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Research Paper

Optimizing bio-methanation by differential zero valent iron (ZVI) particle size in cassava pulp feed CSTRs

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ABSTRACT

The inherent limitation of biogas from Anaerobic Digestion (AD) process is high carbon dioxide (CO_2) which deters and remains as a challenge for ultimate applicability of biogas as bio-energy for multi-purposes. The use of metallic powder as catalyst within AD process is promising to reduce both economic and environmental cost of post biogas upgrading technologies available nowadays. In utilizing Zero Valent Iron (ZVI) in differential particle size, M100 (nano-particle) and R12 (micro-particle), in cassava pulp and wastewater as feedstock materials for bio-methanation, this study attempted to optimize methane (CH_4) concentration of biogas in Continuous Stirred Tank Reactors (CSTRs). Synchrotron X-ray radiations and Scanning Electron Microscopy (SEM) were applied for characterizing the valency, atomic concentration, and particle size. The experiments showed under the optimum dosage values of 1 and 8 g/L for M100 and R 12, while up to 93% bio-methane enhancement was achieved with 67% addition of biogas volume than ZVI free control reactor. The presence of nano-particle M100 ZVI in AD system had instantaneous and stimulatory impact by its readily reactivity with aqueous substrate, but it leads to digestion failure even with few margins of excess. The micro-particle size R 12 amended CSTRs demonstrated ease of better digestion performance, process control and recoverable properties by its slow disintegration and bio-availability in AD system.

Key words: Anaerobic digestion, particle size, bio-methane, ZVI, CSTRs.

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INTRODUCTION

Amid global population growth, food and energy security has been a challenging issue across the globe. As of recent UN's figures, by 2030, world population is expected to surpass 8.6 billion people from current number of 7.8 billion people (UN World Population Prospect, 2017). As a result of the increasing population, attempts are being made to meet mounting challenges triggered by rising food consumption and energy demand (GAP Report, 2017). On the other hand, the dwindling fossil fuel reserves and fluctuated crude oil price exacerbate the energy issues being confronted in every modern society. Despite the fact that the sustainable and renewable energies (that is, solar, wind, hydropower and geothermal) assist the world with energy sufficiency to some extent, each technology holds inherent limitation from scarcity in resources to seasonal or temporal variation (Flohn, 1977). These shortcomings are eliminated in bioenergy technology in which the main substrate could either be biomass form agricultural by-products or wastes from numerous sources as long as feedstock materials consists of mainly carbohydrates, fat and protein (Hermiati et al., 2011; Jain et al., 2015). According to USDA 2018 reports, the global grain-starch export and consumption of top producing countries such as United States and China were steadily increasing from 2.5

million tons in 2007/08 fiscal year to 4.8 million tons in 2017/18 (WAP, 2018). Similarly, the corn starch production alone in Thailand increased by 3~6% in 2018 from that of 2017 figure reaching 5.3 million metric tons. Thailand has also been ranked world 2nd cassava root producer after Nigeria, and 1st producer and exporter of cassava-derived foodstuffs (Piyachomkwan and Tanticharoen, 2011). There is increasing application of cassava products in diverse products, the production surged from 22 million tons in 2010/11 to 33 million tons in 2016/17 (Nguyen et al., 2007; Pingmuanglek et al., 2017; TTSA, 2018). The increased production generates more wastes and by-products which are ideal resource for bioenergy. Therefore, agro-industrial countries benefit green and reliable energy from its agriculture potential to harness attractive renewable bio-energy.

Bioenergy offers heat and biofuels (that is, biogas, bioethanol, hydrogen etc.). However, the practicability varies among two determinants of available resource and best technology. While heat energy could be converted directly by incineration in all region, biofuel production is only feasible in areas where biosynthesis is favourable. The generic advantages of biogas over its counterparts are the ease of workability, management, environmentally friendly and rate of return per resource input (Nguyen et al., 2007; Jain et al., 2015). By means of Anaerobic Digestion (AD) process, biomass is converted into biogas through series of bioconversion phases in orders (that is, hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Paylostathis and Gaialdo-Gomez, 1991; Geradi, 2003). Microbial communities are responsible for each step of symbiosis strictly in anoxic environment under multiple controlling factors such as pH, temperature, organic loading, solid regime, carbon to nitrogen ratio (C/N) and so on (Boone et al., 1993; Veeken and Hamelers, 1999; Monnet 2003; Naik et al., 2014). No matter how these factors could be configured, bio-methane content, the sole energy calorific of biogas regenerated in low quantity, remains as an obstacle. Biogas is the combination of methane (CH_4) - 65% max, carbon dioxide (CO_2) - up to 35% and some trace gases (Rasi, 2009; Appels et al., 2011). Although methane gas could be proliferated by several gas upgrading technologies, the technology itself is still in infancy and most of them are economically unviable for commercial purposes (Chen et al., 2008; Amoah et al., 2019). Since recent decades, research pertinent to new and better digestibility of biomass for higher biogas yield has been emphasized comprehensively. Nevertheless, few efforts have focused on better bio-methanation draws and as such, further research needs to be conducted.

Researchers have mentioned introducing catalyst into the AD process could promote microbial activities and thus further accelerate biochemical reactions within intermediary products and all biogas process as a whole (Waite, 2002; Mohamed and Nageh, 2015; Yun, 2016; Romero et al., 2016). In this regard, metals (that is, Fe, Mn, Co, Cu, Se, and Co) supplementation has been a renewed interest to enhance methane during biogas process (Demirel and Scherer, 2011; Ma et al., 2015; Choong et al., 2016, Cai et al., 2018). Along with a range of metal catalysts, Zero Valent Iron (Fe^0) or ZVI as reducing agent and dominate electron donor is considered to have high potential in contributing reducing CO_2 to CH_4, and foster beneficial effects for microbial communities particularly to acetogenic and methanogenic phase which is responsible for hydrogenotrophic methanation (Feng et al., 2014; Liu et al., 2015; Ganzoury and Allan, 2015; Carpenter et al., 2015; Hao et al., 2017). In spite of the low cost and stimulatory, the impact of metallic ZVI's (Fe^0) impact on anaerobic biota and its mechanism remains ambiguous. The principal kinetic pathway of ZVI with intermediary products within anaerobic digestion processes as reducing agent are at per following key reactions (Karri et al., 2005; Hu et al., 2015; Hao et al., 2017):

$$CO_2 + 4Fe^0 + 8H^+ \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \quad (1)$$

