
The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition

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Abstract: This study aims to investigate the effect of bioaugmentation with enriched methanogenic propionate degrading microbial consortia on propionate fermentation under ammonia stress from total ammonia nitrogen concentration (TAN) of 3.0 g N L^{-1}. Results demonstrated that bioaugmentation could prevent unstable digestion against further deterioration. After 45 days of 1 dosage (0.3 g dry cell weight L^{-1} d^{-1}, DCW L^{-1} d^{-1}) of bioaugmentation, the average volumetric methane production (VMP), methane recovery rate and propionic acid (HPr) degradation rate was enhanced by 70 mL L^{-1} d^{-1}, 21% and 51%, respectively. In contrast, the non-bioaugmentation reactor almost failed. Routine addition of a double dosage (0.6 g DCW L^{-1} d^{-1}) of bioaugmentation culture was able to effectively recover the failing digester. The results of FISH suggested that the populations of *Methanosaetaceae* increased significantly, which could be a main contributor for the positive effect on methane production.

Keywords: Bioaugmentation, ammonia inhibition, propionate degradation, propionate-oxidizing bacteria, microbial community

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Introduction

Anaerobic digestion (AD) is a proven technology that offers many environmental benefits, such as efficient treatment of organic material and the generation of renewable energy. Despite this, research is still required to improve the operational stability and efficiency of AD (Banks et al., 2011; Fisgativa et al., 2016). Bioaugmentation is the practice of adding specific microorganisms to a system to enhance a desired activity and could provide a means to improve the efficiency of AD (Deflaun & Steffan, 2002; Maier et al., 2000; Rittmann & Whiteman, 1994).

Over the past decade, bioaugmentation has successfully reduced the start-up period (Lins et al., 2014), shortened hydraulic retention time (Baek et al., 2016; Neumann & Scherer, 2011) and decreased the recovery time of anaerobic digesters stressed by oxygen (Schauer-Gimenez et al., 2010) or organic overloading (Acharya et al., 2015; Tale et al., 2011; Tale et al., 2015). Furthermore, bioaugmentation also has been studied to improve the performance of AD, including increase in methane production from cellulosic waste (Cater et al., 2015; Lu et al., 2013; Martin-Ryals et al., 2015; Nielsen et al., 2007; Nkemka et al., 2015; Peng et al., 2014; Weiss et al., 2016; Weiss et al., 2010; Yu et al., 2016; Zhang et al., 2015), digested sludge (mainly proteins and polysaccharides) (Lu et al., 2014), lipid-rich wastes (Cirne et al., 2006), ammonia-rich substrate (Fotidis et al., 2014) , and long-chain fatty acids (LCFA) (Cavaleiro et al., 2010).

Compared to enrichment of individual cultures to enhance AD process for each specific substrate, a more practical and time-saving approach may be to target key, ubiquitous intermediates to improve digestion performance (Tale et al., 2015). Propionate and acetate as bioaugmentation targets are of great interest, which at high concentration may cause the deterioration of digester performance. Several studies found that adding propionate-utilizing cultures (Schauer-Gimenez et al., 2010; Tale et al., 2015) or VFA-degrading culture (Acharya et al., 2015) could reduce propionate accumulation and improve digestion. In addition, bioaugmentation has proven an effective way to counteract ammonia inhibition, with the introduction of hydrogenotrophic methanogens showing increased methane production at high ammonia levels (Fotidis et al., 2013; Fotidis et al., 2014). However, not all bioaugmentation cases result in a positive impact on digestion performance. The addition of syntrophic acetate-oxidizing cultures did not affect digestion performance or stability against ammonia inhibition (Fotidis et al.,

2013; Westerholm et al., 2012). This might be due to methanogens playing a more important role than syntrophic acetate-oxidizing culture in anaerobic digestion under high ammonia levels (Fotidis et al., 2013).

