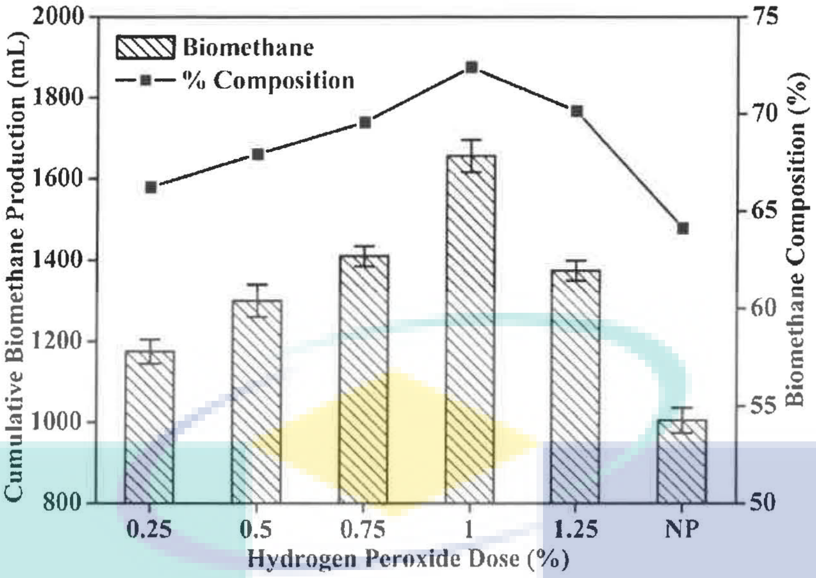

Fig. 6. Cumulative Biomethane Production and Biomethane Composition (%) for OHP Pretreated and Nonpretreated (NP) during 24 Days of Anaerobic Co-digestion (Error Bar is Standard Deviation)7.

to greater amount of 250 − 350 mLCH$_4$/g VS. Yet again, a pretreatment course by hydrogen peroxide was enhanced to optimize the biodegradation of rice straw and enhancement in biogas production [[illegible]]. The cellulose, hemicellulose, and lignin of rice straw were expressively degraded with the increasing concentration of hydrogen peroxide. The ideal conditions for the usage of pretreated rice straw in digestion process were 2.68 % H_2O_2 ans it results in a methane production of 288 mL/g VS. Thus, hydrogen peroxide pretreatment for rice straw could be used to expand methane content during production of biogas.

3.4. Kinetic modeling of biomethane yield

To assess the effect of pretreatment by OHP dosing on methane production from POME co-digestion, a first-order kinetic study is frequently investigated to ACoD process was used to compare the methane production through the co-digestion period [[illegible]].

$$Y = Y_m(1 - e^{-kt}) \tag{5}$$

Where Y is displayed as the cumulative methane production at the time taken t (mL/g VS), Ym is the final methane production (mL/g VS), k is termed as the apparent constant in kinetic (d^{-1}), t is the total co-digestion time (days). So, the final methane production expresses the ultimate value after that no more gas from the bioreactor is collected. The curve fitting by nonlinear regression for the sets of investigational data (Y, t) calculated the apparent constant in kinetic (k).

As presented in [illegible], methane production is well described by the Cheynoweth first-order kinetic model from Eq. 5. The kinetic constants (k) for different OHP from 0.18647 to 0.23029 d^{-1}. The simulated Cheynoweth curve and real data curve of methane production from the 1.00 % OHP doses are shown in [illegible]. Real methane production was entirely analogous to simulated Cheynoweth results.

Table 3
Parameters for the First Order Reaction of Biomethane Production (Cheynoweth Equation) of Palm Oil Mill Effluent and Cattle Manure.

OHP Doses	k (d^{-1})	Y_m (mL)	Adjusted R^2
0.25 %	0.21437	1192.26	0.9917
0.50 %	0.23029	1335.77	0.9878
0.75 %	0.20172	1516.81	0.9927
1.00 %	0.21925	1807.60	0.9976
1.25 %	0.18647	1432.80	0.9906
Non Pretreated	0.20862	1011.69	0.9884

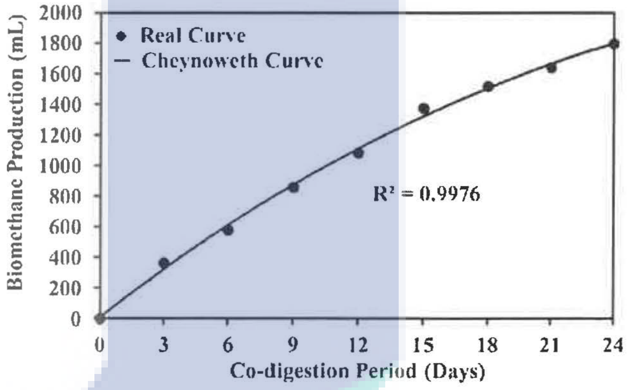

Fig. 7. Biomethane Production and Cheynoweth First Order Kinetic Curve by 1.00 % OHP Doses.

4. Conclusion

Nowadays, the anaerobic bioreactor is the most extensively practiced strategy treating a vast range of wastewaters. Regardlessness of wastewater treatment application and simplicity of installation makes it a unique technology. ACoD of POME with CM (equal proportion) in SABr provided the ultimate degradation efficiency, maintain process stability by controlling temperature and pH, and maximize the bioenergy production compared to conventional treatment systems. The present study proves the biodegradability and bioenergy production potential of OHP pretreated POME with CM with a 50:50 mixture ratio with the constancy and performance of the continual co-digestion procedure. The effects of OHP dose with different concentration was investigated on the physicochemical characteristics of POME. This research discloses that OHP dose of 1.00 % with 1 mM Fe^{3+} having a contact time of 30 min show a remarkable reduction in the physicochemical parameter of POME. The maximum biogas and methane production were obtained at 1.00 % of OHP doses, which was improved by 46.00 % and 64.83 % respectively compared to the untreated group. OHP pretreatment of the POME ensured an improvement to the subsequent ACoD as its digestibility was augmented. According to this result, it is transparent that the OHP dosing enhances the biodegradation of POME in comparison with many other treatment technologies. So, it can be concluded that OHP is an effective, sustainable, and environment-friendly technique for enhanced degradation of POME for enhanced bioenergy production.

Declaration of Competing Interest

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.

