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Effect of micro-aeration on anaerobic digestion of primary sludge under septic tank conditions

James Diak · Banu Örmeci · Kevin J. Kennedy

Abstract Micro-aeration, which refers to the addition of very small amounts of air, is a simple technology that can potentially be incorporated in septic tanks to improve the digestion performance. The purpose of this study was to investigate and compare the effects of micro-aeration on anaerobic digestion of primary sludge under septic tank conditions. 1.6 L batch reactor experiments were carried out in duplicate using raw primary sludge, with 4.1 % total solids, and diluted primary sludge, with 2.1 % total solids. Reactors were operated for 5 weeks at room temperature to simulate septic tank conditions. Micro-aeration rate of 0.00156 vvm effectively solubilised chemical oxygen demand (COD) and improved the subsequent degradation of COD. Micro-aeration also increased the generation of ammonia and soluble proteins, but did not improve the reduction in total and volatile solids, or the reduction in carbohydrates. Experiments using diluted sludge samples showed similar trends as the experiments with raw sludge, which suggest that initial solids concentration did not have a significant effect on the degradation of primary sludge under septic tank conditions.

Keywords Anaerobic digestion · Sludge · Septic tank · Micro-aeration

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Introduction

The septic tank and soil absorption system are an elegantly passive on-site wastewater treatment process that remains to be one of the most common methods of treating household wastewater in rural areas where a complex sewage piping infrastructure is economically unfeasible. Septic systems are suitable for on-site wastewater treatment due to their low energy requirements, small space requirements and simple reactor design [1]. Although the original design of the septic system dates back to 1881 [2], very few changes have been made to the conventional system.

Micro-aeration is a simple technology that can be incorporated in septic tanks to improve the digestion performance. Micro-aeration refers to the addition of very small amounts of air (oxygen) to the system. A large amount of research has focussed on completely aerobic or anaerobic biological treatment systems, but less research has focussed on the transition region between these two extremities [3].

Park et al. [4] conducted a series of 30-day batch reactor experiments at 25 °C to compare the performance of aerobic and anaerobic digesters. Results of the study demonstrated that the reduction in volatile solids (VS) coincides with an increase in ammonia and soluble proteins. The study also demonstrated that some of the VS will only be degraded aerobically and some only anaerobically. Therefore, cycling from one to the other improves VS reductions.

Kumar et al. [5] employed aerobic digestion following anaerobic digestion, resulting in an additional 48–68 % reduction in VS. Aerobic post-treatments also decreased the concentration of proteins and carbohydrates compared to stand-alone anaerobic digestion, which suggests a potential decrease in the polymer demands for sludge dewatering.

Novak et al. [6] also reported an improvement in VS reduction using sequential anaerobic–aerobic digestion and an even greater reduction using a three-stage configuration cycling from anaerobic to aerobic and then back to anaerobic.

Alternate anaerobic–aerobic cycling is used to minimise sludge yield in activated sludge processes [7]. The cycling process is used to uncouple the catabolic and anabolic processes by limiting the amount of energy supplied to anabolism, thereby reducing biomass yield. Subsequently, catabolism and hydrolysis are improved, and ultimately digestion is improved. On the other hand, increased biomass and carbon dioxide production, and reduced methane yield are expected when oxygen is introduced as an electron acceptor in an anaerobic system [3]. Limiting amounts of oxygen to improve hydrolysis, without excessive biomass production, must be achieved for micro-aeration to be effective.

In the experiments by Johansen and Bakke [3], 4-day batch reactor tests with 300 mL samples of chemically precipitated sludge were used to examine the effects of micro-aeration on sludge hydrolysis. The accumulation of volatile fatty acids (VFAs) and methane generation for the samples under micro-aerobic conditions were roughly half of those of the anaerobic samples. Furthermore, the amount of biomass generated under micro-aerobic conditions was more than five times higher than the anaerobic reactors. However, under micro-aerobic conditions, a 50 % increase in hydrolysis was estimated based on carbon, nitrogen and chemical oxygen demand (COD) balancing.