Most of the successful cases of bioaugmentation have occurred in batch AD processes. For continuous reactors the major challenge for bioaugmentation is to ensure that the introduced microorganisms are able to thrive and are not washed out of the reactor (Fotidis et al., 2014; Mohan et al., 2005). In order to favor survival and prolonged activity of the exogenous microbial population, routine bioaugmentation for the continuous reactor might a more effective strategy (Martin-Ryals et al., 2015).

As described above, previous studies have shown that bioaugmentation is effective in enhancing poor digestion performance from either ammonia or propionate inhibition. Implementation of bioaugmentation under synergetic stress of ammonia and propionate has been less well addressed. Further studies are needed in this area since the accumulation of propionate, together with high acetate concentration is considered to be a major problem in digesters with high ammonia concentrations (Westerholm et al., 2015). It is also important to consider the nutrient concentrations, in particular trace elements, as well as the dosage of the bioaugmentation culture, both of which may have a significant effect on the microbial diversity and abundance within the digester.

With consideration of previous work, this study will investigate the routine bioaugmentation with solid methanogenic cultures enriched for propionate degradation to prevent deterioration of digester performance and recovery of the digester from the double stress of ammonia and propionic acid (HPr) accumulation. This work looks at the effect of different culture dosages of bioagumentation and the functional microbial groups as a result of this.

2. Materials and Methods

2.1 Inoculum and bioaugmention seed

The inoculum was taken from an anaerobic digester treating municipal wastewater biosolids (Millbrook Wastewater Treatment works, Southampton, UK). Before use the digestate was sieved through a 1 mm mesh to remove grit and other solids.

The bioaugmentation culture was taken from a propionate-degrading enrichment digester. To

avoid the culture medium impact on the digester, the enriched culture suspensions was centrifuged at 7000 g for 5min at room temperature and resuspended with ddH_2O, then centrifuged again under the same conditions to collect the microbial precipitate for bioaugmentation. The 454 whole genome pyrosequencing data of the bioaugmentation seed was deposited in NCBI Sequence Read Archive database with bioproject accession number PRJNA359412. According to the 454 whole genome pyrosequencing, the bacteria belong to *Proteobacteria , Firmicutes, Chlorflexi, Bacteroidetes, Synergistetes, Actinobacteria* and 24 other phyla, and the dominant archaeal groups are *Methanosaetaceae* (above 90%), *Methanospirillum* (below 5%) *and Methanosphaerula* (below 5%).

2.2 Experimental set-up and procedure

The whole experiment lasted for 125 days with four different experimental phases: phase I (0–50 day), phase II (50–75 day), phase III (75–95 day), and phase IV (95–125 day). The main strategic operational conditions of each experimental reactor are shown in Fig. 1.

During the first phase, the experiment was carried out in a laboratory-scale semi-continuously stirred tank reactor (Reactor 0, R0) with a working volume of 1.5 L. From day 51 the digestate in R0 was divided into two parts homogeneously and maintained in two 1 L conical flasks with 0.75 L of working volume (Reactor1, R1 and Reactor 2, R2). Each flask was connected to a gas sampling bag (Tedlar, SKC Ltd., UK) and connected to the flask by a stainless steel tube inserted through a butyl rubber bung. The flasks were maintained at 36 ± 1℃ in an orbital shaking incubator operating at 100 rpm continuously. They were operated in daily fill-and-draw mode with identical hydraulic retention time (HRT) of 15 days by removing the appropriate volume of reactor content and replacing it with the same volume of feed once per day.

The feed comprised a certain amount of sodium propionate and the volume was made up with nutrient medium. The nutrient medium contained the following [mg/L]:NH_4Cl [400]; $MgSO_4 \cdot 6H_2O$ [250]; KCl[400]; $CaCl_2 \cdot 2H_2O$ [120]; $(NH_4)_2HPO_4$ [80]; $FeCl_3 \cdot 6H_2O$ [55]; $CoCl_2 \cdot 6H_2O$ [0.5]; $NiCl_2 \cdot 6H_2O$[0.5] the trace metal salts $MnCl_2 \cdot 4H_2O$,$CuCl_2 \cdot 2H_2O$, $AlCl_3 \cdot 6H_2O$, $Na_2WO_4 \cdot 2H_2O$, H_3BO_3, Na_2SeO_3 and $ZnCl_2$ [each at 0.5]; $NaHCO_3$ [5000] (Tale et al., 2011).