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References

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Co-digestion of palm oil mill effluent for enhanced biogas production in a solar assisted bioreactor: Supplementation with ammonium bicarbonate

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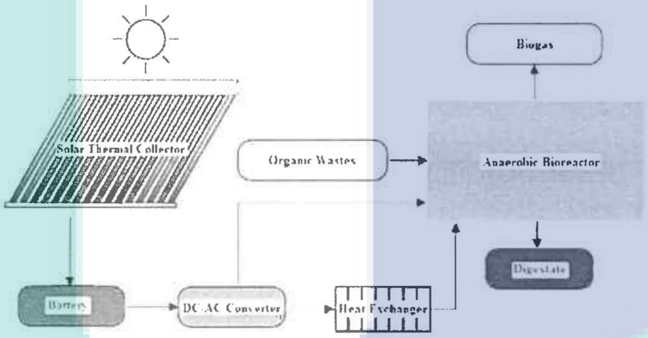
HIGHLIGHTS

- A novel and successful strategy was developed for ammonium bicarbonate dosing.
- Ammonium bicarbonate was supplemented for improving biogas production.
- Biogas production increased by 29.80% with 10 mg/L ammonium bicarbonate dosing.
- Biomethane production increased by 42.30% with 10 mg/L ammonium bicarbonate dosing.
- Application of specific mathematical equation fitted biogas production curves as well.

GRAPHICAL ABSTRACT



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Biogas production

ABSTRACT

Lack of sufficient nitrogenous substrate and buffering potential have been acknowledged as impediments to the treatment of palm oil mill effluent through co-digestion processes. In this study, ammonium bicarbonate was used to provide the nitrogenous substrate and buffering potential. To regulate the impact of ammonium bicarbonate toxicity on the anaerobic co-digestion system, dosages from 0 to 40 mg/L were supplemented. The biogas yield was used to indicate the effects of NH_4^+ toxicity. In a solar-assisted bioreactor, solar radiation was first collected by a solar panel and converted into electricity, which was then used to heat a mixture of palm oil mill effluent and cattle manure to maintain the reactor in the mesophilic temperature range. This co-digestion operation was performed semi-continuously and was analyzed at a 50:50 mixing ratio of palm oil mill effluent and cattle manure. The results indicate that the additional dosing of ammonium bicarbonate can significantly enhance biogas production. Maximum cumulative biogas and methane productions of 2034.00 mL and 1430.51 mL, respectively, were obtained with the optimum addition of 10 mg/L ammonium bicarbonate; these values are 29.80% and 42.30% higher, respectively, than that obtained in the control co-digestion operation without addition of ammonium bicarbonate. Utilization of a mathematical equation ($G = Gm^{kt}$) to describe a kinetic analysis of the biogas yield also indicated that the optimum ammonium bicarbonate dose was 10 mg/L. The results of this study suggest that supplementation with ammonium bicarbonate doses of up to 40 mg/L can be used to provide nitrogenous substrates and buffering potential in anaerobic co-digestion processes. The determination of the

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optimal dose provides an alternative and efficient option for enhanced biogas production, which will have obvious economic advantages for feasible industrial applications.

1. Introduction

Palm oil mills produce vast quantities of wastewater, which is a combination of the undergrowth wastewater produced during various steps of the palm oil yield procedure and the clean water used for washing; this wastewater is referred to as palm oil mill effluent (POME). POME contains several suspended and dissolved elements that can produce unpleasant odors after organic breakdown by microbes (Nasrullah et al., 2017). POME has acidic characteristics, and the degradation is relatively complex (Zaied et al., 2019a). Through pumping and distributing water, unindustrialized actions consume a greater amount of energy, and the palm oil industry is responsible for the production of a large amount of extremely contaminating waste substances that contain valuable biological substances and moisture. If POME is discharged directly into the waterways, the environment can be polluted owing to high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Zaied et al., 2019c). Moreover, feedlot farming with slaughterhouse incorporation represents a relatively new issue, and this is necessary to administrate these carefully for waste management. Though the cattle are kept for three months to make them more fatty before slaughter, the amount of manure and effluent originating from these slaughterhouses is approximately 200–300 t per day (Omar et al., 2008).

Anaerobic co-digestion (ACoD) is a wastewater treatment method that processes two or more substrates, with consequences for the development of system competence (Álvarez et al., 2010; Zaied et al., 2019a). This process leads to greater biogas yields and improved system steadiness compared to digestion of a single substrate (Astals et al., 2011). Co-digestion is the simultaneous digestion of more than two substrates to improve process efficiency (Zaied et al., 2019c). This is a promising option to overcome the difficulties of single digestion and expand the economic feasibility of anaerobic digestion (AD) owing to improved biogas yields. Consequently, the implementation of AD with POME as the inoculum is favorable because the biogas source is delivered and the pollution risk is simultaneously resolved when the POME is accompanied by cow manure (CM) (Haiman et al., 2011). Previously, many researchers have reported the co-digestion of POME with several inoculums (Poh and Chong, 2009). However, the co-digestion of POME with CM for biogas production is inadequate. At present, studies are being conducted on the prospective use of CM as an inoculum with POME for biogas production under controlled pH and temperature conditions.

On the other hand, solar energy is used in less costly bioreactors for the treatment of organic wastewater. The solar heating system of the bioreactor is related to enhanced biogas production. In lieu of using electrical or diesel heating for organic wastewater, a solar-assisted bioreactor (SABr) can also enhance the biogas yield, allowing the cost of biogas production to be reduced. An SABr comprises a solar thermal radiation collector, temperature regulator, stirred tank reactor, and produced gas collector (Rasi et al., 2007). Solar radiation from sunlight is collected by the solar panel and converted to electricity with the use of a battery and DC-AC converter; this can then provide the appropriate temperature for organic waste breakdown and maintain a steady bioreactor temperature (Ahmad et al., 2003). The main tank reactor at the required temperature then yields biogas with an enhanced total biogas production (Siedlecka and Stepnowski, 2005). A previous study has shown that mesophilic (35 °C) and thermophilic (55 °C) conditions provide stable functioning temperatures for biogas production with the anaerobic digestion method (Siddique et al., 2014).

An experimental trial of treating POME using ACoD with CM has previously been carried out (Zaied et al., 2019b). The experimental results indicated that POME treatment necessitates additional substrates to maintain important functioning constraints such as the pH, alkalinity, and biomass. Investigation during the ACoD operation revealed that these constraints were below the suggested levels. Subsequently, after the volume of substrates reached 1.75 L in the main tank reactor after 12 d, the entire trial operation failed. This failure occurred as a result of an unusual pH drop and increase in volatile fatty acids (VFAs). This is suggested that the pH and VFAs are essential to the ACoD treatment process, particularly for the critical methanogen set of microbes. Yet, the substrate amount of 1.75 L was found to be insufficient to become a reason for failure in the SABr. Hence, this was reported that an inadequate buffering capacity and disturbance of the bacterial activity stability between methanogens and non-methanogens for the conversion of carbon materials to methane (CH_4) were the main reasons for the operational failure. Difficulties during ACoD trials should be identified, as this procedure has been demonstrated to be an effective alternative for treating highly organic wastewater to produce CH_4 as a form of biogas (Borja and Banks, 1994).