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \quad (2)$$

Based on the aforementioned reaction kinetics, since ZVI's serves as lowering oxidation-reduction potential (ORP), and acid buffer, it is being widely used for the purpose of methane enhancement especially in activated sewage waste sludge and wastewater. ZVI was proved to enhance methane production capability up to 27% from primary sludge of wastewater treatment plant in batch test with varying ZVI concentration with 0.2 mm diameter size when 0-20 g/L added (Meng et al., 2013; Carpenter et al., 2015). As demonstrated in laboratory Biochemical Methane Potential (BMP) tests, ZVI as chemical addictive resulted in enhanced methane production and de-waterability but without specifying CH_4/CO_2 fraction (Wei et al., 2018). Using same substrate, Zhang et al. (2015) suggested that ZVI helps in VSS removal up to 60% through 0 – 5 g/L ZVI powder addition and consequently generated increased methane yield by 91.5%. ZVI was also investigated and found to have positive impact with 2.46 -3.53 times increase in the growth of methanogens only under 10 g/L dose by 16s rRNA genetic sequencing (Antwi et al., 2017). However, controversy over verifying reliable ZVI sources, forms and dose applied in AD system exists, and few studies have emphasized the effect of ZVI on organic biomass (Liu et al., 2015; Wei et al., 2018; Cai et al., 2018). Although, highly purified ZVI could be synthesized at laboratory scale, its low output is unfeasible for commercial purpose (Li et al., 2006; Zhang et al., 2006). In addition, though iron powders are readily available in variable particle sizes (μm – mm diameter) and sizable quantity (Hu, 2005), it oxidizes itself upon exposure to humidity, and thus requires cautious handling prior to application. The reactivity and response of ZVI in anaerobic process largely depend on its

Table 1: Substrate characteristics.

Parameter (Units)	Fresh cassava wastewater	Synthesized cassava wastewater
pH	4.5 ± 0.2	4.2 ~ 4.5
Total Suspended Solids (TSS - g/l)	2 ~ 3	5 ± 0.5
Volatile Suspended Solids (VSS – g/l)	2.55 ~ 2.82	3.25 ± 0.25
Total Chemical Oxygen Demand (TSCD – mg/l)	20,000 ~25,000	10,000 ~ 12,500
Soluble Chemical Oxygen Demand (SCOD – mg/l)	5,000 ~ 8,000	5,500 ± 500
Volatile Fatty Acids (VFAs – mg/l)	4,000 ~ 6,000	4,500 ±500
NH_3-N (mg/l)	100 ~ 300	N.D.
TKN (mg/l)	700 ~1,000	N.D.

*N.D. = Not detected.

Table 2: Classification of graded Iron powders.

Products	Apparent density (g/cm³)	Specific surface BET (m²/kg)	Particle size (%)			Chemical composition	
			+60 mesh / 250 µm	+100 mesh /150 µm	-325 mesh / 45 µm	H_2-loss (%)	C (%)
M100	2.4	130	0	2	20	0.82	0.21
R12	1.4	225	46	79	3	1.8	0.02

particle size with respect to substrate characteristics (Li et al., 2006; Mansoori et al., 2008). However, digestion response with respect to the particle size and dose remains uncertain especially in agro-industrial biomass, and prevailing ZVI literatures investigated predominantly activated waste sludge.

In this study, using cassava pulp and wastewater, the impacts of two different sizes of ZVI powder (nm and µm) on bio-methane production were evaluated through validating selected ZVI sources and alternative doses. Our previous work, using scrap iron and same feedstock materials, concluded that characterization of ZVI is crucial in verifying valency Zero in iron sources to ensure ultimate ZVI's function, and found that scrap iron supplementation in CSTRs resulted slightly in higher biogas and more resilient to temperature stress. This study intends to investigate the response of anaerobic digestion by two differential ZVI particle size and examine optimum dose for optimum bio-methane enhancement.

MATERIALS AND METHODS

Substrate and inoculum source

Main substrate (5% TS w/v) for anaerobic digestion was prepared from major by-products of commercial cassava starch mill, cassava pulp and its wastewater. The characteristic of cassava wastewater was analysed and synthesized further for extended use throughout the digestion period. Synthesized in large polypropylene barrel, the process included fermentation by cassava wastewater from parent wastewater pond and newly blended cassava pulp mixture, and active fermentation was maintained by intermittent aeration arrangement. Table 1 shows the physiochemical characteristic of both fresh and synthesized feedstock substrate. Prior feed loading into the reactor, synthesized substrate was neutralized to pH 7.0 by $NaHCO_3$ as buffer chemical. Seeds (inoculum) were collected from covered lagoon and anaerobic digestion was executed within 24 h. Inoculum characteristics were found as pH 7.75 ± 0.2, TS 12.15 (g/l) ± 2.50, VS 10.55 (g/l) ± 0.5, and C/N 25.02 ± 3.5.

Zero valent iron (ZVI)

Powder ZVI - M100 and R12 utilized in this study was obtained from Swedish metal powder manufacturer, Höganäs AB. Table 2 shows typical properties of selected products. From the disparity of particle size distribution, it could be graded M100 as extremely fine powder (nm) and R12 as coarse powder (µm) (Hu, 2005). Since iron powders are in commercial grade, they were subjected to heat treatment prior to application to eliminate impurities and self-oxidation by atmospheric humidity (Table 2).