The organic loading rate (OLR) started at 0.5 g VS $L^{-1}d^{-1}$ in HRT1 and was then increased to 0.625 g VS $L^{-1}d^{-1}$ by adding the appropriate amount of sodium propionate. Ammonium chloride

was added in the daily feeding medium to keep NH_4-N of the digestate at approximately 3.0 g L^{-1} during the whole experimental period except for one week washout (from day 27 to 34).

For R1, the bioaugmentation was conducted during periods II and III with the addition of 1 dosage of bioaugmentation cultures daily (the precipitate of centrifuged 100ml of microbial culture suspensions, around 0.23 g DCW). For R2, the bioaugmentation started in period III with the addition of 2 daily dosages of bioaugmentation culture (the precipitate of centrifuged 200ml of microbial culture suspensions, around 0.45 g DCW). The bioaugmentation was stopped for both reactors during period IV.

2.3 Analytical methods

Reactor contents were regularly sampled using syringes and VFA analysis were conducted at certain intervals to monitor the propionate degradation profiles. Biogas was collected with gas bags and the production and composition of biogas was measured every 3 days. Alkalinity and concentration of ammonia were measured weekly. Microbial community structure was analyzed using the fluorescent in situ hybridization (FISH) technique. Total solids (TS) and volatile solids (VS) were measured using Standard Method 2540 G. pH was determined using a Jenway 3010 meter (Bibby Scientific Ltd., UK) with a combination glass electrode calibrated in buffers at pH 7 and 9.2 (Fisher Scientific, UK). Alkalinity was measured by titration with 0.25 N H_2SO_4 to endpoints of pH 5.7 and 4.3, allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA). Total Kjeldahl Nitrogen (TKN) was determined using a Kjeltech block digester and ammonia by steam distillation unit according to the manufacturer's instructions (Foss Ltd., Warrington, UK). Volatile fatty acids (VFA) were quantified in a Shimazdu GC-2010 gas chromatograph (Shimadzu, Milton Keynes, UK), using a flame ionization detector and a capillary column type SGE BP-21. Biogas composition (CH_4 and CO_2) was determined using a Varian star 3400 CX Gas Chromatograph, calibrated with 65% (v/v) CH_4 and 35% (v/v) CO_2.

2.4 Fluorescent in situ hybridization

The digesters were sampled on day 47 and day 75 for microbial community structure analysis using the FISH technique. One milliliter of digestate was mixed with 9 ml of 1xPBS (phosphate buffer saline) solution in a Waring blender for 1 min. One milliliter of this diluted digestate was

transferred into a 2-ml centrifuge tube. After centrifugation at 10,000g for 10 min, the microbial biomass was suspended with 0.3 ml of 1xPBS, and then fixed with 4% of paraformaldehyde (Sigma–Aldrich, UK) solution for FISH analysis. The oligonucleotide probes (Thermo Electron Biopolymers, Ulm, Germany), as detailed in Table 1, and the hybridization stringency were chosen based on previous studies (Ariesyady et al., 2007). Hybridized samples were viewed using a Leica TCS SP2 confocal laser scanning microscopy, and 15 different microscope fields were randomly selected for each hybridization treatment. The laser wavelengths to excite the fluorochrome dyes 6-Fam, Cy3, and Cy5 were 488, 561 and 633 nm, respectively.