Nevertheless, the ability of ammonium bicarbonate to improve SABr systems has not been investigated comprehensively. This study investigates supplementation with ammonium bicarbonate (NH_4HCO_3), which can provide the required buffering capacity and nitrogen availability to prevent VFA accumulation during the ACoD treatment process; this also benefits the bacterial population. Ammonium bicarbonate can play significant roles in the anaerobic digester as the preferred nitrogenous substrate for microbes and to provide an important buffering potential. However, high ammonium bicarbonate doses can cause ammonia toxicity, particularly for methanogens. Thus, the optimum dosage for ammonium bicarbonate supplementation in ACoD processes needs to be resolved.

2. Materials and methods

2.1. Feedstock collection and preparation

A 100.00 L POME sample was collected from the anaerobic pond of the LKPP Corporation Sdn. Bhd., No. 45/4, Jalan Teluk Sisek, 25000 Kuantan, Pahang, Malaysia. In addition, 100.00 kg of partly fermented CM was collected from an average-sized cattle farm in Gambang, Malaysia. The POME and CM were placed in compact frost containers and transferred to a cold room. The temperature was maintained at 4 °C during storage. Before the ACoD trials, the POME sample was subjected to a simple screening to remove coarser constituents. The CM was diluted with water at a volume ratio of 1:25 and sieved through a 20 μm sieve to eliminate debris.

2.2. Reactor design and fabrication

The conventional design of a bioreactor is not sufficiently capable of maintaining the pH and temperature in a stable condition. Therefore, a pH (0–14) and temperature (0–55 °C) regulator was introduced to achieve good control of the system stability. A two-unit battery (48 V, 0–100 A) stored solar radiation obtained from direct sunlight using a solar panel (150 W, 147 cm × 67 cm) and converted the solar energy to electrical energy with a DC-AC inverter and controller (0–220 V output). The instrumentation parameters of the SABr are listed in Table 1. The total volume of the SABr was 5.00 L, with a working volume of 3.50 L. The bioreactor was configured as a cylindrical global system fabricated of glass and stainless steel. This was closely covered with a steel frame containing four nuts. The agitating motor was installed over the top of the main reactor. A device for controlling the agitation speed in

Table 1
Instrumental parameters of solar assisted bioreactor (SABr).

No.	Tools	Range	Accuracy	Parameter
1	Total Radiometer	1500 W/m^2	5%	Total Solar Irradiation
2	Scattering Radiometer	1500 W/m^2	5%	Scattering Solar Irradiation
3	Radiant Data Collector	–	–	–
4	Thermocouple	−200 to 350 °C	1 °C	Slurry Temperature
5	Thermometer	−50 to 50 °C	0.2 °C	Air Temperature

the range of 0–450 rpm was also affixed. A water jacket surrounded the main reactor to ensure the desired temperature for microbial degradation was maintained. Two feeding injectors were used to feed the sample into the main reactor. The produced gas was collected by installing a gas bag. Fig. 1 shows a photograph of the SABr used for the ACoD treatment process in these experiments.

2.3. Reactor operation

The POME and CM mixture was slowly added to the reactor as this was required few times for the microorganisms to adjust to the new anaerobic environment. During operation, the temperature was kept at 35 °C for 24 d, while 437.50 mL of the substrates mixture (50:50) was added gradually every 3 d until the working volume of 3.50 L was reached (Zaied et al., 2019c). Mixing was performed at 60 rpm by direct electric motors powered by the collected solar energy. In the meantime, the anaerobic microbes consumed organic substances in the reactor and created a suitable anaerobic environment for the stable growth of microbes. The properties of fed samples were examined every 3 d, while the pH was measured daily. The indexes of the ACoD process were the effects of co-digestion on the biodegradation, yield of biogas, and, most importantly, system steadiness.

2.4. Supplementation with ammonium bicarbonate

Each digester contained inoculum, substrate sludge, and varying amounts of the ammonium bicarbonate additive depending on the test conditions. Initially, the reactor containing only POME and CM at a 50:50 mixing ratio without ammonium bicarbonate addition was designated as the control operation. Other reactor conditions were designated as R1, R2, R3, and R4, which were supplemented with ammonium bicarbonate at doses of 10, 20, 30, and 40 mg/L, respectively. To confirm adequate mixing and to facilitate the biogas yield, all reactors were stirred for 30 min before the sample was poured into the reactor. The optimal ammonium bicarbonate dosing was determined based on the total biogas production. The impact of ammonium bicarbonate toxicity, particularly on the methanogen activity in the reactor system, could be determined based on the maximum production of biogas.

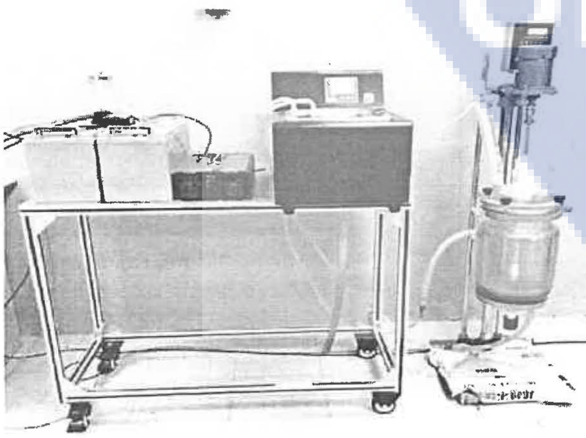

Fig. 1. Experimental photograph of the solar assisted bioreactor.

2.5. Analytical methods

The total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), and soluble ammonia nitrogen (SAN) of the sludge supernatant were measured according to the standard methods (Association et al., 1915). The pH and temperature in the digester were controlled and measured as described previously (Hansen et al., 1998). The concentration of VFAs was detected using gas chromatography (GC; GC-2010, Shimadzu, Japan) equipped with a flame ionization detector (FID). Biogas production was measured using a water displacement system under a fixed temperature and ideal pressure condition (Wang et al., 2014). The regular and cumulative production of biogas was measured in this study with the water displacement system. The biogas yield was thus reported as a volume yield expressed in units of mL. The amount of water displaced was equivalent to the amount of biogas generated. The methane composition was investigated using an Agilent gas chromatograph equipped with a thermal conductivity detector (TCD). Helium gas was utilized as the carrier gas with a flow rate of 30.00 mL/min. The oven, inlet, and detector temperatures were maintained at 70 °C, 120 °C, and 200 °C, respectively, and the ideal condition for the pressure was 0–150 psi.

2.6. Statistical analysis

The triplicate experimental data were analyzed using Microsoft Excel 2016. All essential mathematical and statistical analyses were performed in this software. The mean, standard deviation, and standard error results for the data were computed based on replicates using OriginPro 9.1 and adjusted for each figure and table value.

