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Impact of Sudden Change in Feed Substrate Types on Steady Response of Suspended Growth Anaerobic Reactors

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ABSTRACT: Industries in developing countries add new production lines producing altogether different waste stream. This newer waste invariably finds its way to existing anaerobic treatment system causing gradual/sudden change in feed substrates affecting its steady performances. This study investigates steady response of anaerobic reactors subjected to sudden change in feed substrate types, assesses its impact on biomass and explores possibility of restoring pre-changed steady responses again. Three suspended growth batch anaerobic reactors (R1, R2 and R3) were started-up and operated in three different phases. In Phase–I, all the reactors were operated for 65 d on jaggery feed at an organic loading rate (OLR) of 1.40 kg COD/m^3 d to obtain steady responses. In Phase–II, the change in feed substrate types were applied suddenly to R2 (from jaggery to cerelac) and R3 (from jaggery to neutralized acetic acid) while keeping R1 as the control and operated for 64 d on the same OLR. R2 gave steady response with reduced biogas production whereas complete cessation of biogas production was observed in R3. The total methanogenic activity of R3 biomass yielded comparable values with R1 and R2 biomasses indicating preservation of biomass integrity. When R2 and R3 were restored suddenly again with jaggery feed in Phase–III at the same OLR, R3 recovered quickly and all the reactors gave similar steady responses comparable to Phase–I.

Key words: Anaerobic Biomass, Batch Reactor, Biogas, Substrate, Methanogenic Activity, Steady Response, Suspended Growth

INTRODUCTION

Methanogenic species types and their relative population levels in anaerobic biomass depend on feed substrate as well as operational and/or environmental conditions maintained (Novaes, 1986). Many researchers have investigated anaerobic processes with short duration sudden/ gradual change in operational and/or environmental conditions without changing feed substrate types (Leitao *et al*., 2006; Singh and Pandey, 2009). Chua et al. (1997) studied response of an anaerobic fixed–film reactor to short duration hydraulic shock loadings while maintaining a constant organic loading rate (OLR) using the same type of feed substrate and observed that the immobilized biofilm was tolerant of up to five times hydraulic shock loadings. Tay and Zhang (2000) attempted to rank three high–rate anaerobic reactors on the basis of their stability under short duration (3 h) shocks of seven different types using the same synthetic feed substrate. Angenent et al. (2002) studied effect of 42 h organic shock load on stability of an anaerobic migrating blanket reactor by doubling organic load from 27 to 50 g COD/L d using same type of feed substrate while maintaining a constant hydraulic retention time (HRT) which resulted in decreased soluble COD removal efficiency but the performance returned to pre-shock-load levels after organic load restored to 25 g COD/L d. Sanchez et al. (2005) investigated the effect of influent strength on performance of down-flow anaerobic fixed bed reactor treating piggery wastewater at HRTs in the range of 1-6 d and observed that the process performance improved with increase in influent strength from 2 to 8 g total COD/L at a HRT of