3. Results and discussion

3.1 Digestion performance

The performance of R0 is shown as phase I in Fig.2. The VMP of R0 decreased from 0.27 L L^{-1} d^{-1} on day 0 to 0.93 L L^{-1} d^{-1} on day 26, and the methane recovery rate (the percentage of the real VMP: the theoretical VMP) dropped from 82.91% to 28.09%, and propionic acid started to accumulate at the end of HRT 1, which increased to 1.6 g L^{-1} on day 26, indicating that 3.0 g L^{-1} of TAN concentration inhibited propionate degradation to methane. In an attempt to recover the performance of R0, ammonium chloride was excluded from the daily feeding medium from day 27 to day 34 to wash NH_4-N out from the digestate. While the VMP and methane recovery rate still decreased to 0.06 L L^{-1} d^{-1} and 20.59% respectively on day 31, although propionic acid concentration kept dropping during the TAN washout period. The VMP started to increase slightly on day 32 when TAN was below 2.0 g. It seems that the recovery of methane production lagged behind propionate oxidation step, which might be because the growth period of methanogenic archaea are longer than that of bacteria and they need more time to recover from the inhibition of ammonia stress. At the end of phase I (day 35-50), TAN concentration was back to 3.0 g L^{-1}, which led to the propionic acid accumulated again. The average HPr degrading rate was about 45% (Table 2) .The VMP was relatively stable around 0.10 L L^{-1} d^{-1} , and the methane recovery rate was about 30% (Table 2). The methane production was stable while the propionic acid kept increasing might because part of methane generated from the accumulated acetic acid since during this period the acetic acid concentration decreased slightly.

In order to investigate the effect of bioaugmentation on the propionic acid degradation

performance, R0 was divided into two reactors homogeneously on day 51. Phase Ⅱ of Fig.2 (Table 2) compares two reactors with and without bioaugmentation. For R1 with bioaugmentation methane production was increased, with no increase in propionate. Contrary to R1, without bioaugmentation, the concentration of propionic acid of R2 accumulated to 8500 mg L^{-1} on day 72. This resulted in the biogas production and methane percentage both decreasing substantially, with almost no methane produced by day 75. To better evaluate the effect of bioaugmentation, statistical analyses of digestion performance were performed (Table 2). Within 25 days of introducing 1 dosage of special microbial consortia, R1 exhibited higher performance parameters than R2. The average VMP, methane percentage, methane recovery rate and propionic acid degradation rate were higher of 0.13 L L^{-1} d^{-1}, 40%, 39% and 55%, respectively. Without bioaugmentation, non-bioaugmention reactor almost failed. It suggests that using bioaugmentation microbial seed can avoid digestion process failure caused by both stress from the accumulation of VFA and ammonia.

To examine the impact of higher bioaugmentation dosage, from day 76 (Phase III from Fig. 2) the dosage in R2 was doubled compared to R1. After 3 days, R2 showed a sharp recovery of digestion performance with significantly enhanced methane production. The VMP increased from below 0.001 L L^{-1} d^{-1} to 0.24 L L^{-1} d^{-1}. The methane percentage increased from 1.18% to 75.00 %. Moreover, the accumulated propionate was degraded, with a concentration change from 8400 mg L^{-1} to 3000 mg L^{-1} during phase III. Meanwhile the bioaugmentation dosage in R1 remained constant at 1 dosage of microbial seed during phase III. The performance of R1 was enhanced compared to the control R0. For R1 the VMP increased from 0.22 L L^{-1} d^{-1} at the beginning of phase III to 0.27 L L^{-1} d^{-1} on day 83, and then decreased slightly. The propionic acid concentration decreased from 2800 mg L^{-1} to 150 mg L^{-1}, while acetic acid started to accumulate from 780 mg L^{-1} to 2600 mg L^{-1}, indicating the reaction rate of propionate oxidization was higher than the conversion of acetic acid to methane. Moreover, statistical analyses show that the digestion performance of R1 phase III was better than that for the phase Ⅱ (Table 2). This illustrates that a prolonged bioaugmentation time is helpful to improve digestion performance when there is still propionic acid accumulation.