3. Results and discussions

3.1. Characterization of substrates

The characteristics of the POME, CM, and POME:CM (50:50) mixture before co-digestion are summarized in Table 2. The concentrations of total solids (TS) and volatile solids (VS) in the POME were 39.75 g/L and 32.56 g/L, respectively. These high TS and VS concentrations indicate that microbes are abundantly available in the POME sample. Generally, POME contains carbohydrates, lignin, cellulose, hemicellulose, and sugars, whereas CM contains significant buffering potential, a sufficient amount of anaerobic bacteria, and a wide-ranging diversity of important nutrients for maximum microbial growth (Ahmadi-Pirlou et al., 2017; Liu et al., 2013). The pH of the POME and CM were 4.60 and 5.40, respectively. The COD of the POME was 28.34 g/L. The ammoniacal nitrogen content was greater in the CM than the POME. The ammonia from the CM supported the enhanced process equilibrium throughout the ACoD process.

3.2. Biogas and methane production

Operational failure can be caused by VFA accumulation, which may occur as a result of additional constraints such as the availability of

Table 2
Compositions and characteristics of palm oil mill effluent and cattle manure.

Parameter	Raw POME	Raw CM	POME:CM (50:50)
pH	4.60 ± 0.40	5.40 ± 0.50	5.48 ± 0.50
COD	28.34 ± 0.30	16.72 ± 0.20	24.58 ± 0.30
BOD	15.28 ± 0.20	9.28 ± 0.10	13.40 ± 0.20
TS	39.75 ± 0.30	2.38 ± 0.10	22.79 ± 0.10
VS	32.56 ± 0.30	1.17 ± 0.10	18.23 ± 0.10
VFA	0.30 ± 0.10	1.40 ± 0.05	0.85 ± 0.05
SCOD	0.56 ± 0.12	2.48 ± 0.13	1.64 ± 0.13
NH_3-N	0.037 ± 0.004	0.84 ± 0.07	0.48 ± 0.02

Note: All parameters are in g/L except pH.

micronutrients (Fe, Mg, Ni, Cu, Co, and P). However, insufficient micronutrients can be prevented on the basis of the mineral proportions. As the CM was obtained from partly digested cow manure, the phosphorus content should be satisfactory. Currently, the presence of ammoniacal nitrogen is considered as the most important source of macronutrients in a fermented sludge and is also recognized as a noteworthy concentration after de-nitrification is accomplished (Metcalf et al., 2007). Sodium nitrate is also considered as an alternative choice to overcome a lack of nitrogenous substrates. However, during the application in the co-digestion process in the reactor, the NO_3^{-1} discharge will increase the oxidation-reduction potential. The oxidation potential of the reactor is likely to be maintained above −300 mV; methanogenesis is hindered at the lower values of the oxidation potential (Szostková and Vítěz, 2010). To finalize the buffering potential in the ACoD process, the proper chemical choice is an important factor. Undesirable solid substances can be formed as a result of $CaCO_3$ precipitation.

Biogas and methane production were the key indicators for the utilization of resources for the POME, and the results are presented in Fig. 2 The cumulative biogas (P_{biogas}) and methane ($P_{methane}$) production had stabilized after 24 d of co-digestion. P_{biogas} and $P_{methane}$ were improved by supplementation with ammonium bicarbonate at doses of 10 to 40 mg/L, and the optimum production was found at an ammonium bicarbonate dose of 10 mg/L. Compared to the control co-digestion, ammonium bicarbonate addition of 10, 20, 30, and 40 mg/L resulted in an increase in P_{biogas} of 29.80%, 17.55%, 11.42%, and 3.64%, respectively, and an increase in $P_{methane}$ of 42.30%, 26.16%, 16.98%, and 6.38%, respectively; 10 mg/L was the optimal dosage. In comparison, Siddique and Zularisam also applied ammonium bicarbonate to the sludge anaerobic digestion of petrochemical wastewater under mesophilic temperature conditions and found that the methane production was enhanced by 27.77% compared to the control digestion (Siddique and Zularisam, 2012). This divergence can be attributed to the species present and their specific mechanisms for enhanced methane yield. The

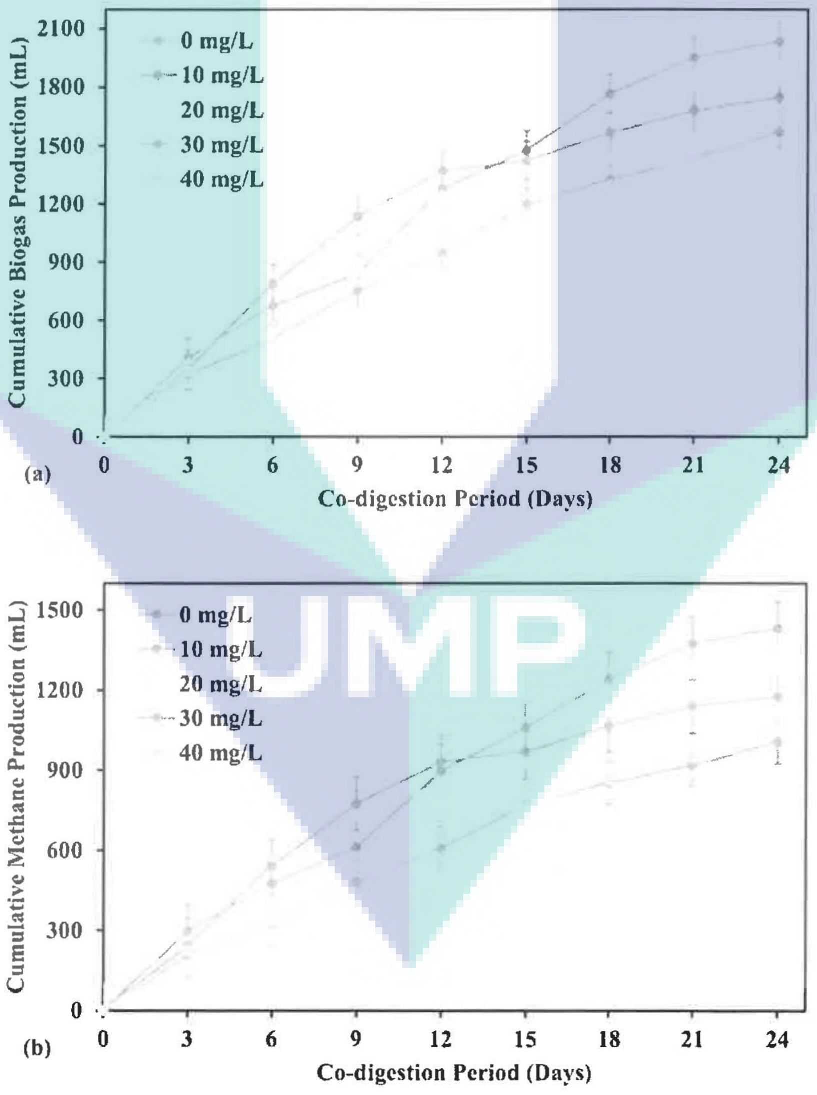

Fig. 2. Cumulative biogas production (a) and methane production (b) during ACoD process.

experimental outcomes showed that an appropriate supplementation of ammonium bicarbonate (10 mg/L) enhanced the biogas and methane production, whereas dosing with more than 10 mg/L may not have a significant impact.