Experiments by Zhu et al. [8] also demonstrated that sufficient micro-aeration rates improve the hydrolysis of carbohydrates and proteins during anaerobic digestion of vegetable and flower waste. Results of the study also suggest that micro-aeration promotes acidogenesis and helps prevent the build-up of lactic acid, but should be stopped following the early stages of digestion to maintain efficient solubilisation rates.

Díaz et al. [9] conducted 20-day batch reactor experiments at 35 °C, using 500 mL sludge seeds from an anaerobic digester, to evaluate the effects of micro-aeration on the degradation of amorphous cellulose. Experiments demonstrated that although the hydrolytic activity and maximum methane production were unaffected by micro-aeration, the time taken to reach maximum methane production was reduced as a result of the micro-aeration treatments.

Experiments by Jenicek et al. [10] evaluated the effect of micro-aeration on the quality of digested sludge using 10 L bench-scale digesters, operated at 40 °C. Although micro-aeration did not improve the reduction in total or volatile suspended solids (TSS or VSS), the dewaterability of the sludge was improved, the foaming potential was reduced and the accumulation of soluble COD (sCOD) was reduced.

Experiments by Díaz et al. [11] demonstrated that micro-aeration is also effective for the removal of hydrogen sulphide from digester gas. 200 L pilot-scale digesters were operated at 35 °C with a retention time of 20 days. Experiments tested micro-aeration of the feed sludge, recirculated sludge and headspace gas, demonstrating greater than 98 % removal of hydrogen sulphide through micro-aeration of the headspace gas. Similarly, experiments by Jenicek et al. [12] using 10 L bench-scale digesters, operated at 40 °C, reported a higher concentration of sulphate oxidising bacteria (SOB) as a result of micro-aerobic treatments. Jenicek et al. [13] also reported that micro-aeration does not harm sensitive anaerobic bacteria, such as methanogens, as long as the facultative microorganisms consume the available oxygen.

Micro-aeration in anaerobic digesters under mesophilic and thermophilic conditions has been shown to improve solubilisation of organics, reduce hydrogen sulphide concentrations in biogas, and improve digested sludge quality [3, 8, 10–13]. The purpose of this study was to investigate the effects of micro-aeration treatment under septic tank conditions. The research intends to determine if hydrolysis and biodegradation rates can be enhanced using a simple technology, such as micro-aeration, that is easy to maintain and does not require expensive or complex modifications to septic tanks.

Materials and methods

Sludge samples

Primary sludge samples were collected from the Robert O. Pickard Environmental Centre (ROPEC) in Ottawa, Canada. The initial characteristics of the raw primary sludge are presented in Table 1. In order to evaluate the effect of initial solids concentration on digestion rates, both raw primary sludge, with 4.1 % total solids (TS) and diluted primary sludge, with 2.1 % TS, were used in batch reactor experiments. Sludge samples were diluted with 50 % distilled water, therefore, the initial characteristics of the diluted batch reactors were one half the raw primary sludge values presented in Table 1.

Reactor setup and operation

Batch reactor experiments were carried out at 23 °C in 2.0 L Erlenmeyer flasks with a total working volume of 1.6 L. The flasks were sealed with a #10 rubber stopper which housed a 1/4 inch gas exit line and a glass Pyrex #16 stopper for the sampling port. For the anaerobic (control)

Table 1 Characteristics of the primary sludge used for the batch reactor experiments

Parameter	Unit	Batch reactor experiments AVG ± SD[a]
TS	g/L	41.0 ± 1.4
VS	g/L	32.3 ± 2.9
Total COD	mg/L	52,200 ± 5,091
Soluble COD	mg/L	3,930 ± 125
Ammonia	mg/L as NH$_3$-N	199 ± 18
Proteins	mg/L	1,890 ± 3
Carbohydrates	mg/L	10,500 ± 136