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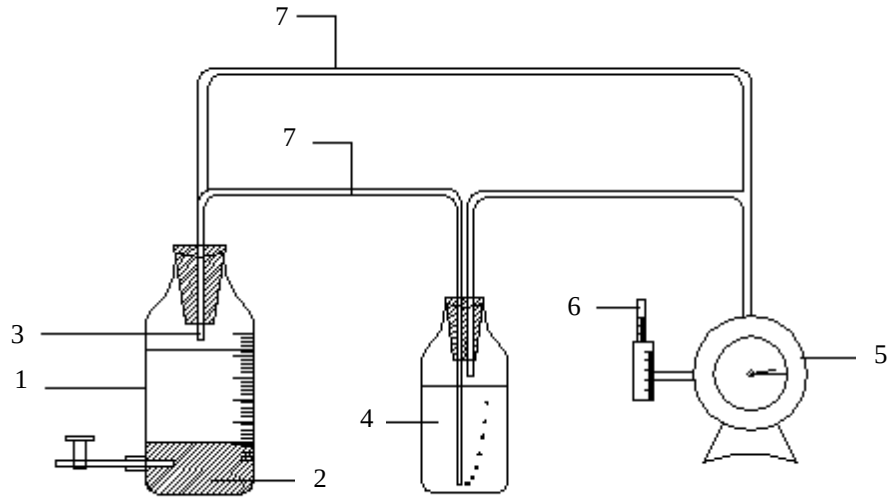
1d, whereas performance deteriorated at higher influent strengths. Lu *et al.* (2009) investigated effects of stress of pH and/or acetate on the fermentation product formation of polysaccharide-rich organic waste in an anaerobic fermentor and observed that acetate stress on product formation was stronger than pH stress. In contrast, no studies are available on the steady response of anaerobic reactors subjected to stress/shock conditions by gradual/sudden change/modification in feed substrate types as well as its impact on anaerobic biomass. The results of such studies are likely to be useful for developing countries where polluting industries are installing treatment systems for the existing waste streams to meet the regulatory discharge limits. While industries expands its activities by starting newer production lines producing additional waste streams with altogether different composition than the existing wastes, but fail to take into consideration the treatment aspect of additional waste streams and requirement of up-gradation/augmentation of existing treatment system at the planning stage of expansion. Once the additional waste stream is generated, the possibility of this stream finding its way to existing treatment system cannot be ruled out. This might make the existing biological treatment system – especially anaerobic reactor vulnerable to stress/ shock conditions by gradual/sudden change/ modification in feed characteristics due to merging of additional waste stream(s). Under such situations, the anaerobic reactor is likely to show an immediate impact and might settle for a new steady response if the changed/modified feed continued for long (Nain and Jawed, 2006). In the field, the existing treatment system is bound to receive changed/modified feeds due to addition of newer waste stream leading to its stabilization to a new but a deteriorated steady response. Therefore, it is important in the field to know the new steady response of reactors when subjected to sudden/gradual change/modification in feed substrate along with its impact on biomass so as to explore the possibility of restoring the reactor responses to the pre-changed conditions. Keeping this requirements in mind, the objectives of this study are to: (a) investigate the impact on steady response of anaerobic reactors subjected to extreme condition of sudden change in feed substrate types while maintaining similar sets of operational and environmental conditions, (b) assess its impact on biomass under changed feeding conditions and (c) explore the possibility of restoring reactor responses to pre-changed feeding conditions.

MATERIALS & METHODS

The study was carried out with three different types of feed substrate: (a) jaggery – a simple soluble feed substrate (unrefined sugar, which is known as gur in the local market), (b) cerelac – a complex feed substrate with high suspended fraction (available as a baby feed in the local market) and (c) neutralized acetic acid (acetate) – another simple soluble feed substrate preferred by acetoclastic methanogens. The experiments were carried out at room temperature (18–29 ^{o}C) in three suspended growth batch anaerobic reactors (designated as R1, R2 and R3) fabricated with glass aspirator bottles (5 L capacity) as shown in Fig. 1. All three reactors were started-up with synthetic feed substrate prepared using jaggery. Reactor R1 was designated as the control, while reactors R2 and R3 were used to study the steady responses when subjected to sudden change in feed substrate types. The characteristics of feed substrate types are presented in Table 1. The synthetic feed substrate was prepared by taking required amounts of jaggery/cerelac/ neutralized acetic acid (the main source of organic carbon), urea and KH_2PO_4 (to yield a COD:N:P ratio of 100:14:1 while ignoring the amount of N and P present in jaggery and cerelac), $NaHCO_3$ (the external source of alkalinity to maintain reactor pH near 7), and all dissolved in 250 mL of tap water. A wet-type gas flow meter (Model: INSREF-IRI 08B, M/S Instrumentation and Refrigeration of India, Madras, India) was directly connected to reactors for biogas measurement, whereas methane was measured after scrubbing the biogas of CO_2 using 11.2% w/v KOH trap. The anaerobic seed (total solids = 81.325 g/L and volatile solids = 39.547 g/L) was obtained from a biogas plant located within IIT Guwahati campus running for more than 2 years on animal droppings and kitchen wastes. All three reactors were charged with 1.5 L of anaerobic seed and 3.5 L of tap water (purged with N_2) to make total volume to 5 L. Reactors were water-sealed and its contents were mixed thoroughly – manually tilted