For phase Ⅳ bioaugmentation of both reactors was ceased. Subsequently the methane production

of both reactors initially decreased and then stabilised. The propionic acid concentration in R2 increased and then dropped slightly to approximately 2000 mg L^{-1} at the end of the experiment. For R1 there was almost no propionic acid accumulation but the concentration of acetic acid fluctuated around 2000 mg L^{-1} in the later stage of the experiment. The ratio of intermediate alkalinity: partial alkalinity (IA: PA) was below 0.6, indicating digestion stability (Ripley et al., 1986). The average VMP of both reactors during phase Ⅳ were higher than before bioaugmentation (R0). In comparison to R2, R1 underwent a longer period of bioaugmentation, thus it showed higher ability of propionate degradation and methane production (Table 2).

The above result demonstrates that bioaugmentation with the enriched methanogenic propionate degrading culture was an effective method to keep the unstable digestion process from failure, by preventing the further accumulation of VFA, which at high concentrations could cause the deterioration of digester performance (Aydin, 2016; Regueiro et al., 2015). Bioaugmention can also recover the digester from severe inhibition under organic loading 0.625 g propionic acid L^{-1} d^{-1} and 3.0 g NH_4-N L^{-1} conditons with 11150 mg L^{-1} VFA accumulation（propionic 8500 mg L^{-1}, acetic acid 3000 mg L^{-1}. As previous studies have suggested, the addition of propionate-utilizing enrichment cultures can accelerate the conversion of acetate and propionate to methane, which leads to improved digestion performance (Acharya et al., 2015; Tale et al., 2015).

3.2 Bioaugmentation efficiency

To better elucidate the improvement of digestion performance before and after different dosages of bioaugmentation, statistical analysis was used to calculate the average performance characteristics over several stable periods (Table 3). From this it is clear that the performance of the augmented reactors was considerably enhanced. This effect of bioaugmentation lasted for the remainder of the experimental period of both reactors without any further addition of microbial cultures. For R1, after 45 days of 1 dosage bioaugmentation, the average VMP yield, methane recovery rate and propionic acid degradation rate during the period of day 103-123 was enhanced by 70 mL L^{-1} d^{-1}, 21% and 51% than before bioaugmentation (R0, day 38-50), respectively. After 20 days of 2 dosages of bioaugmentation, R2 (day 103-123) exhibited a pronounced increase in methane production and propionic degradation than that before bioaugmentation during day 59-75. The average VMP yield, methane percentage, methane recovery rate and propionic acid degradation

rate were higher at 158 mL L^{-1} d^{-1}, 48% and 62%, respectively than that before inoculation.

In total, approximately 10.12 g DCW of augmented microbial community was added over 45 days to R1 for the single dosage of bioaugmentation, while approximately 9 g DCW in total was inoculated during 20 days for the double dosage of R2. Table 3 shows the bioaugmentation efficiency (enhanced performance value per day per gram of microbial community) of double dosage (R2) was higher than for a single dosage in terms of VMP, methane recovery rate and propionic degradation rate. However, the two reactors do not share the same staring point (i.e. VFA concentration, IA: PA, etc.) when the different dosages of microbes were introduced into the reactors (Fig.2), so it is difficult to compare them directly. In comparison to R0 (days 35-50), the improvement of methane production of both reactors after bioaugmentation during days 103-123 were at the same level, while R2 took shorter augmentation time (20 days) than R1 (45 days). Besides, R2 was under more severe inhibition when bioaugmentation was started. Therefore, it is possible to deduce that a higher dosage will result in a more efficient bioaugmentation. Considering the economic value, the minimum amount of bioaugmentation sufficient to avoid culture washout would typically be required in a continuous reactor (Fotidis et al., 2014). The suitable dosage of bioaugmentation culture may depend on the inhibited level of the AD process.