Since metal iron exists in numerous oxidation states, powders were further investigated for valency and atomic concentration by synchrotron radiation lights for X-ray Absorption Spectroscopy (XAS) and X-Ray Photoelectron Spectroscopy (XPS) at beamline 6.1 and 6.2 of synchrotron light research institute, Thailand, and metal surface crystallinity structure and size by Scanning Electron Microscopy (SEM). Peaked with binding energy of 712ev,

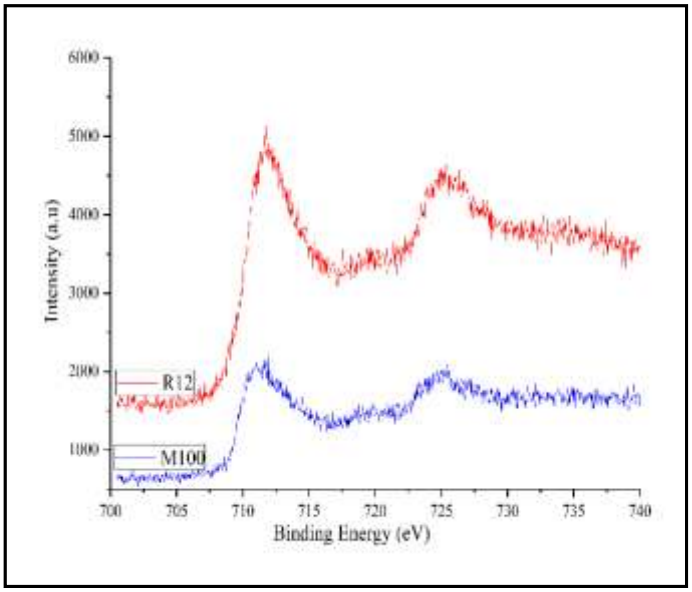


Figure 1: XPS spectrum of R12 and M100.

Table 3: Atomic Concentration of M100 and R12.

Samples	Atomic Concentration (%)			
	Fe	O	C	Others
M100	93.01	4.85	0.66	0.48
R12	96.86	1.26	0.92	0.96

and XPS spectrum of both M100 and R12 were verified as they predominantly consist of elemental iron (Figure 1). The atomic concentration was mainly found in Fe with trace of O and C (Table 3). Generated from XAS spectra of different iron oxides, M100 and R12 fall on zero as per relative valency of iron(s) (Figure 2). Finally, surface morphological and crystallographic state of the sample was observed as per SEM images (Figure 3).

AD set up and configuration

Bio-methanation in this study was conducted in Continuous Stirred Tank Rectors (CSTRs) as shown in Figure 4. Divided into two phases, the first phase includes equalization process in which 6 CSTRs operated in parallel in order to determine Hydraulic Retention Rime (HRT) in optimum Organic Loading Rate (OLR). Reactors were set up in 2-L sized 6 CSTRs in equally food to microbial ratio (F/M) under ambient environmental condition where ambient temperature ranged from 24°C min ~ 32°C max throughout the day. Stirring or mixing was controlled at the rate of 150 rpm with 15 min operation in every 2 h. Stable organic loading rate (OLR – 3.25 ± 0.25 g VSS L^{-1} day^{-1}) among CSTRS with ± 10% variance biogas yield (600 ± 50 ml/OLR/day) was achieved at hydraulic retention time (HRT) 16 days. Volatile Fatty Acids (VFAs) to Total Alkalinity (TA) ratio (VFA/TA) was maintained below 0.3 as per the limitation for healthy reactor.

In the second phase, ZVI supplementation was proceeded up to the next 30 Operation days. Initially, M100 and R12 ZVI powder in 5 different fixed dosages (1-2-4-8-15 g/L) was introduced to assess inhibition threshold of each differential particle size. Based on initial assessment for digestion response of M100 and R12 ZVI particle, further iron concentration adjustment was made. The performance of with and without ZVI input CSTRs was comparatively examined. This study covered the second phase on the response of differential particle size upon ZVI addition with regard to bio-methanation.

Analytical methods

The APHA standard methods (2005) were applied for Soluble Chemical Oxygen Demand (SCOD). For Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) profiles, 3 points GLP titration method was employed by Titroline 7000 automatic SI analytic machine. pH and buffer were measured using HORIBA Scientific® pH meter. Analytical

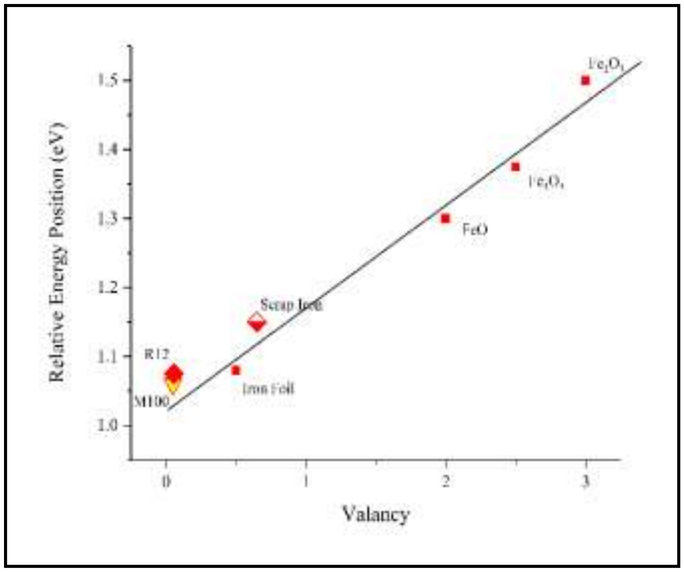
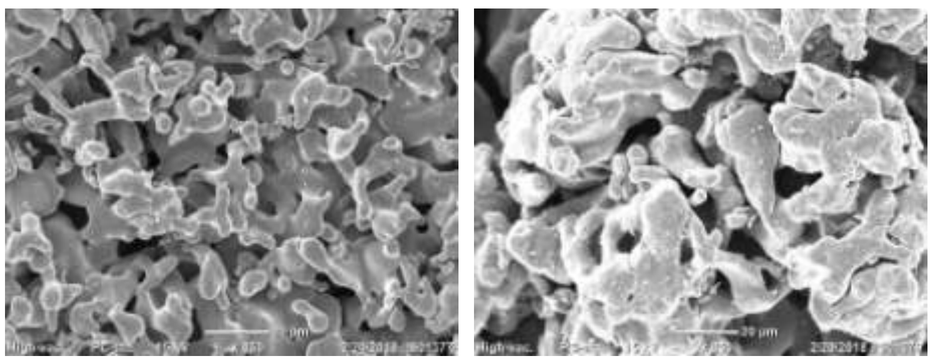


Figure 2: Relative valency of iron(s).