by neck (without lifting) and shaken to give a swirling type motion to liquid and then allowed to stand for 48 h. Thereafter daily feedings were started by withdrawing 250 mL of supernatant liquid with minimal loss of seed from the reactor and the synthetic feed prepared was poured into the reactor and mixed thoroughly. The withdrawn supernatant was used for estimation of total COD, volatile fatty acid (VFA) and bicarbonate alkalinity (BA). Liquid samples were centrifuged (Model: R-24, M/S Remi India Ltd., Bombay, India) at 10000 rpm for 20 min to separate out suspended solids from liquid. The liquid portion was used to estimate soluble COD. The reactor contents were mixed thoroughly to draw reactor biomass samples for methanogenic activity tests and solid analysis. COD and solid analysis were carried out as per Standard Methods (1998). VFA and BA were estimated directly by titration method (DiLallo and Albertson, 1961).

Methanogenic activity tests on withdrawn biomass were carried out at room temperature (18–29 ^{o}C) as per method suggested by Jawed and Tare (1999) with selected feed substrate types



1. Aspirator Bottle 2. Anaerobic Biomass 3. Gas Outlet 4. KOH Trap 5. Gas Flow Meter 6. Thermometer 7. Rubber Tubes

Fig. 1. Schematics of laboratory scale suspended growth batch reactors

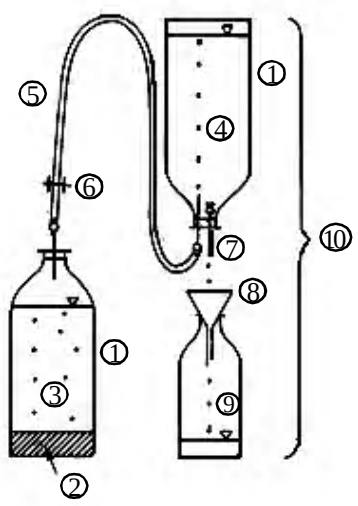
Table 1. Characteristics of feed substrate types used

Feed Substrate	Characteristics	Source
Jaggery	per 100 g: Sucrose sugar = 65-85 g, Reducing sugar = 5-15 g, Protein = 0.4 g, Fat = 0.1 g, Calcium = 8 mg, Phosphorous = 3-4 mg, Total minerals = 0.6-1 g, Moisture content = 3-8 g, Carotene (Vitamin A) = 280 μg, Nicotinic acid = 1 μg, Thiamine (Vitamin B) = 20 μg, Colour = Golden yellow to brown, Energy = 383 kcal.	Gehlawat (1996)
	COD = 957±22 mg/g Jaggery	Nain (2005)
Cerelac	per 100 g: Carbohydrate = 67.9 g, Protein = 15 g, Fat = 9 g, Calcium = 480 mg, Potassium = 500 mg, Sodium = 150 mg, Phosphorous = 370 mg, Moisture content = 2.5 g, Folic acid = 25 μg, Thiamine (Vitamin B) = 0.8 mg, Ash = 3.2 g, Colour = White, Energy = 413 kcal.	Nestle (2004)
	COD = 1055±49 mg/g Cerelac	Nain (2005)
Neutralized acetic acid (Acetate)	100 mL analytical grade acetic acid + 50 mL distilled water + analytical grade NaOH pellets to obtain a pH of 7 and made-up the final volume to 500 mL with distilled water to give a stock acetate solution.	Nain (2005)
	COD = 161±2 mg/mL of stock solution	