[a] Average (AVG) and standard deviation (SD) based on a minimum of 3 replicates

reactors, the gas exit line was connected to 3.0 L Tedlar gas sampling bags (CEL Scientific Corp, Santa Fe Springs, CA, USA). For the micro-aerobic reactors, the flasks were closed with a rubber stopper (#10) which housed a 1/4 inch gas inlet line connected to the diffusing stone and a gas exit line which was vented to the laboratory exhaust system. Each flask was fitted with a Pyrex coarse grained diffusing stone mounted on an 8 mm diameter glass rod, so that the diffusing stone was 2.5 cm from the bottom of the flask. House air was used to micro-aerate samples at a rate of 2.5 mL/min, equivalent to 0.00156 volumes of air per volume of reactor liquid per minute (vvm). The air was passed through a pressure regulator, then a disc-shaped HEPA filter, then to an air flow meter (Tube No. 032-41-N, glass float, Cole-Palmer Instrument Co., IL, USA). The regulated flow was then split into four lines and sent to each of the four micro-aeration flasks. At each flask, the flow was regulated with an individual flow valve to get an equal amount of air discharge from each flask, visible in the gas flow monitor. A diagram of the setup used for the anaerobic and micro-aerobic batch reactors is presented in Fig. 1.

Micro-aeration was tested on both diluted and undiluted primary sludge, in duplicate, for a total of four micro-aeration flasks. The reactors were operated for 5 weeks at room temperature (23 °C) which is in the operational temperature range for septic tanks. Batch reactors were filled with 1.6 L of primary sludge. Reactors using diluted sludge samples were filled with 0.8 L of primary sludge and 0.8 L of distilled water.

Chemical analyses

Sludge from batch reactors was sampled weekly to measure the total COD (tCOD), sCOD, NH$_3$, protein, carbohydrate, TS and VS concentrations in duplicate. Prior to sampling, the flasks were mixed by hand using a stern orbital motion, and samples were withdrawn using a modified wide-mouth 10 mL pipette inserted through the sampling port on the rubber stopper. The reactors could not be opened in order to maintain anaerobic conditions, and difficulties were experienced in taking uniform samples with the pipette. Some of the samples were homogenised with a mortar and pestle and diluted if necessary, depending on the test.

Ammonia and COD concentrations were measured using HACH methods 10031 and 8000, respectively, with a HACH DR2800 spectrophotometer (HACH Company, Loveland, CO, USA). Samples for sCOD were centrifuged at 3,400 rpm [relative centrifugal force (RCF) 1,200g] for 10 min prior to being filtered through a washed 0.45 μm retention cellulose nitrate membrane filter (Whatman Ltd, Piscataway, NJ, USA). Soluble protein concentrations were determined using a Coomassie Brilliant Blue G-250 reagent (Pierce Biotechnology Inc., Rockford, IL, USA) with the Bradford method [14]. A colour response curve (wavelength 595 nm) was generated using bovine serum albumin at concentrations from 0 to 1 g/L (Pierce Biotechnology Inc., Rockford, IL, USA). Carbohydrate concentrations were determined using the Anthrone method [15]. A colour response curve (wavelength 620 nm) was

Fig. 1 Batch reactor setup used for (**a**) micro-aeration reactors with raw and diluted primary sludge and (**b**) the control reactors with raw and diluted primary sludge
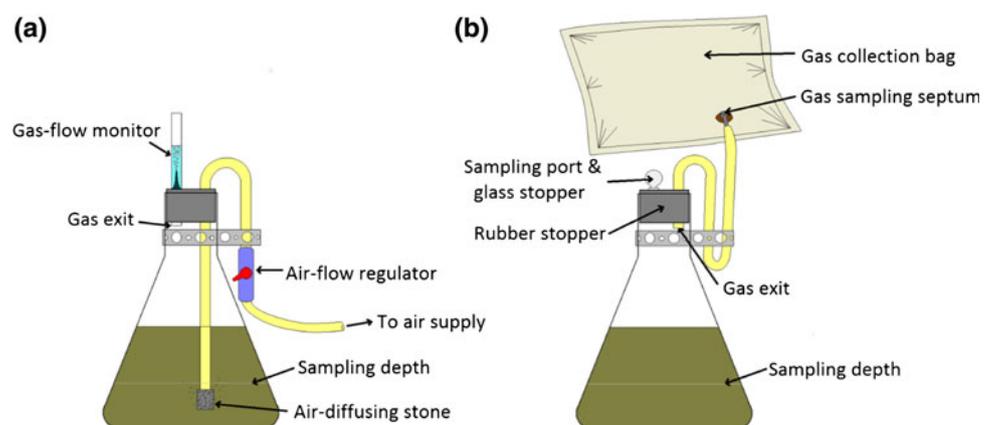
generated using galactose powder (Acros Organics, Fair Lawn, NJ, USA) dissolved in distilled water to concentrations from 0 to 200 mg/L. TS and VS concentrations were measured using standard methods 2540 B and 2540 E, respectively [16].