(a) (b)

Figure 3: SEM images of M100 (a) and R12 (b).

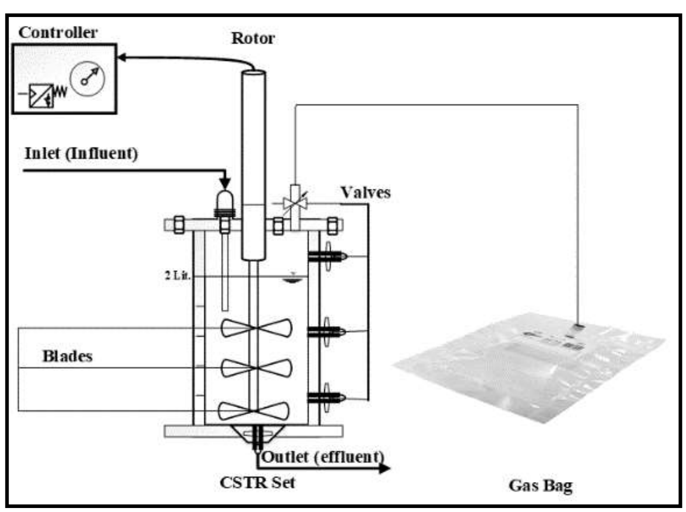
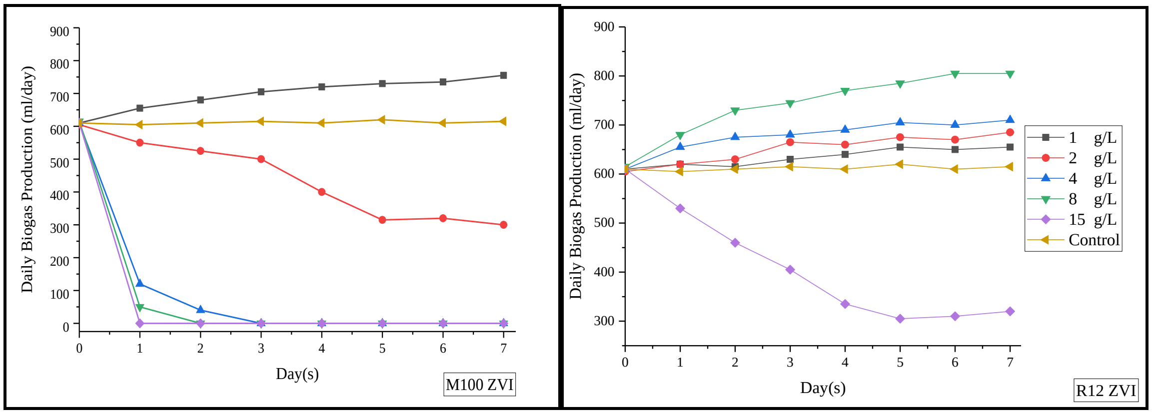


Figure 4: Schematic diagram of AD CSTR set.



(a) (b)

Figure 5: Digestion response of ZVI addition (a) M100 ZVI (b) R12 ZVI.

samplings were conducted in triplicate for each sample on daily basis throughout the period of experiment. Quantitative biogas volume was examined in equivalence of specific water replacement method in pressure head swing. Bio-methane was collected in 1-L sized SKC Tedlar® sample bag, then the relative content of the gas was analysed in Agilent 7890A GC system. Chromatographic gas demarcation was performed in every other day throughout bio-methanation period.

RESULTS AND DISCUSSION

Initial assessment on fixed ZVI dose for digestion response

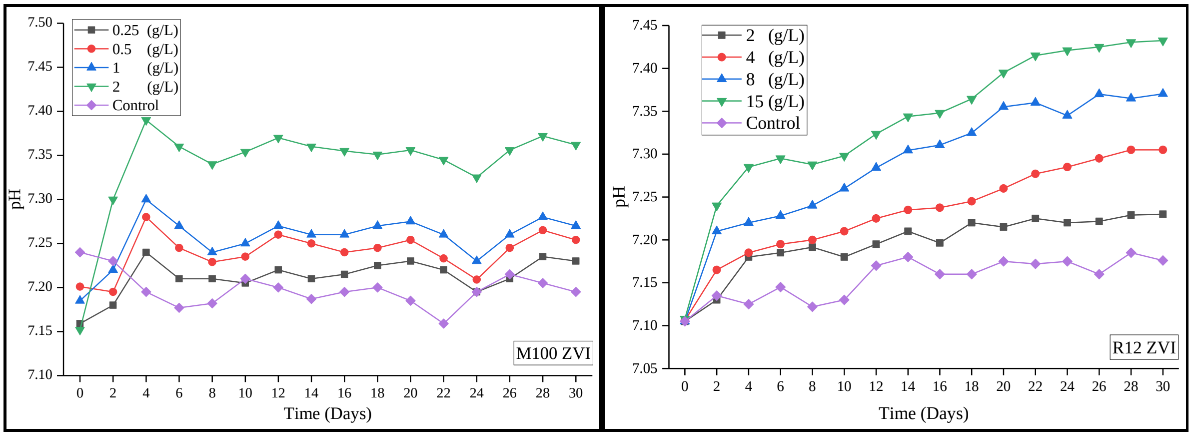
Since early studies on effect of ZVI on bioenergy were mostly related to Waste activated Sludge (WAS), few references relating ideal dose for agricultural biomass is available. By applying ZVI in fixed concentration (1-2-4-8-15 g/L) for both M100 and R12, and comparing against ZVI free control reactors, the proximate stimulatory and inhibiting concentration thresholds of each ZVI class for cassava pulp substrate were identified (Figure 5). After ZVI addition into bioreactors, digestion response in M100 ZVI nanoparticle supplemented sets encountered inhibition when concentration exceeded 2 g/L (Figure 5a). Iron toxicity occurred when the dosing was beyond 2 g/L causing digestion failure in the later days. A compromised new M100 dose (0.25-0.5-1-2 g/L) was established to determine optimum bio-methanation potential with 1.0 g/L ZVI concentration found to be ideal (Figure 5a). Identically, using WAS in biochemical methane potential (BMP) assay, Zhen et al. (2015) and Jia et al. (2017) suggested that 1.0 g ZVI/g VSS dose achieved highest methane production. Suanon et al. (2016) studies also stressed that the effect of nanoscale ZVI addition at 0.75 g/150 g dewatered waste sludge led to higher substrate degradation and methane formation. In a separate BMP test of Yang et al. (2013), at the concentration 30 mM (1.68 gm/ L) of Nano Zerovalent Iron (nZVI), methane production was noticeably reduced. Therefore, cassava pulp substrate of about 1 g/L margin of nano ZVI supplementation improved digestion and reaction response in bioreactors.