If a rapid reaction occurs under specific environmental conditions, the biogas production will reach a maximum. For the conversion of organic substances into methane during the ACoD process, methanogenesis is the important operational process. Hydrogen and carbon dioxide are used by hydrogenotrophic methanogens, whereas acetic acid and carbon dioxide are used by acetoclastic methanogens to produce methane as a final product (Lee et al., 2012). This is recommended to avoid the accumulation of VFAs by supplementation with effective and appropriate strong bases in a co-digestion process with other waste substances (Chen et al., 2012; Lahav and Morgan, 2004). This approach maintains a suitable carbon–to-nitrogen ratio and sufficient buffering potential to ensure a stable pH. Therefore, the occurrence of methanogenesis with improved steadiness will successfully lead to enhanced methane generation.

The maximum methane production increased with an ammonium bicarbonate dosage of 10 mg/L compared with the control but decreased at dosages of 20 mg/L to 40 mg/L compared with the 10 mg/L dosage. A higher dosage of ammonium bicarbonate leads to an increased hydrolysis reaction rate, and a noticeable improvement in hydrolysis with the addition of ammonium bicarbonate is supported by the results of a similar study with corn stover (Li et al., 2013) and a study of the co-digestion process with pig manure and dewatered sewage sludge (Zhang et al., 2014). However, dosages of greater than 40 mg/L may not progressively increase the hydrolysis rate. A 10 mg/L addition of ammonium bicarbonate provided the best performance and improved the ACoD process in terms of the following features: an increased hydrolysis period, enhanced hydrolysis and methanogenesis effectiveness, and enhanced biogas and methane production. These outcomes reveal that supplementation with ammonium bicarbonate accelerated the hydrolysis rate, and rapid methanogenesis at the higher hydrolysis rate improved the methane yield; however, an overdose of ammonium bicarbonate may hinder methanogenesis in the co-digestion process.

3.3. Soluble COD and volatile fatty acids

The concentration of SCOD (Fig. 3a) illustrates the hydrolysis rate, and the results indicate the occurrence of rapid hydrolysis at the start of the process, which then progressively decreased. The highest SCOD values with the addition of ammonium bicarbonate occurred within the first 6 d of co-digestion (with maximum values in R1 of 5486.40 mg/L, R2 of 6472.80 mg/L, R3 of 7324.50 mg/L, and R4 of 8792.30 mg/L), resulting in a fast decline. The positive relationship between the maximum SCOD and the ammonium bicarbonate dose reveals the enhancement and rate of the hydrolysis, which echo the fitting results for the hydrolysis rate. In particular, the SCOD was quite high from days 3 to 6 in the test digesters. However, for the control operation, the SCOD reached maximum value (7054.40 mg/L) at day 6 of co-digestion and rapidly decreased without a longer preservation phase. Considering the enhanced methane production observed in the reactors, the existence of such a preservation stage may indicate faster formation and utilization of SCOD as well as accelerated hydrolysis. After 6 d of co-digestion, the SCOD value fluctuated significantly, and the next fluctuation was observed after day 15, which may indicate the conformity of the hydrolysis and methanogenesis actions at the mid-stage of the ACoD process to produce a gradual increase in the SCOD; meanwhile, a continuous increase in the biogas yield (Fig. 2a) was found throughout this duration in R1 and the control co-digestion.

The concentration of accumulated VFAs signified the acidification phase, as shown in Fig. 3b. Usually, the value is approximately 50% of the SCOD, and the value also has similar production and degradation behavior to that of the SCOD. The highest concentrations of VFAs in the reactor were accumulated before day 6 (with maximum VFA concentrations in R1 of 2846.00 mg/L, R2 of 3674.00 mg/L, R3 of 4324.00 mg/L, and R4 of 4796.00 mg/L), and a positive correlation between the maximum VFA value and the ammonium bicarbonate dosage was observed. In contrast, the highest VFA concentration in the control operation was 4855.60 mg/L, which occurred on day 9. In particular, at day 3, the VFA accumulation increased with increasing ammonium bicarbonate supplementation, with a greater increase rate with respect to the control co-digestion, indicating that the addition of ammonium bicarbonate accelerated the production of VFAs production and enhanced the acidification. Moreover, the highest VFA accumulation in the control operation was greater than that in R1, and the decrease in the VFA concentration in R1 operation was more gradual than that in the control operation. These results can be attributed to the previous utilization of VFAs by methanogenesis resulting in the decrease in the observed outcomes; at the same time, the continuous formation of VFAs in the reactors kept the observed outcomes from decreasing so rapidly. The accumulation of VFAs in the five reactors were all affected by biodegradation after reaching the maximum value, and were almost fully biodegraded after 24 d of co-digestion.

3.4. pH and soluble ammonia nitrogen

Supplementation with ammonium bicarbonate induced a sudden rise in the pH (as shown in Fig. 4a). Increasing the dose of ammonium bicarbonate resulted in an increased initial pH value. However, after 3 d of operation, the pH was approximately 6.4 in all reactors, representing a rapid offsetting of the pH increase and the utilization of the supplemented ammonium bicarbonate. After 3 d of co-digestion, varying pH trends were observed: the pH in R1 was nearly the same as that in the control co-digestion operation, whereas the other three reactors exhibited a faster pH gradient, and a positive correlation was observed between the pH increase rate and the ammonium bicarbonate dose. After 18 d of operation, the rebound slowed, and the pH values were steady within approximately 6.8–7.1.

The formation and utilization trends in the VFA accumulation (Fig. 3b) may be considered along with the pH variations: higher production of VFAs occurred with increasing dosage of ammonium bicarbonate. The VFA accumulation was degraded rapidly from days 6 to 12 of co-digestion, and the degradation rate slowed after day 12. Similar variation trends in the pH were observed at a similar period of operation. In previous studies, an alkaline pH was considered to increase sludge digestion (Zhang et al., 2011; Zhang et al., 2010), while also resulting in increased reformation of hydrolytic microbes, which additionally produces extracellular proteases, depolymerises, and Clostridium (Chen et al., 2017; Zhang et al., 2010; Zheng et al., 2013). The fast pH rebound produced by supplementation with ammonium bicarbonate enhanced the effectiveness of the methanogens, and the improvement in the acidic environment facilitated the degradation of hydrolytic microbes.