Statistical analyses

All measurements were carried out in duplicate for each duplicate reactor. The data points in the figures show the average and the error bars show one standard deviation. Two-tailed t test was used to determine the p values and statistical significance ($p < 0.05$) of results.

Results and discussion

During the initial stages of the batch reactor operation, a typical solubilisation period occurred, at which time bacterial populations acclimatised and the system approached anaerobic conditions. Primary sludge is readily degradable, and as a result, dramatic changes were observed in the early stages of reactor operation. The following sections present and discuss the trends in the solids concentrations, the COD, proteins, carbohydrates and ammonia.

Effect of micro-aeration on total and volatile solids

Figure 2 shows the TS and VS of raw sludge samples during batch reactor operation. The profiles for the micro-aeration and control reactors show almost identical trends throughout the testing period. Within the first 8 days of reactor operation, there was a large degree of solubilisation, in which the TS and VS decreased by 26 and 31 % in the micro-aeration reactors and by 30 and 35 % in the control reactors, respectively. Profiles of the sCOD, which are discussed in more detail later, also show an increase during this period. Following the initial solubilisation period, there was very little decrease in TS and VS for all reactors, and after 26 days, the total VS reductions were 35 % in the micro-aeration reactors and 37 % in the control reactors. This suggests that the micro-aeration treatments on raw sludge samples did not have any significant impact on the degradation of TS and VS relative to the control reactors ($p > 0.05$). Micro-aeration experiments by Jenicek et al. [10], using 10 L bench-scale anaerobic digesters, also reported no difference in TSS and VSS reductions relative to the control reactors.

Micro-aeration treatments on diluted sludge seemed to have delayed the solubilisation of organic solids compared to the control reactors (Fig. 3). Furthermore, the VS/TS ratio of the micro-aerobic reactors was slightly higher than the control reactors on days 8 and 16. This suggests an
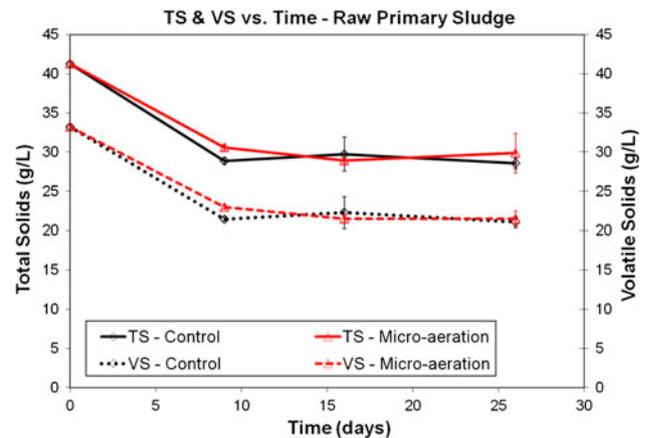

Fig. 2 Profiles of the total and volatile solids concentrations during the batch reactor tests with raw primary sludge treated with micro-aeration (TS: $p = 0.371$, VS: $p = 0.486$)
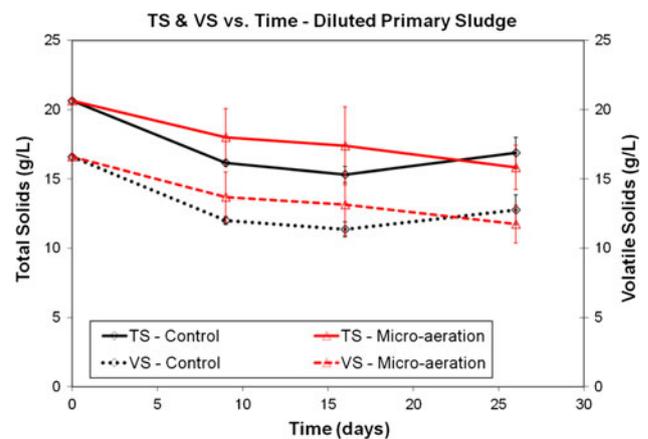