In contrast to M100 ZVI nanoparticles, the initial digestion response of R12 was detected at 8g/L concentration (Figure 5b). While at the lower dose (4 g/L), the stimulatory performance of AD was insignificant, and the negative impact of R12 increased at 15 g/L dose. Unlike nanoparticles (M100), the inhibition of R12 micro-particle in initial response of digester was found gradual, while those of nanoparticles were rapid. Zhao et al. (2018) showed that at 10 g/L ZVI powder (0.2mm diameter) addition, 30~35% methane enhancement was achieved than ZVI free reactor. For dosing of 10 g/L micro ZVI particle, Liu et al. (2012) explained that powder ZVI helps in COD removal and process stability.

The authors further suggested that powder ZVI (0.2mm diameter) accelerates acidogenesis and thus subsequent treatment receives beneficial impact. 43.5% of methane productivity was proved by addition 20 g/L in waste activated sludge (Feng et al., 2014). In the case of Ibrahim and Abdulaziz (2016), 15g/L addition led to 82% increase in biogas production. This figure contradicts that of the present study and thus R12 dosage was set at 2-4-8-15 g/L in extended studies.

Extended study to ZVI impacts during bio-methanation process

In applying optimized concentration of (0.25-0.5-1-2 g/L) and (1-2-4-8-15 g/L) for M100 nano-particle and R12 micro-particle, respectively, the effect of particle size on several key indicators during biogas processes was analysed for extended hydraulic retention time (HRT)



(a) *(b)*

Figure 6: Long-term pH response of CSTRs by ZVI. (a) M100 (b)R12.

(i) pH Buffer
(ii) VFAs /TA Ratio
(iii) Process Efficiency

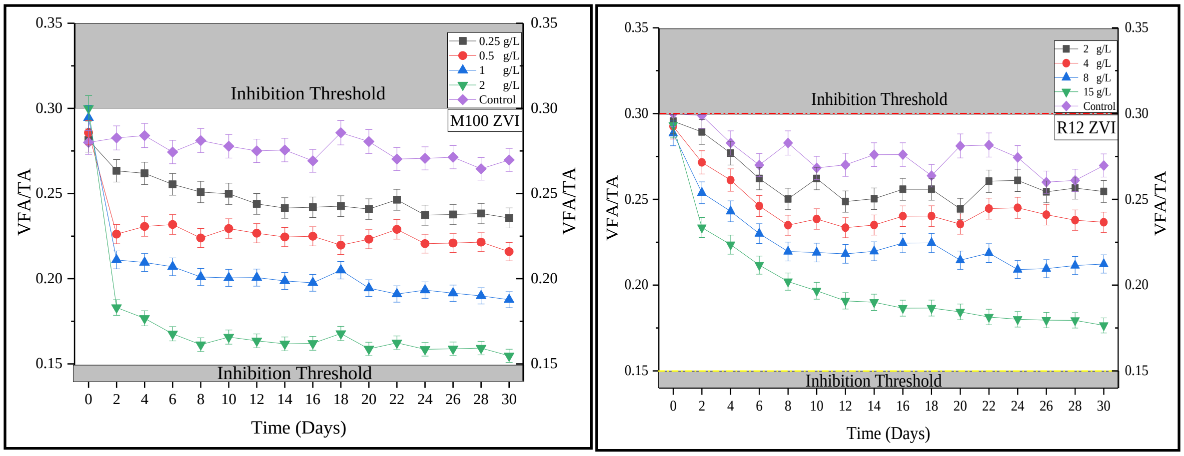
pH buffer

Since M100 and R12 ZVI supplementation were not conducted in parallel under same period, relative performance for comparative evaluation is inaccessible. However, by applying the compromised stimulatory doses, the result from the separate investigation uncovered the effect of each type ZVI particles on pH in extended digestion period (Figure 6). ZVI had been proved to encourage buffer resistance in AD system (Li et al., 2013; Carpenter et al., 2015; Wu et al., 2015), the current study is satisfied with the hypothesis that ZVI assists buffer resistance capacity. Despite there was slightly difference in initial pH (7.15 ~7.25) prior to M100 ZVI addition, pH surged as ZVI concentration increased and the increments were observed to be most active during the first 4 days, then stabilized in the case of M100 nano-particles (Figure 6a), but steadily increased in R12 micro-particle (Figure 6b). In Carpenter et al. (2015) studies, intense pH changes occurred at the first 3 days of iron amendment. While the rate of pH increase in 2 g/L dose supplementation was most dynamic, the rates of pH in 0.25, 0.5 and 1 g/L dosage were found to marginally increase. After day 4, the trend of pH fluctuation in M100 ZVI amended reactors was almost corresponding to that ZVI free (control) reactor, this is due to the fact that nanoparticles ZVI has been completely disintegrated under instant reaction with less resistance to stresses impending by other AD variables.

In contrast, pH response in micro-particle ZVI (R12) were gradually increased after day 2, maintaining pH below 7.5 at the end of digestion period. All compromised doses of R12 satisfied under optimum pH for healthy digestion are between pH 7.0 and 7.5 (Clark and Speece, 1971). Under this scenario, the frequency of ZVI addition becomes a justification factor for workability and ease of process monitoring in commercial operation. With lower concentration, nanoparticles M100 ZVI require daily addition upon next organic loading. However, in micro-particle R12, though higher dose, it requires only single amendment for the whole digestion period. Therefore, R12 ZVI addition saves more chemical consumption for buffer resistance and daily chemical consumption in neutralizing acidic substrates against M100. Excess ZVI can also lead to digestion failure immediately in nanoparticles, volatile fatty acids (VFAs) depletion, and balance growth of microorganisms (Yang et al., 2013; Wu et al., 2015). The slow atomic iron release and surface decomposition in R12 ZVI lacks those limitations and thus offers the advantage on ease of application and process control over M100.