using the test set-up shown in Fig. 2. A known amount of biomass was transferred in 500 mL serum bottles. Tap-water (purged with N_2) was added upto 500 mL mark. The biomass amount was taken such as to get the final concentration of volatile suspended solids [VSS(F)] in the range of 1 to 2 g/L. Sufficient amount of substrate (jaggery, cerelac and acetate) was added to serum bottles to get the initial COD level in the range of 2000–2500 mg/L. The $NaHCO_3$ was also added to buffer the system at neutral pH conditions. The serum bottles were properly capped connected to liquid displacement system and then content of serum bottles mixed by swirling manually. A shorter time interval (0.5 to 2 h) was selected for noting gas production in the first 12 h after feeding and longer time interval (4 h or more) afterwards up to 48 h after feeding. After every reading, contents of the serum bottles were mixed by swirling manually. When gas production for the first feeding had been recorded, supernatant of the serum bottles were decanted. Tap water (purged with N_2) was immediately poured in to serum bottles and volume was again made up to 500 mL mark. Same amount of substrates were fed as in the first feeding, the bottles capped and connected to the liquid displacement system, and then the content were mixed manually. The gas production was recorded for next 48 h. This constituted the second feeding. Likewise the procedure was repeated for the third feeding. On completion of the tests, amount of the biomass [VSS(F)] in the serum bottles were estimated. Slope of cumulative methane gas production versus time graph for the third feeding were used to estimate the methanogenic activity of the anaerobic biomass.

RESULTS & DISCUSSION

All three reactors were operated under similar sets of environmental and operational conditions (except change in feed substrate types) in three different phases: (a) Phase–I was aimed to obtain similar steady responses before application of sudden change in feed substrate types. Reactors were operated at an OLR of 1.40±0.03 kg COD/m^3 d for 65 d on synthetic feed prepared using jaggery and monitored for steady responses, (b) Phase–II was devoted for application of sudden change in feed substrate types to obtain the steady response under such condition. The control



1. Serum Bottle 2. Anaerobic Biomass
3. Reaction Mixture
4. CO_2 Scrubber (11.2% w/v KOH + thymol blue indicator)
5. Rubber Tubing 6. Pinch-cock7. Hypodermic Needle
8. Conical Funnel 9. Displaced Liquid
10. Liquid Displacement System

Fig. 2. Schematics of methanogenic activity test set-up

(reactor R1) was maintained on jaggery feed, while reactor R2 was subjected to sudden change in feed substrate type – from jaggery to cerelac and reactor R3 – from jaggery to neutralized acetic acid feed. All three reactors were operated at an OLR of 1.42±0.03 kg COD/m^3 d and monitored for 64 d, and (c) Phase–III was aimed to restore suddenly the synthetic feed prepared using jaggery in reactors R2 and R3 and to assess the possibility of restoring steady reactor responses to pre-changed conditions. All three reactors were operated at an OLR of 1.43±0.01 kg COD/m^3 d and monitored for 54 d. For the purpose of this study, some of the selected operational and environmental parameters (namely influent total COD levels, OLR and room temperature) and steady responses (namely effluent total COD level, total COD removal, biogas and methane produced) for the three reactors have been segregated phase-wise and presented in Fig. 3. The first, second and third column of blocks in (0.59±0.02 L/d for R1, 0.73±0.01 L/d for R2 and 0.67±0.12 L/d for R3) and total COD removal (39.54±5.29% for R1, 42.43±3.81% for R2 and 2.15±0.14 L/d for R3), methane production 44.18±2.56% for R3) when operated on synthetic feed prepared using jaggery during Phase–I.