Fig. 3 Profiles of the total and volatile solids concentrations during the batch reactor tests with diluted primary sludge treated with micro-aeration (TS: $p = 0.333$, VS: $p = 0.283$)

increase in biomass content due to cellular activities which may have been stimulated by the micro-aerobic conditions. However on day 26, VS reductions were 29 % in the micro-aeration reactors, and 23 % in the control reactors, with no significant difference among reactors ($p > 0.05$). This indicates that the micro-aeration treatments did not cause a significant increase in biomass, which is often linked with aerobic treatments. It may also be that the small amount of excess biomass produced as a result of the micro-aerobic environment was offset by improved enzymatic hydrolysis, as observed in the micro-aeration experiments by Johansen and Bakke [3].

Effect of micro-aeration on COD

Micro-aeration treatments on raw primary sludge resulted in a 40 % reduction in tCOD after 39 days of batch reactor
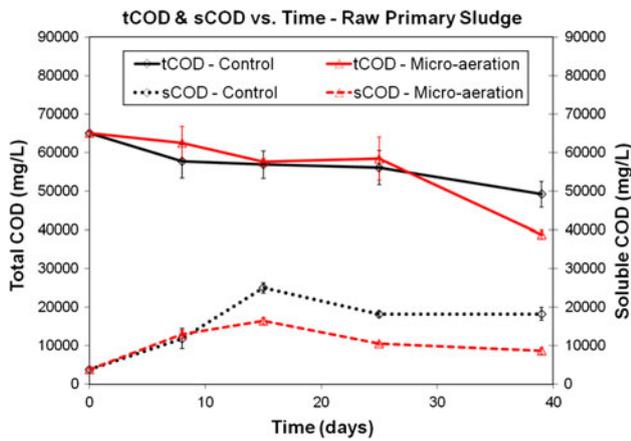
Fig. 4 Profiles of the total COD (tCOD: *solid lines*) and soluble COD (sCOD: *dashed/dotted lines*) during the batch reactor tests with raw primary sludge treated with micro-aeration (tCOD: $p = 0.00535$, sCOD: $p = 0.00029$)

Fig. 5 Profiles of the total COD (tCOD: *solids lines*) and soluble COD (sCOD: *dashed/dotted lines*) during the batch reactor tests with diluted primary sludge treated with micro-aeration (tCOD: $p = 0.00151$, sCOD: $p = 0.0000013$)

operation, whereas the tCOD of the control reactors decreased by only 23 % (Fig. 4).

During the first week, the sCOD of the micro-aeration and the control reactors increased to around 12,500 mg/L, which coincided with the reduction in TS and VS (Fig. 2) as discussed earlier. After 2 weeks, maximum sCOD was recorded in all reactors; however, the maximum sCOD of the control reactors was 25,000 mg/L, whereas the maximum sCOD of the micro-aerobic reactors was only 16,000 mg/L. In the following weeks, the sCOD of all reactors decreased, and by day 39, the sCOD of the control reactors was 18,000 mg/L, whereas the sCOD of the micro-aeration reactors was less than 9,000 mg/L. Similar trends were observed in the 10 L bench-scale anaerobic digestion experiments by Jenicek et al. [10], who reported lower sCOD concentrations using micro-aeration compared to the conventional anaerobic digester, despite no differences in the reduction in TSS and VSS.