VFA/ TA ratio

The state of Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) in equilibrium is one of the essential criteria in ensuring healthy digester in bioconversion process of biogas (Drosg, 2013; Li et al., 2014). While TA changes are triggered by buffering substrate to neutral pH by bases chemicals, VFAs are an intermediary product formation from decomposition of organic substances by acids formers, acidogenic and acetogenic bacteria community. The methane forming archaea (methanogens) favours alkaline conditions and they consume VFA and generate terminal products, Bio-methane (CH_4) and Carbon dioxide (CO_2). High VFA formation leads to rapid drop of pH and consequently intoxicates them. Vice versa, high alkalinity condition acidogenic bacteria diminish VFA formation leading to foods shortage for proper symbiosis to all sort of anaerobic bio communities. Therefore, VFA to TA ratio (VFA/TA) must be maintained between 0.15 ~ 0.3 for diversification of both acidic and basic favoured microbial



(a) *(b)*

Figure 7: VFAs/TA Profile of CSTRs in 30 Days HRT (a) M100 (b) R12.

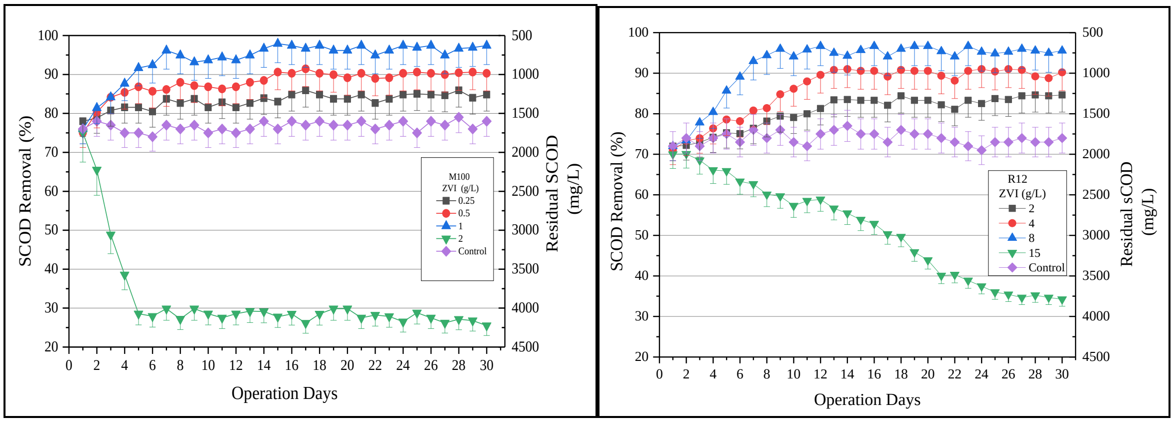
communities in equilibrium between pH 6.8~7.5 (Mata-Alvarez, 2002; Khalid et al., 2011; Appels et al., 2011). In spite of saponification by $NaHCO_3$ in synthesized substrate, VFAs could be reduced to ideal range below 500 mg/L (Mouneimne et al., 2003; Battimelli et al., 2009), ZVI was also found actively aided in driving down the VFAs mass by mean of a reactive media.

In this experiment, instantaneous decline in VFA/TA was observed upon ZVI supplementation, while ZVI free control reactor's VFA/TA remained closer upper inhibition threshold of 0.3 in both nano-particle (M100) and micro-particle (R12) cases (Figure 7). The higher the ZVI concentration, the lower the VFA/TA ratio was observed leading to another inhibition threshold underneath under ideal VFA/TA condition. However, concentration of nanoparticle 1-2 g/L abruptly dropped VFA/TA from 0.3 to under 0.2 margin (Figure 7a). This could lead digestion anomaly by immediate release of iron particle for rapid reaction among iron and organic chemical compounds within digester (Yang et al., 2013; Abdelsalam et al., 2017). For nanoparticle concentration of 2 g/L in extended digestion, the VFA/TA was found critically low ending up in VFA exhaustion. Consequently, there is likely low gas yield for food source deficiency for methanogens. It is therefore important to maintain the condition of VFA/TA in-between upper and lower inhibition threshold as provision for fluctuation by daily OLR and persistent anaerobic biochemical reactions (Ahring et al., 1995; Duan et al., 2012). Thus, nanoparticle ZVI (M100) dose ranging from 0.5-1 g/L could be considered as optimal. With slow disintegration R12 micro iron particle, VFA/TA responded contrarily to its counterpart. Once injected, VFA/TA varied just slightly from 0.3 to 0.25. Iron particle deposit found at the bottom of the digester are evidence that dissolution of micro-particle was slower than those of nanoparticle in which it transformed into sludge, changing substrate's colour into pitch back.

In addition, the time taken for noticeable VFA/TA changes took longer than nanoparticle. The investigation uncovered that since R12 micro particle was added, VFA/TA variation was active from first 8 days, then stabilized on later days. In term of VFA/TA changes, with the exception to the highest R12 dose (15 g/L), the remaining micro-particle amended CSTRs reactor performed at the mid of both inhibition thresholds (Figure 7b). This led CSTRs to be more resilient to impending stresses derived from variable anaerobic digestion setting (Aquino and Stuckey, 2007; Romero et al., 2016; Choong et al., 2016). While nanoparticle M100 required sustained daily supplementation to maintain ZVI function, the frequency of micro-particle R12 dosing could be save by recycling it into the CSTRs till fully disintegrated. As a result, despite nanoparticle demanded in large quantity in initial stage, over 30 days HRT period, the chemical consumption for nanoparticle M100 was higher in each dosage level.