Fig.3 represent operational/environmental parameters and steady responses in Phase – I, II and III respectively, whereas the top row of blocks represent the operational/environmental parameters maintained in three reactors in all the three phases of studies. The second and third rows of blocks from top in Fig. 3 represent the steady response for control reactor R1, while fourth-fifth and sixth-seventh rows of blocks give the same for reactors R2 and R3.

All three reactors yielded comparatively similar steady responses in terms of biogas yield (2.09±0.07 L/d for R1, 1.91±0.11 L/d for R2 and

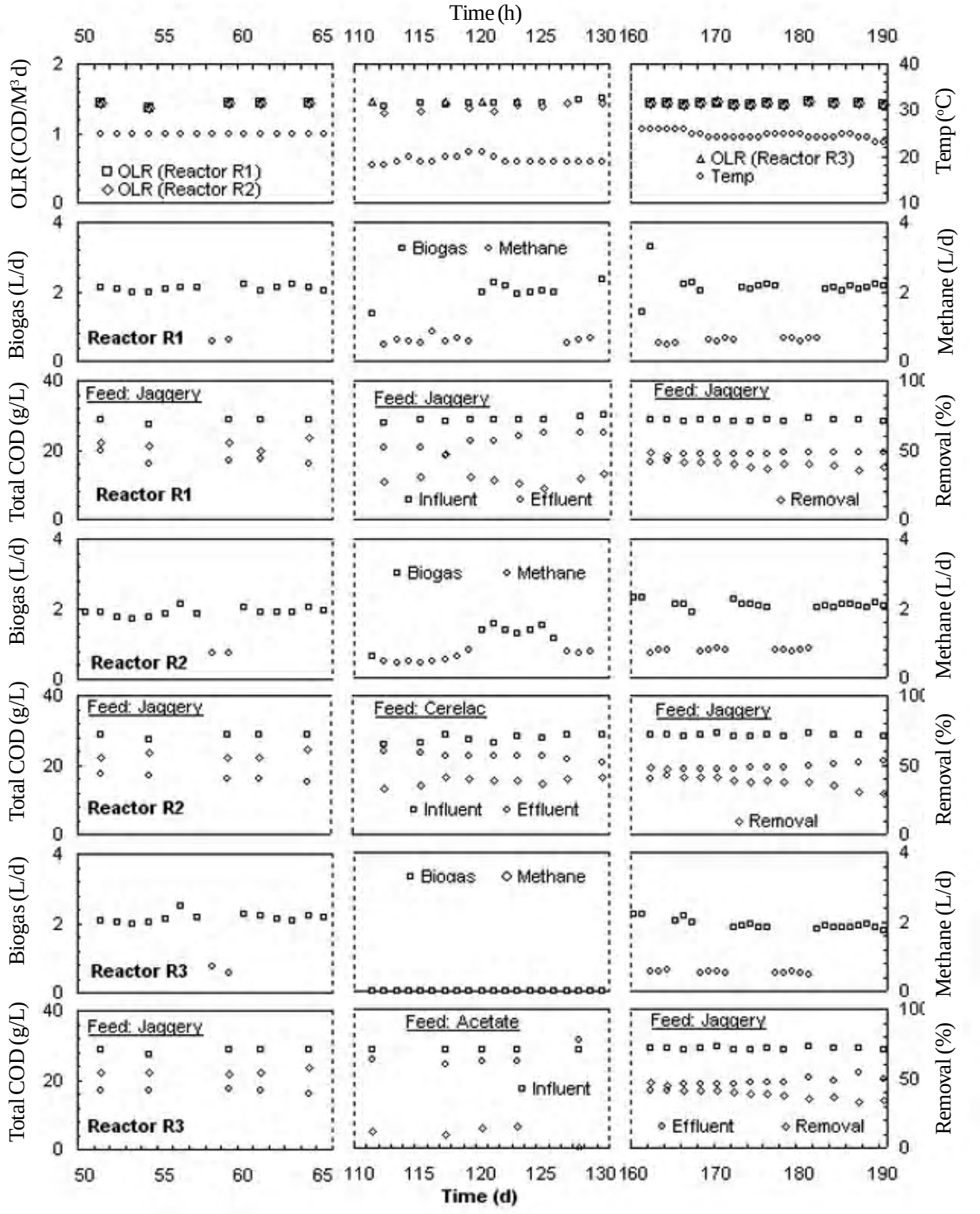


Fig. 3. Spatial variations in selected steady response of reactor R1, R2 and R3

Reactors R2 and R3 were then suddenly fed with changed feed substrate types (keeping reactor R1 as the control) in the beginning of Phase–II (i.e. on 67th d of operation) while maintaining similar operational and environmental conditions and operated for steady responses. As seen in Fig. 3. the steady response of reactor R2 deteriorated in respect of biogas yield (decreased to 1.27±0.30 L/d in Phase–II from 1.91±0.11 L/d in Phase–I and 1.96±0.31 L/d in Phase–II of the control reactor R1) and methane production (decreased to 0.58±0.15 L/d in Phase–II from 0.73±0.01 L/d in Phase–I and 0.60±0.11 L/d in Phase–II of the control reactor R1). However, the steady response of reactor R3 deteriorated very much with complete cessation of biogas production (decreased to zero in Phase–II from 2.15±0.14 L/d in Phase–I and 1.96±0.31 L/d in Phase–II of the control reactor R1). Product inhibition was also observed by Ma et al. (2009) when an UASB reactor was exposed suddenly to propionic acid as a feed. As the response of reactor R3 deteriorated completely, daily feedings were modified – fed every second day during 91st to 98th and then every third day during 99th to 123rd d of operation. However, it did not improve the responses.One of the notable changes in environmental conditions during Phase–II was the reduction in room temperature to 18–20 ^{o}C compared to 25 ^{o}C in Phase–I.Since steady responses of reactors R2 and R3 were also compared with respective responses of the control taking into account the impact of variation in temperature and therefore, the obtained trend was not likely to be affected much.

The methanogenic activity tests were carried out on biomass withdrawn during steady response period on 116th d of operation in Phase–II using three different substrates – jaggery, cerelac and neutralized acetic acid (or acetate) in separate but parallel test set-ups to assess the impact of changed feeding conditions on the biomass. The activity test with acetate reflected activity of acetoclastic methanogen – termed as acetoclastic methanognic activity (AMA) whereas tests