3.3 Microbes targets with FISH

Figure 3 shows the distribution of the groups of mainly methanogens targeted by probe ARC 915 (green colored) and *Methanosaetaceae* (red colored) targeted by probe MX 825. It is clear that the populations of both methanogens and members of *Methanosaetaceae* increased significantly after bioaugmentation. The morphology of mainly methanogens showed diverse, including coccoid, filamentous and small rod cells. The groups of targeted *Methanosaetaceae* were mainly coccoid-shaped, which overlaid the great proportion of methanogens, the rest were filamentous-shaped methanogens. The low performance of methane production of R0 during phase Ⅰ might be due to the low population density of methanogens under ammonia stress. After bioaugmentation, *Methanosaetaceae*, the strict acetoclastic methanogens were the main contributor towards the improvement in methane production. Therefore, although it was more vulnerable to ammonia than hydrogenotrophic methanogen (Poirier et al., 2016), the routine addition of the culture might be the key to culture survival from ammonia stress and prolong their

methanogenic activity. These FISH results clearly show that bioaugmentation can enhance the diversity of methanogens, which could explain the alleviation from TAN and propionate inhibition.

4. Conclusions

This study demonstrates that bioaugmentation is not only able to prevent the further deterioration of a poor performing digester, but can also recover from anaerobic digestion failure under the double stress from propionate and ammonia accumulation. After bioaugmentation the reactors showed better performance in terms of methane production, methane recovery rate and VFA degradation since the density of functional microbes was enhanced. Moreover, prolonging the bioaugmentation period is helpful to improve digestion performance when there is still VFA accumulation. In addition, a higher dosage of bioaugmentation is able to shorten the recovery time when inhibition is severe.

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Figure captions

Fig. 1. Main strategic operational conditions of experimental reactors. Phase I (0–50 d), Phase II (50–75 d), Phase III (75–95 d), Phase IV (95–125 d)). 1x presented 1 dosage of bioaugmentation seed (0.3 g DCW L^{-1} d^{-1}), 2x presented 2 dosage of bioaugmentation seed (0.6 g DCW L^{-1} d^{-1}).

Fig. 2. The digestion performance of the reactors. R1, 1 dosage bioaugmentation (before bioaugmentation Phase I ; bioaugmentation, Phase II , III; after bioaugmentation, Phase IV) ; R2, 2 dosage bioaugmentation (before bioaugmentation, Phase I, II; bioaugmentation, Phase III; after bioaugmentation Phase IV)

Fig. 3. Results of FISH for microorganisms in propionate-fed CSTR exposed to ammonia (3 g L^{-1} TAN) before (R0, day 47) and after bioaugmentation (R1, day 75). Fluorescent probes: ARC915 (red), EUB338 (green) and overlay of probe ARC915 and probes EUB338

Table 1

Oligonucleotide probes used for the microbe population identification

Probe	Phylogenetic group	Functional group	Probe sequence (5’ – 3’)
ARC915	*Archaea*	Mainly methanogenic.	GTGCTCCCCCGCCAATTCCT
MX825	*Methanosaetaceae*	Aceticlastic methanogenic.	TCGCACCGTGGCCGACACCTAGC

Table 2

Statistical analyses of digestion performance during different experimental periods.

Reactor	Period	BA dose (g DCW L^{-1} d^{-1})	VMP (mL L^{-1} d^{-1})	CH_4 percentage （%）	CH_4 recovery rate （%）	HPr degradation rate（%）
R0	day 35-50	0	98.40（0.30）	72.20 (0.02)	29.73 (0.28)	44.96 (0.81)
R1	Phase Ⅱ	0.30	151.66（0.87）	74.56（0.05）	45.81 (0.79)	66.01 (3.66)
	Phase III	0.30	243.06（0.37）	75.63 (0.06)	73.42 (0.34)	97.79 (0.06)
	PhaseⅣ	0	189.35（0.61）	72.85（0.01）	57.19 (0.56)	93.79 (0.23)
R2	Phase Ⅱ	0	23.39（0.86）	34.53 (5.64)	7.06 (0.79)	11.38 (1.53)
	Phase III	0.60	172.13（5.97）	68.88 (1.78)	51.99 (5.44)	77.42 (7.58)
	PhaseⅣ	0	166.91（0.14）	71.99（0.01）	50.42 (0.13)	71.55 (4.48)

Values are expressed as mean values with the standard deviation shown in parentheses

Table 3

Efficiency of different dosage of bioaugmentation (BA)