Process efficiency

The rate of Soluble Chemical Oxygen Demand (SCOD) removal and its residual fraction represents the efficiency of anaerobic digestion process of bio-reactors in converting digestible biomass into bio-methane. Chemical Oxygen Demand (COD) comprises the combination of indigestible or un-biodegradable solid, and digestible or biodegradable soluble solid, among which only soluble solid is accessible by microorganisms as food source as SCOD (Rossle and Pretorius, 2001). With the exception of 2 and 15 g/l in M100 and R12 respectively, the presence of ZVI in both cases promoted active bio-conversion process in long term investigation up to 95% removal rate with lesser daily residual SCOD upon next loading. While ZVI free control reactor's removal rate was 75%, process efficiency



(a) *(b)*

Figure 8: SCOD removal rate and Residual SCOD in CSTRs along 30 operation days (a) M100 (b) R12.

increased with different rate in each ZVI concentration rates (Figure 8). However, the response of finer particle size in M100 ZVI cases was found to be faster (Figure 8a) than those of R12 ZVI injected CSTRs (Figure 8b). Optimum efficacy in improving SCOD removability of respective concentration of M100 ZVI was achieved from just first 4 - 7 days after introduction, whereas for R12 ZVI, the peak bioconversion rate occurred after day 12 through lower rate. Although, finer particle size M100 ZVI provided shorter duration in increasing reactors' productivity, it led to immediate failure by ZVI toxicity when M100 ZVI of more than 1 g/L was present, and as such, in the reactor irrecoverable state, the microbial communities could sustain bio-conversion process. With higher difference in concentration margin of between 8 and 15 g/L in R12 coarser ZVI particle, the rate of ZVI inhibition resulted in gradual drop in SCOD removal leaving time. This alarmed the need for troubleshooting in order to recover active digestion from excessive ZVI introduction. Therefore, comparison between finer M100 ZVI and coarser R 12 ZVI shows that the latter has more ease of process control for ensuring optimal conditions under other anaerobic digestion variables (that is, temperature, mixing rate, OLR, solid content, F/M, etc.).

The relative SCOD removal efficiency with reference to control reactor in this experiment was up to 35% increase in optimum conditions when both M100 and R12 ZVI were present in CSTRs. In the retrospective studies of similar investigation, COD removal efficiencies increased up to 22% from 54% by finest nano-particle ZVI (nZVI) and 66.2% by grain size iron powder (IP) against control reactor at 44.6 %, suggesting grain size ZVI for enhancing methane (Suanon et al., 2017). High level of iron addition along with essential trace elements resulted in elevated COD removal rates of 70-80% process efficiencies when reference reactor's achievement was only 53% (Ortner et al., 2015). Under the 20g/L ZVI dosage, COD removal declined to 25% within 6 h in an effort to enhance anaerobic wastewater treatment (Liu et al., 2012). However, under controlled variables addition of ZVI according to reactors' real-time performance, COD removal was about at least one time higher as compared with that of ZVI free reactor (Liu et al., 2012; Yang et al., 2013). The results of the current study demonstrated that the difference in particle size distribution has strong influences on rate of process efficiency and stability.

Bio gas yield and methane content

According to the investigation throughout 30 operation days after steady state conditions in all parallel CSTRs, upon ZVI introduction, the results (Figure 9a-c) showed that the impact of gas volume and methane enhancement of nano-particle size M100 ZVI advanced immediately, whereas the trend of micro-particle size R12 ZVI progressed gradually. While the time taken for optimizing bio-methane was achieved within first few days in M100 ZVI, in the case of R12, optimum methane improvement occurred on day 15. As a result of M100 ZVI toxicity in 2 g/L concentration, it disrupted overall bio-methanation process ending up into digestion failure. Similarly, in 15 g/l R12 ZVI added reactor, in contrast to that of M100 ZVI, although methane optimization continued till operation period, the daily biogas yield decreases gradually. This is due to the fact that the excessive presence of ZVI unnecessarily elevated pH and buffer within the reactors, then it interrupts proper synthrophy of microorganisms, especially those bacteria responsible for acidogenesis and acetogenesis of anaerobic digestion process (Yang et al., 2013; Suanon et at., 2017). Therefore, the optimum concentration for methane enhancement in cassava pulp bio-methanation of M100 and R12 ZVI was observed at 1 and 8 g/L respectively. In comparison with reference control reactor, under unit daily OLR rate, gas yield increased from 600 ± 25 ml to 920 ± 20 ml (67%) in both R