with jaggery and cerelac in which both hydrogen oxidizing and acetoclastic methanogens contributed towards methane production – termed as total methanogenic activity ($TMA_{Jaggery}$ and $TMA_{Cerelac}$). Prior to subjecting the reactors to change in feed substrate types, activity tests were carried out on biomass withdrawn from all three reactors with jaggery and acetate substrates on 9th d of operation in Phase–I. It yielded $TMA_{Jaggery}$ values of 0.078, 0.091 and 0.095 g CH_4 COD/g VSS d for reactor R1, R2 and R3 biomasses respectively indicating similar conditions of biomass in Phase–I operation. However, the biomass did not yield any measurable amounts of methane with acetate substrates and hence AMA values could not be estimated. As the reactor R3 had shown extreme steady responses – complete cessation of biogas production in Phase–II, the typical activity test results for reactor R3 biomass withdrawn on 116th d of operation in Phase–II are presented in Fig. 4 where the top, middle and bottom blocks show the activity results with jaggery, cerelac and acetate substrates respectively. It was observed that the activity tests with jaggery and cerelac substrates yielded consistently higher amounts of cumulative methane in 2nd and 3rd feedings whereas the tests with acetate substrate yielded very small amounts of methane in all three feedings. Similar trends were observed in cumulative methane production for reactor R1 and R2 biomass during activity tests. The estimated activity values for reactor biomasses are presented in Table 2. It was observed that reactor biomass yielded comparatively higher TMA values, whereas AMA values were consistently very low. Since jaggery was the feed substrate for control reactor R1, ratio of $AMA/TMA_{Jaggery}$ and $TMA_{Cerelac}/TMA_{Jaggery}$ were also considered. The ratio of $AMA/TMA_{Jaggery}$ was consistently low for all three-reactor biomasses indicating lower population levels of acetoclastic methanogens. However, $TMA_{Cerelac}/TMA_{Jaggery}$ ratio was comparatively higher for all three-reactor biomasses indicating presence of sufficient population of microorganisms to carry out the hydrolysis, fermentation and acidification steps. Even when reactor R3 biomass was maintained on neutralized acetic acid (acetate) feed substrate and biogas production had ceased completely in Phase–II, TMA values obtained were comparable to reactor R1 and R2 biomasses. It indicated that anaerobic biomass of reactor R3 was capable to preserve its integrity during adverse feed conditions maintained over a relatively longer period of time and also hinted that if the adverse feed conditions replaced with favorable feed conditions, the biomass might start producing biogas and methane again. Though the anaerobic seed was obtained from the same source