Compared subjects	Before and after 1 dosage BA	Before and after 2 dosage BA
Compared reactor (period)	R0 (day38-50) *vs.*R1 (day103-123)	R2 (day59-75) *vs.* R2 (day103-123)
BA period (d)	45	20
Total BA dose (g)	10.12	9.00
VMP (mL L^{-1} d^{-1})	105.98(0.02) *vs.*176.20(0.10)	4.25(0.02) *vs.* 161.95(0.08)
Enhanced VMP (mL L^{-1} d^{-1})	70.22	157.70
[a]BA efficiency of VMP (mL L^{-1} $d^{-1}g^{-1}$ DCW d^{-1})	0.15	0.88
CH_4 recovery rate (%)	32.01(0.02) *vs.*53.22(0.10)	1.28 (0.02) *vs.*48.92(0.07)
Enhanced CH_4 recovery rate (%)	21.21	47.64
[a]BA efficiency of CH_4 recovery rate (% g^{-1} DCW d^{-1})	0.05	0.26
HPr degradation rate (%)	41.49 (0.12) *vs.* 92.55 (0.22)	9.50(0.67) *vs.* 71.86(5.38)
Enhanced HPr degradation rate (%)	51.06	62.36
[a]BA efficiency of HPr degrading rate (% g^{-1} DCW d^{-1})	0.11	0.35

Values are expressed as mean values with standard deviation shown in parentheses

For a: bioaugmentation efficiency is calculated by enhanced VMP (or CH_4 recovery rate, HPr degradation rate) / bioaugmentation time (as a day)/ inoculated microbes mass (as a gram)

Fig. 1.

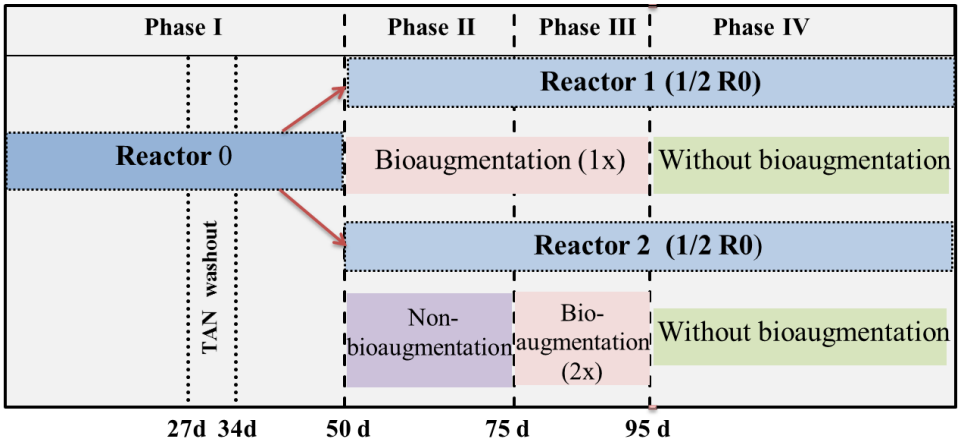
Phase I
Phase II
Phase III
Phase IV
Reactor 1 (1/2 R0)
Reactor 0
Bioaugmentation (1x)
Without bioaugmentation
Reactor 2 (1/2 R0)
TAN washout
Non-bioaugmentation
Bio-augmentation (2x)
Without bioaugmentation
27d
34d
50 d
75 d
95 d

Fig. 2.