The soluble ammonia nitrogen (SAN) concentration (Fig. 4b) was correlated with the pH variation because the release and accumulation increased with the pH. Consistent with the change in pH, SAN increased from day 3 to day 15. At day 3, the concentration of SAN in all five reactors was as follows: control (242.00 mg/L) > R1 (178.00 mg/L) > R2 (170.00 mg/L) > R3 (152.00 mg/L) > R4 (62.00 mg/L), indicating that supplementation with ammonium bicarbonate may hinder the hydrolysis of protein-like substances in the primary stage. Subsequently, the SAN concentrations in the reactors with added ammonium bicarbonate became greater than that in the control operation. Higher ammonium bicarbonate dosages resulted in higher SAN values, such as that of R4 (658.00 mg/L) at day 12. After 12 d of operation, SAN hardly varied and remained steady. Overall, the SAN concentration in the five reactors exhibited a similar trend to that of the SCOD and VFA concentration, in which increased supplementation with ammonium bicarbonate

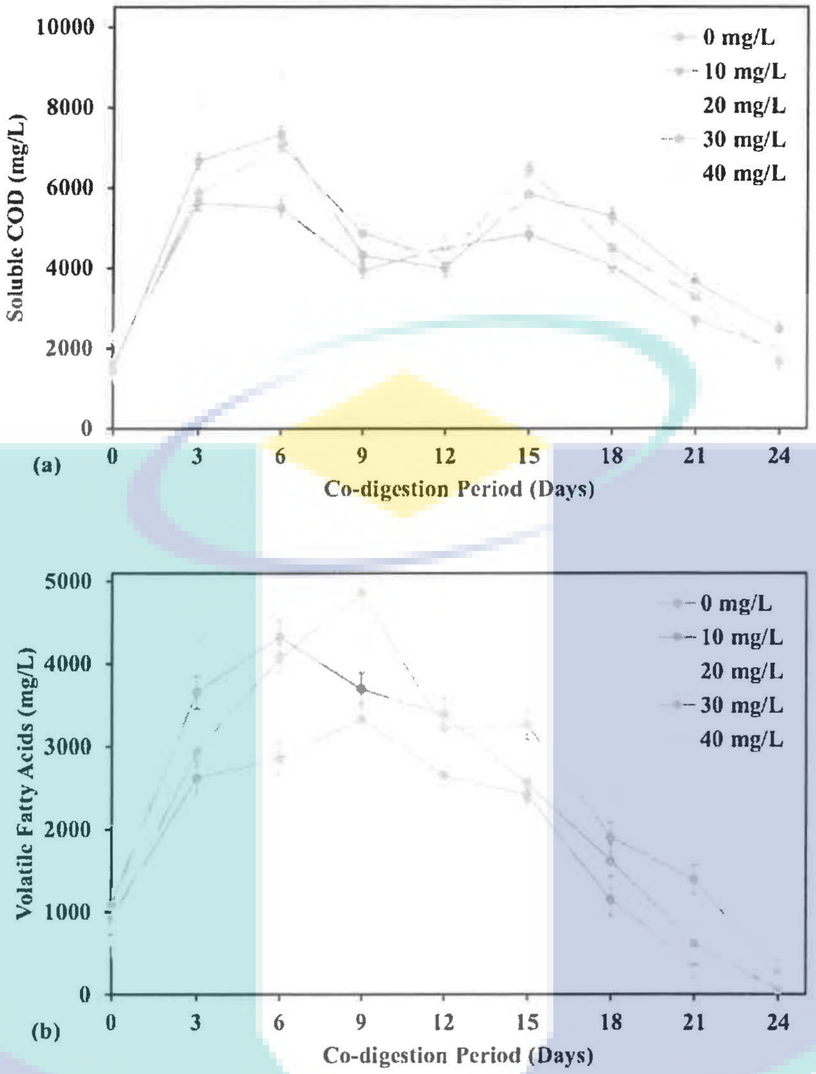

Fig. 3. Changes of soluble COD (a) and VFSs (b) concentrations during ACoD process.

promoted the hydrolysis, whereas the SAN with lower ammonium bicarbonate supplementation (e.g., in reactor R1) remained low with respect to the control operation.

3.5. Use of a specific mathematical equation

Conventional statistical methods, i.e., ANOVA and *t*-tests, are not appropriate for analysis of a small number of trials. The data explanation presented in Fig. 2 was not clearly able to indicate the effect of ammonium bicarbonate on the biogas production. Thus, a numerical study of the volumetric biogas production was carried out using the specific mathematical equation given in Eq. (1). This mathematical equation was presented and later modified to provide a kinetic description of methane production by anaerobic digestion processes (Daniel and Wood, 1980; Redzwan and Banks, 2004). Similarly, this equation can be used to measure the maximum biogas production (G_m) without continuing the experiment to exhaustion. Eq. (1) is linearized and rearranged as Eq. (2). Table 3 summarizes the quantity of biogas produced (G) at time t, and these data were used to determine the coefficients in Eq. (2). The linear plot of lnG vs. 1/t is presented in Fig. 5. The G_m value can be obtained, validating the efficiency of ammonium bicarbonate supplementation to the process.

$$G = G_m^{k/t}. \quad (1)$$

$$\ln G = \ln G_m + k/t. \quad (2)$$

where G is the cumulative biogas yield, G_m is the maximum biogas yield, k is the slope coefficient in the linear equation, and t is the time.

All of the data plotted in Fig. 5 show that G_m can be calculated using the specific mathematical equation, as the plotted data exhibit a linear correlation. Table 4 summarizes the projected maximum production of biogas. This is calculated by inversing the stoppage values at the y-axis in Fig. 5, as described in Eq. (2). This projection gives a maximum biogas production value when 10 mg/L of ammonium bicarbonate is used in the co-digestion. The maximum biogas production from this experiment is 2034.00 mL, while the projected maximum biogas production obtained using the specific mathematical equation (Eq. (2)) ranged between 1658.00 and 2150.00 mL, as indicated in Table 4.