and was acclimatized with the same simple soluble synthetic feed substrate prepared using jaggery prior to change in feed substrate types, the reactor biomass behaved differently leading to different steady responses. When the biomass in reactor R2 was subjected to sudden change in feed substrate – from jaggery to cerelac, the reactor biomass was able to adjust to the new feed substrate type mainly due to availability of diverse groups of anaerobic microorganisms required to carry out hydrolysis, fermentation and acidification of suspended type of feed substrate before methanisation. It might be possible that products of hydrolysis and fermentation yielded smaller amounts of acetate and hydrogen on a continuous basis till the next feeding, which were utilized continuously by methanogens without experiencing any adverse impact. However, in the case of biomass in reactor R3 with change in feed substrate – from jaggery to neutralized acetic acid, the reactor ceased to produce biogas and utilize COD. It was mainly due to non-availability of adequate population of methanogens required to undertake methanisation from acetate feed substrate as observed from the results of activity tests in Phase–I.

At the end of Phase–II, effluent total COD values in all three reactors were observed to be high. Therefore, it was felt appropriate to minimize effluent total COD before initiating Phase–III studies by decanting settled supernatant from all three reactors and then made–up reactor volume to 5 L with N_2 purged tap water. These operations were carried out on 134th and 138th d for reactors R1 and R2 and on 123rd, 134th and 138th d for reactor R3. Reactors were not fed during this period. This helped in bringing down supernatant total COD values to 4.8 g/L for reactor R1, 6.4 g/L for reactor R2 and 3.2 g/L for reactor R3 as against total COD levels of 20–30 g/L before the start of Phase–III studies. The reactors were then fed suddenly with synthetic feed prepared using jaggery (same as Phase–I feed) from 138th d of operation onwards in Phase–III. Reactor R3 responded with biogas production along with other two reactors and all three reactors started giving steady responses from 162nd d of operation onwards. The steady response of reactors R2 and R3 were comparable with the control in terms of biogas yield (2.16±0.33 L/d for R1, 2.11±0.10 L/d for R2 and 1.92±0.14 L/d for R3), methane production (0.60±0.10 L/d for R1, 0.79±0.04 L/d for R2 and 0.54±0.03 L/d for R3) and total COD removals (34.80±3.60% for R1, 32.46±2.45% for R2 and 34.85±4.73% for R3) as shown in Fig. 3. It is important to note that reactor R3 gave an extreme response of complete cessation of biogas