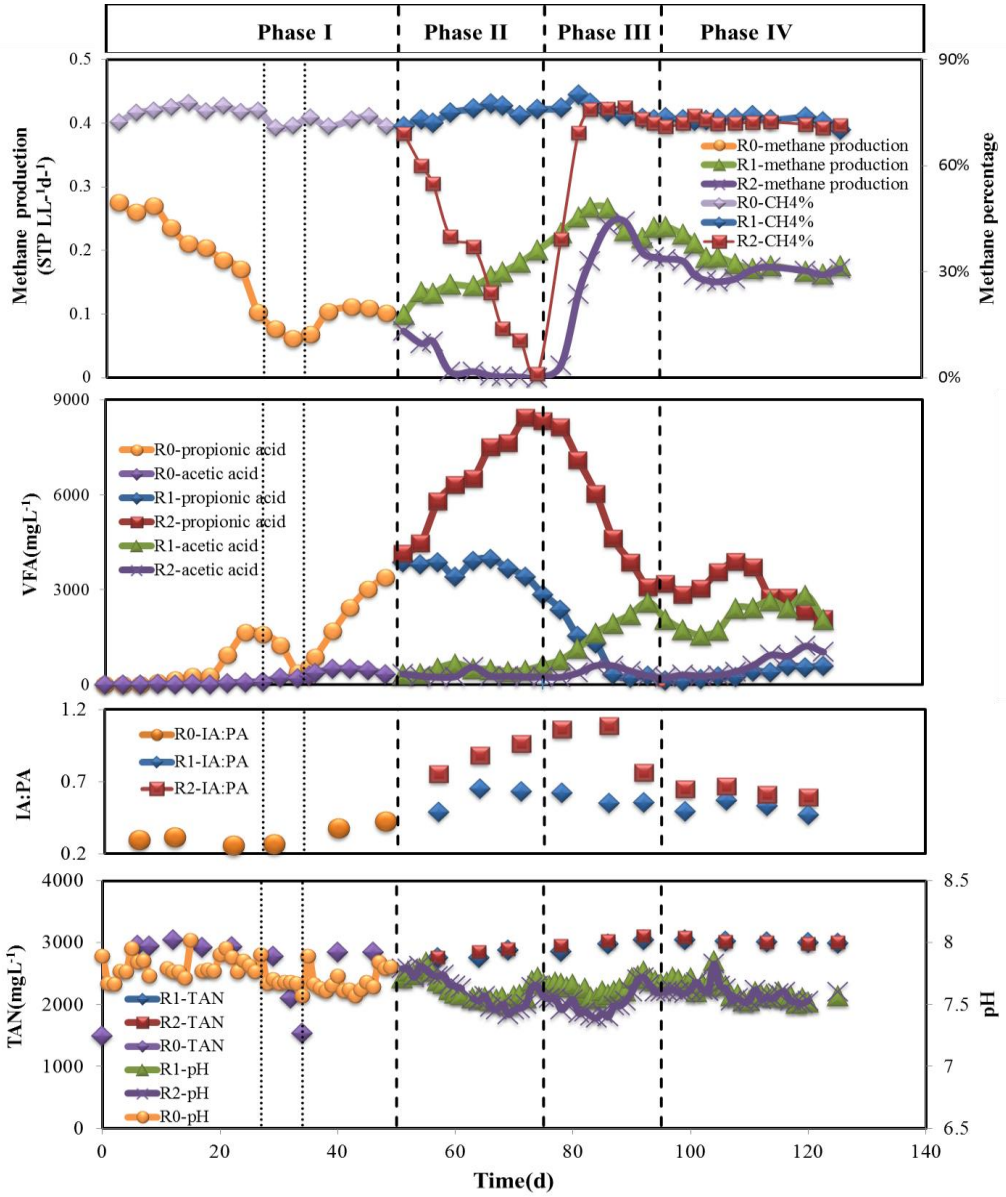
Phase I
Phase II
Phase III
Phase IV
Methane production (STP LL-1d-1)
Methane percentage
R0-methane production
R1-methane production
R2-methane production
R0-CH4%
R1-CH4%
R2-CH4%
VFA(mgL-1)
R0-propionic acid
R0-acetic acid
R1-propionic acid
R2-propionic acid
R1-acetic acid
R2-acetic acid
IA:PA
R0-IA:PA
R1-IA:PA
R2-IA:PA
TAN(mgL-1)
pH
R1-TAN
R2-TAN
R0-TAN
R1-pH
R2-pH
R0-pH
Time(d)

Fig. 3.