Sustaining the methanogen activity in the ACoD procedure is the key process for removing carbon-based materials in the POME. Reproduction of a specific substance in the POME is also

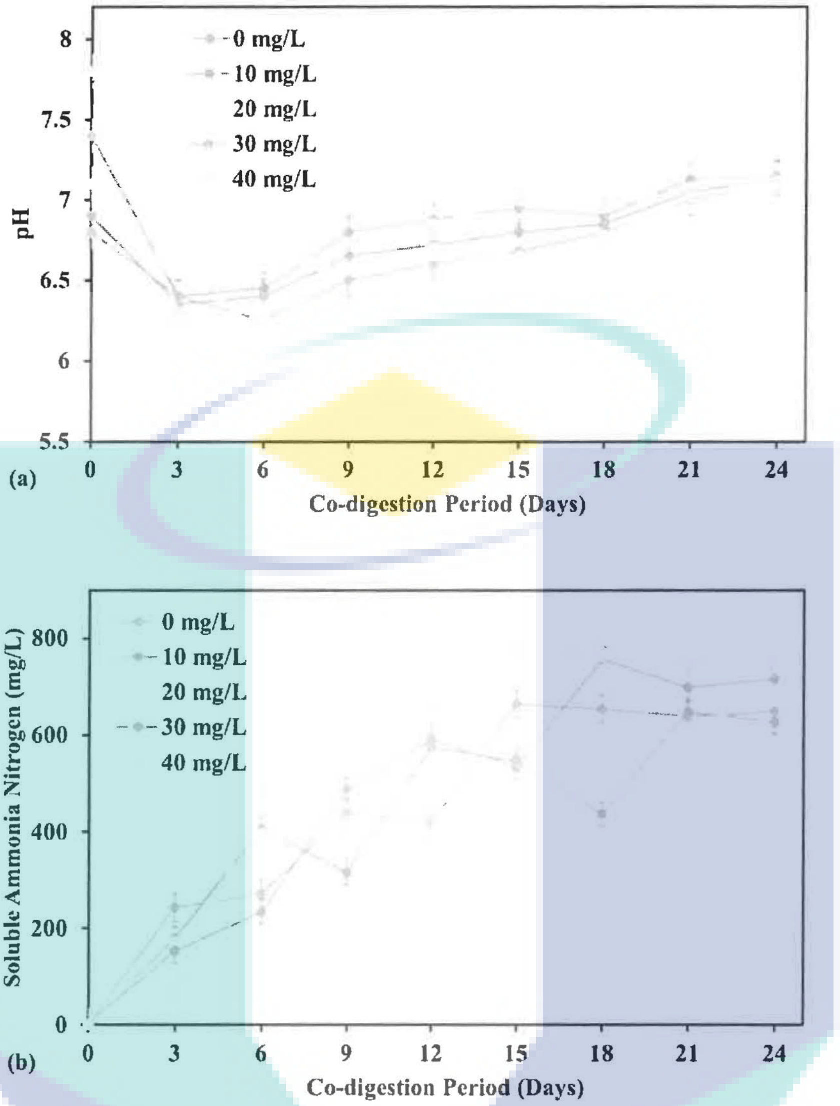

Fig. 4. Changes of pH (a) and soluble ammonia nitrogen during ACoD process.

significant. This is highly recommended that water effluent having a high carbon content should not be mixed with a low-carbon water effluent for treatment by the same procedure, even if both are produced at the same locality (Chmiel et al., 2003). Thus, suitable POME treatment methods for the specific strength and wastewater content should be used. Separating the wastewater treatment procedures with respect to their features can be cost-effective if the treated water effluent is reused (Miyaki et al., 2000). For wash waters generated from every palm oil production process, such as pre-washing of the oil palm and dusting of process containers, channels, and related equipment, using ACoD in every processing system for the treatment of the wastewater might be

Table 3
Cumulative biogas production for serial concentration of ammonium bicarbonate in anaerobic co-digestion system.

Time (days)	Average cumulative biogas production (mL)				
	Ammonium concentration (mg/L)				
	0	10	20	30	40
3	317 ± 20	407 ± 20	369 ± 20	346 ± 20	337 ± 30
6	502 ± 20	672 ± 30	694 ± 30	638 ± 30	512 ± 30
9	749 ± 20	839 ± 30	1026 ± 25	917 ± 25	849 ± 25
12	944 ± 20	1274 ± 20	1154 ± 25	1142 ± 25	1024 ± 20
15	1197 ± 20	1477 ± 20	1338 ± 20	1302 ± 30	1147 ± 25
18	1324 ± 20	1764 ± 20	1568 ± 20	1439 ± 30	1294 ± 25
21	1431 ± 20	1952 ± 30	1693 ± 25	1518 ± 20	1438 ± 25
24	1567 ± 20	2034 ± 30	1842 ± 30	1746 ± 25	1624 ± 20

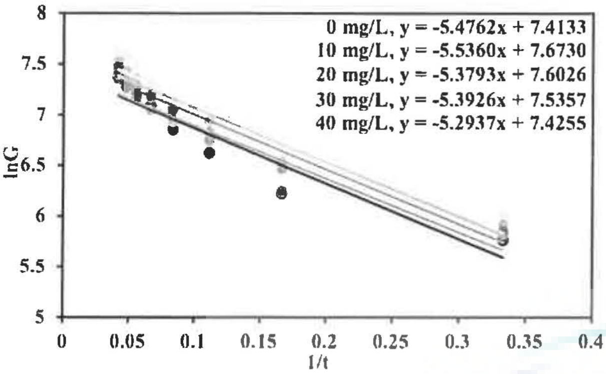

Fig. 5. Determination of coefficients for G_m and k by linearizing Eq. (2) for each of ammonium bicarbonate concentrations.

established based on the wastewater characteristics, composition, and strength. The treatment system would then provide the use of tertiary treatment for improved possibility of the recycling and reuse of treated wastewater.

4. Conclusion

This study confirmed that appropriate supplementation with ammonium bicarbonate can enhance hydrolysis and biogas production from POME. The results indicate that the ammonium bicarbonate accelerates the ACoD efficiency of POME for biogas production. An ammonium bicarbonate dosage of 10 mg/L was found to be the optimal dose for the substrate compared to higher doses of up to 40 mg/L. This is clear that the addition of strong bases like ammonium bicarbonate during ACoD operation can markedly improve the steadiness of the pH and successfully maintain the system stability. Accordingly, the cumulative biogas production was also successfully enhanced. The efficiency of ammonium bicarbonate addition was also described by a specific mathematical equation. According to the mathematical calculation, supplementation with 10 mg/L of ammonium bicarbonate can produce up to 29.80% more biogas compared to the control POME codigestion without ammonium bicarbonate addition. This result can add notable economic benefits to make the POME treatment strategy more feasible for large-volume industrial applications.

Declaration of competing interest

There are no conflict of interest among the authors. All are willingly agree to submit, check and make equal contribution to prepare the manuscript and revised form to this journal.

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Table 4
Estimated maximum volumetric biogas production with serial concentration of ammonium bicarbonate.

Parameters	Ammonium concentration (mg/L)				
	0	10	20	30	40
lnG_m	7.4133	7.6730	7.6026	7.5357	7.4255
G_m (mL)	1658.00	2150.00	2003.00	1874.00	1678.00

References

Ahmad, A.L., Ismail, S., Ibrahim, N., Bhatia, S., 2003. Removal of suspended solids and residual oil from palm oil mill effluent. J. Chem. Technol. Biotechnol. 78, 971–978.

Ahmadi-Pirlou, M., Ebrahimi-Nik, M., Khojastehpour, M., Ebrahimi, S.H., 2017. Mesophilic co-digestion of municipal solid waste and sewage sludge: effect of mixing ratio, total solids, and alkaline pretreatment. Int. Biodet. Biodeg. 125, 97–104.