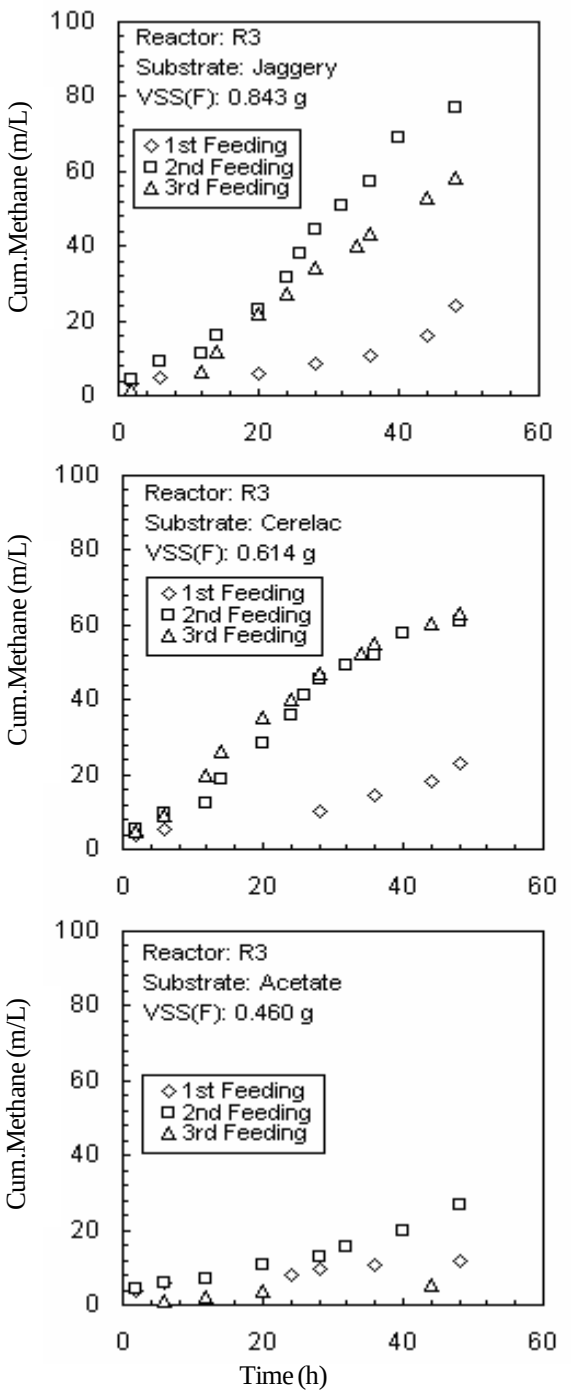


Fig. 4. Typical activity test results with reactor R3 biomass withdrawn on 116th d of operation in Phase–II

production in Phase–II when fed with synthetic feed prepared using neutralized acetic acid but recovered quickly with restoration of pre-changed feed condition of synthetic feed prepared using jaggery in Phase–III. All three reactors gave steady responses similar to Phase–I. The methanogenic activity tests were also carried out on biomass withdrawn during steady response period on 181st d of operation in Phase–III and results are summarized in Table 2 indicated similar trend as was obtained in Phase–II. This clearly indicated that if the adverse feeding conditions were replaced with the favorable feeding, then the steady response of existing reactor could be restored without much difficulty. This gave a positive ramification for the treatment plant receiving changed/modified influents impacting the steady response of the plant.

CONCLUSION

The steady response of reactor R2 was observed to deteriorate in respect of biogas production, while that of reactor R3 deteriorated with complete cessation of biogas production in comparison to the control when reactors were subjected to sudden change in feed substrate types in Phase–II. Even when the reactor R3 biomass was maintained on neutralized acetic acid feed substrate and biogas production had ceased completely in Phase–II, TMA values of R3 biomass were comparable with reactor R1 and R2 biomasses indicating preservation of biomass integrity during adverse feeding conditions. With sudden restoration of synthetic feed of Phase–I, reactor R3 responded quickly by producing biogas along with other two reactors and all three reactors gave comparatively similar steady responses in Phase – III. The results of this study gave a positive ramification for the treatment plant receiving changed/modified influents impacting the steady response of the plant.

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