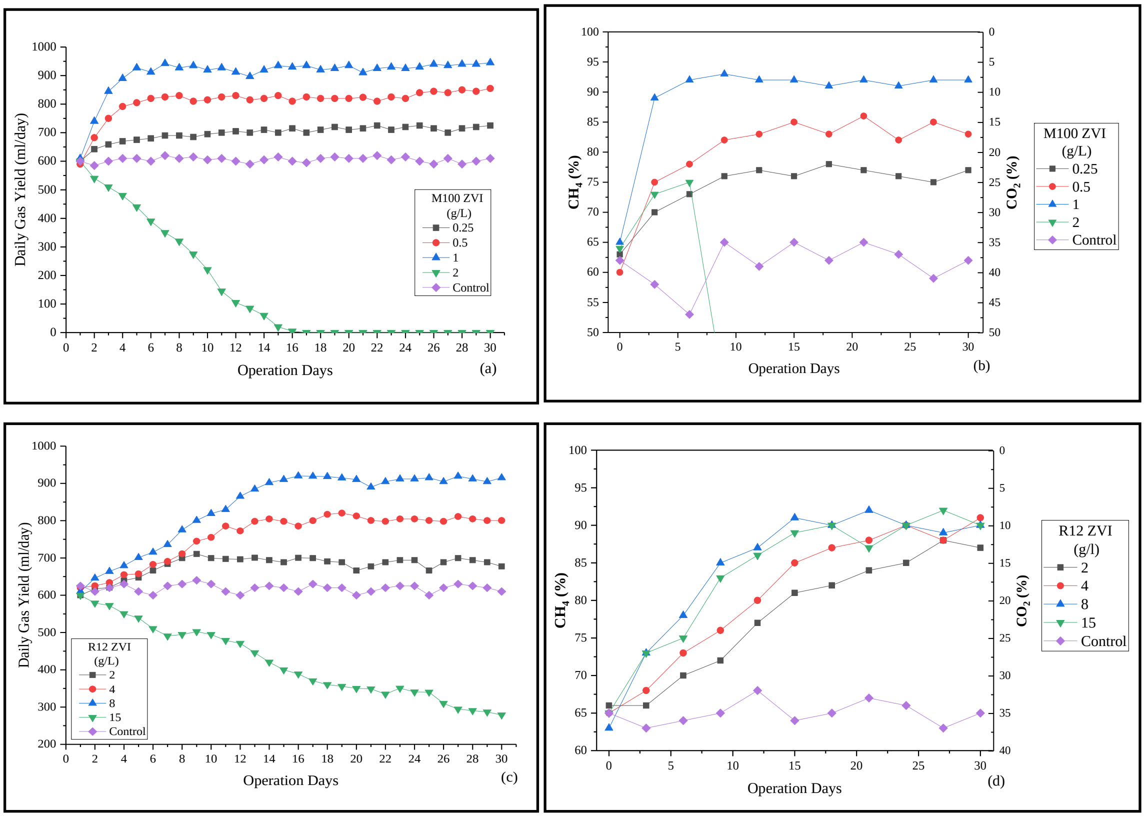


Figure 9: Daily biogas yield and biomethane content (a-b: M100 ZVI) (c-d: R12 ZVI).

12 and M100 ZVI addition (Figure 9a and c). Similarly, methane enhancement was found up to 93% CH_4 concentration from 60 ± 5% of control reactor (Figure 9b and d). However, under the flux of mixing mechanism of CSTR set, particle size in optimizing bio-methane was present as a factor over ease of process control and duration of anaerobic digestion system. Extremely minute M100 ZVI particle unable timely recovery effort because of its promptly reactivity with aqueous substrate once it has been overdosed, while coarser R12 ZVI lacks this limitation and promotes bio-availability for methane producing archaea in the course of its disintegration along mixing action.

Employing 4 different grades of ZVI nanoparticle (1.120, 0.149, 0.044 and 0.010-mm diameter) in enriching methanogenesis and sulphate reduction in anaerobic sludge, the study suggested that the finest particle grade achieved highest rate of methane formation at 0.310 mmol CH_4/mol Fe^0.day and 0.804 mmol SO_4^{2-} reduced/mol Fe^0.day (Karri et al., 2005). In this investigation, micrometre size R12 ZVI had proved 35% more methane formation than ZVI free reactor. The same increment followed in the case of nano meter size M100 ZVI at 1 g/L optimum concentration with identical methane enhancement. Carpenter et al. (2015) reinforced this finding by using commercial ZVI with 150 nm diameter and found 28% increase in biogas with 5% increase in methane than synthesized ZVI in brewery waste water. In comparison with the effect among nano and micro meter size ZVI particles for biogas production, Su et al. (2013) reported nanoparticles ZVI performed superior in methane forming in activated sludge over prolonged digestion with more than 36% (up to 40.4%) enhancement than that of micro ZVI particles. Nevertheless, this finding contradicts the current finding since larger particle size resulted better process control and sustainably of the reactor. Yang et al. (2013) stressed that potential negative impact on methanogenesis with more than 20% inhibition both biogas and methane production in anaerobic digestion when extremely small ZVI particle size (>60 nm) is applied. Liu et al. (2015) demonstrated that granular scrap iron was more effective than ZVI powder (0.2 mm diameter) for bio-methanation in waste activated sludge obtaining 10% more CH_4/ kg VS. In the same way, Feng et al. (2014) mentioned that larger particle size (up to decimetre level) could have higher possibility in chelation and promotes slow release in the course of prolonged biochemical processes. Hence, ZVI with larger particle size could be recommended when it is designed to apply as catalytic agent in the bio-reactors for methane enhancement.

The incident of ZVI toxicity of M100 and R12 in this initial assessment indicates how ZVI particle size influences bioconversion process of both microbial communities and intermediary products. While toxicity took place immediately in nanoparticles (M100 ZVI) injected reactors, those of micro particle (R12 ZVI) were noticed after several days of addition. This is due to the fact that the instantaneous dissolution of nanoparticle and its highly reactivity, which transforms substrate from grey to pitch black colour and more sludge accumulation, led to digestion failure. Some literatures reported the toxicity mechanism of nano ZVI in diverse anaerobic microbial communities as the consequence of disruption to the cell membrane integrity, interference with respiration, and damage of DNA or enzymatic proteins activated by released metal ions from nanoparticles (Chen et al., 2011; Hajipour et al., 2012; Xie et al., 2017). In a comparison between nanoscale and microscale ZVI, Abdelsalam et al. (2016) reported that micro scale ZVI treated biota promoted bacterial growth significantly than nanoscale ZVI. Even under anoxic condition, nano ZVI triggered corrosion and surface oxidation by dissolved oxygen, consequently reduced redox activity (Lee et al., 2008; Lin et al., 2010). Microscale ZVI possesses intrinsic characteristic of surface integrity, higher contact surface and slow atomic exchange (Carpenter et al., 2015; Zhen et al., 2015). Hence, microscale ZVI (R12) amended reactors were more resistance to reaction kinematic stress and longevity for bioavailability. Since R12 ZVI residues were found almost unchanged in both mass and weight on next dose, thus microscale particle also saves dosing frequency by recycling or salvaging the ZVI deposit.

CONCLUSION

Catalytic use of metal element and trace element supplementation during anaerobic digestion process will become future technology to reduce the environmental cost and elevate load in biogas upgrading systems in enriching methane concentration of biogas. Nevertheless, metal powder requires characterization and verification to ensure its kinetic reaction with dedicated substrate. In the investigation of XAS, XPS and SEM technologies, the application of low-cost elemental iron powder as reducing agent for bio-methanation in this experiment showed that ZVI addition optimal concentration proved stimulatory for methane enhancement and gas yield up to 93 and 67%, respectively. However, when ease of process control and sustainability are weighed for long-term operation for cassava pulp feed anaerobic bioreactor against differential ZVI particle size, micro-particle size R12 offers superior characteristics than nano-particle size M100.

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