Álvarez, J.A., Otero, L., Lema, J.M., 2010. A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresour. Technol. 101, 1153–1158.

Association APH, Association AWW, Federation WPC, Federation WE, 1915. Standard Methods for the Examination of Water and Wastewater. 2. American Public Health Association.

Astals, S., Ariso, M., Galí, A., Mata-Alvarez, J., 2011. Co-digestion of pig manure and glycerine: experimental and modelling study. J. Envo. Mgmt. 92, 1091–1096.

Borja, R., Banks, C., 1994. Kinetics of an anaerobic fluidized bed system used for the purification of fruit processing waste water. The Chemical Engineering Journal and the Biochemical Engineering Journal 54, B25–B32.

Chen, Y., Wen, Y., Zhou, J., Xu, C., Zhou, Q., 2012. Effects of pH on the hydrolysis of lignocellulosic wastes and volatile fatty acids accumulation: the contribution of biotic and abiotic factors. Bioresour. Technol. 110, 321–329.

Chen, Y., Jiang, X., Xiao, K., Shen, N., Zeng, R.J., Zhou, Y., 2017. Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH increase – investigation on dissolved organic matter transformation and microbial community shift. Water Res. 112, 261–268.

Chmiel, H., Kaschek, M., Blöcher, C., Noronha, M., Mavrov, V., 2003. Concepts for the treatment of spent process water in the food and beverage industries. Desalination 152, 307–314.

Daniel, C., Wood, F.S., 1980. Fitting Equations to Data. Computer Analysis of Multifactor Data. John Wiley & Sons, Inc.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: inhibition by ammonia. Water Res. 32, 5–12.

Hniman, A., O-Thong, S., Prasertsan, P., 2011. Developing a thermophilic hydrogen-producing microbial consortia from geothermal spring for efficient utilization of xylose and glucose mixed substrates and oil palm trunk hydrolysate. Int. J. Hydrog. Energy 36, 8785–8793.

Lahav, O., Morgan, B., 2004. Titration methodologies for monitoring of anaerobic digestion in developing countries—a review. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology 79, 1331–1341.

Lee, J.C., Kim, J.H., Chang, W.S., Pak, D., 2012. Biological conversion of CO2 to CH4 using hydrogenotrophic methanogen in a fixed bed reactor. J. Chem. Technol. Biotechnol. 87, 844–847.

Li, Y., Feng, L., Zhang, R., He, Y., Liu, X., Xiao, X., et al., 2013. Influence of inoculum source and pre-incubation on bio-methane potential of chicken manure and corn stover. Appl. Biochem. Biotechnol. 171, 117–127.

Liu, Z., Zhang, C., Lu, Y., Wu, X., Wang, L., Wang, L., et al., 2013. States and challenges for high-value biohythane production from waste biomass by dark fermentation technology. Bioresour. Technol. 135, 292–303.

Metcalf, E.I., Asano, T., Burton, F.L., Leverenz, H., Tsuchihashi, R., et al., 2007. Water Reuse. McGraw-Hill Professional Publishing.

Miyaki, H., Adachi, S., Suda, K., Kojima, Y., 2000. Water recycling by floating media filtration and nanofiltration at a soft drink factory. Desalination 131, 47–53.

Nasrullah, M., Singh, L., Mohamad, Z., Norsita, S., Krishnan, S., Wahida, N., et al., 2017. Treatment of palm oil mill effluent by electrocoagulation with presence of hydrogen peroxide as oxidizing agent and polialuminum chloride as coagulant-aid. Water Resources and Industry 17, 7–10.

Omar, R., Harun, R.M., Mohd Ghazi, T., Wan Azlina, W., Idris, A., Yunus, R., 2008. Anaerobic treatment of cattle manure for biogas production. Proceedings Philadelphia, Annual Meeting of American Institute of Chemical Engineers, pp. 1–10.

Poh, P.E., Chong, M.F., 2009. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Bioresour. Technol. 100, 1–9.

Rasi, S., Veijanen, A., Rintala, J., 2007. Trace compounds of biogas from different biogas production plants. Energy 32, 1375–1380.

Redzwan, G., Banks, C., 2004. The use of a specific function to estimate maximum methane production in a batch-fed anaerobic reactor. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology 79, 1174–1178.

Siddique, N.I., Zularisam, A., 2012. Renewable methane from ammonium bicarbonate supplemented petrochemical wastewater treatment during anaerobic co-digestion in CSTR. Procedia Engineering 50, 134–141.

Siddique, M.N.I., Sakinah Abd Munaim, M., Zularisam, A.W., 2014. Mesophilic and thermophilic biomethane production by co-digesting pretreated petrochemical wastewater with beef and dairy cattle manure. J. Ind. Eng. Chem. 20, 331–337.

Siedlecka, E., Stepnowski, P., 2005. Phenols degradation by Fenton reaction in the presence of chlorides and sulfates. Pol. J. Environ. Stud. 14.

Szostková, M., Vítěz, T., 2010. Microbial contamination of the sand from the wastewater treatment plants. Res. Agric. Eng. 56, 147–153.

Wang, X., Lu, X., Li, F., Yang, G., 2014. Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. PLoS One 9, e97265.

Zaied, B., Siddique, M., Zularisam, A., Ahmad, M., Salih, Y., 2019a. Biomethane production from anaerobic codigestion of palm oil mill effluent with cattle manure: a review. Asian J. Chem. 31, 2413–2424.

Zaied, B.K., Siddique, M.N.I., Nasrullah, M., Singh, L., Wahid, Z.B.A., Ahmad, M.F., 2019b. Application of solar assisted bioreactor for biogas production from palm oil mill effluent co-digested with cattle manure. Environmental Technology & Innovation 16, 100446.

Zaied, B.K., Siddique, M.N.I., Nasrullah, M., Singh, L., Wahid, Z.B.A., Ahmad, M.F., 2019c. Application of solar assisted bioreactor for biogas production from palm oil mill effluent co-digested with cattle manure. Environmental Technology & Innovation 100446.

Zhang, P., Chen, Y., Zhou, Q., Zheng, X., Zhu, X., Zhao, Y., 2010. Understanding short-chain fatty acids accumulation enhanced in waste activated sludge alkaline fermentation kinetics and microbiology. Environmental Science & Technology 44, 9343–9348.

Zhang, D., Yan, X., Zhang, X., Liu, C., Dang, R., Xiao, T., et al., 2011. Synchrotron radiation phase-contrast X-ray CT imaging of acupuncture points. Anal. Bioanal. Chem. 401, 803–808.

Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z., et al., 2014. Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. Appl. Energy 128, 175–183.

Zheng, X., Su, Y., Li, X., Xiao, N., Wang, D., Chen, Y., 2013. Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. Environmental Science & Technology 47, 4262–4268.