The COD profiles of the micro-aerobic reactors with diluted sludge follow very similar trends as the micro-aerobic reactors with the raw sludge samples. The micro-aeration treatments on diluted sludge samples resulted in a tCOD reduction of approximately 40 % by the end of the experiments, whereas the tCOD of the control reactors decreased by only 20 % (Fig. 5). Diluted sludge samples with lower initial TS and VS concentrations were used to represent sludge samples with a lower biomass concentration and substrate load. Although a lower initial solids concentration did not have an effect on the overall percent reduction in tCOD after 39 days, micro-aeration of diluted sludge samples seemed to have prolonged the anaerobic acclimatisation period during the first week. The percent reductions in TS, VS and tCOD during the first week were all lower for the reactors with diluted sludge (initial TS 20.5 g/L). Conversely, batch reactor experiments by Díaz
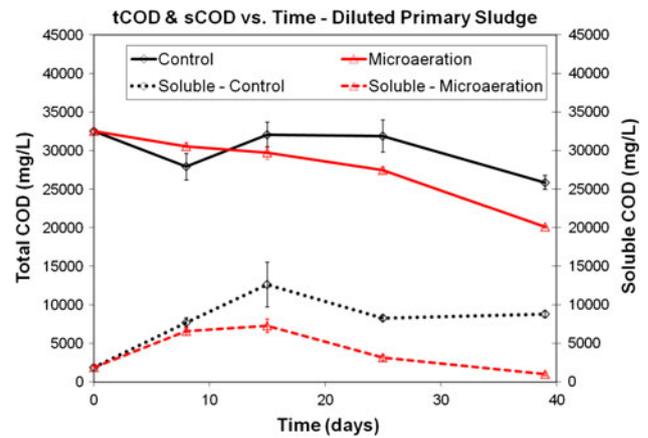
et al. [9] showed that micro-aeration reduced the time required to achieve maximum methane production for the anaerobic digestion of cellulose, using biomass from a full-scale anaerobic digester.

During the initial acclimatisation period, the degradation of tCOD was very small, while at the same time, the generation of sCOD was quite high. During the first week, the sCOD of reactors with diluted sludge increased from 1,950 to 6,600 mg/L for the micro-aeration reactors, and from 1,950 to 7,700 mg/L for the control reactors, with no significant difference among the reactors. Similar to the experiments with raw sludge, micro-aeration treatments reduced the maximum sCOD concentration measured on day 15. Maximum sCOD of the control reactors was 12,700 mg/L, whereas the maximum sCOD of the micro-aerobic reactors was only 7,300 mg/L. After day 15, the sCOD of the reactors decreased, and after 39 days, the sCOD concentration of the control reactors was 8,800 mg/L, whereas the sCOD concentration of the micro-aerobic reactors was only 1,000 mg/L. As was seen in the experiments with raw sludge samples, micro-aeration significantly improved the degradation of sCOD during batch reactor experiments with diluted sludge samples. Pirt and Lee [17] suggested that micro-aeration of algae samples in batch reactors enhanced the growth of facultative anaerobes, which could maintain a low redox potential and provide better growth conditions for the sensitive anaerobes, namely the methanogens. This could explain why micro-aeration improved the reduction of COD during batch reactor experiments with raw sludge and diluted sludge samples.

Effect of micro-aeration on protein concentrations

Proteins represent roughly 50 % of the dry weight of cells and are the most structurally complex macromolecules

known [18]. Protein concentration measurements provide an indication of the microbial activity and level of digestion. During digestion, soluble proteins are released by bacteria and eventually degraded through microbial and enzymatic activities. Proteins contain large amounts of nitrogen containing compounds, which will contribute to the ammonia levels when degraded anaerobically.

During the first 13 days of reactor operation, the soluble protein concentrations of all reactors with raw sludge increased from 2,500 mg/L to well above 25,000 mg/L (Fig. 6); however, the micro-aeration treatments resulted in roughly 15 % more soluble protein generation than the control reactors during this period. The increase in protein concentration within the first 13 days coincides with the reduction in TS and VS, which was also observed in the experiments by Park et al. [4]. From day 13 to 30, soluble protein concentration of the control reactors varied between 27,000 and 28,000 mg/L, whereas the soluble protein concentration of the micro-aeration reactors increased slightly more, from 31,000 to 35,000 mg/L. Experiments by Zhu et al. [8] also reported that sufficient micro-aeration rates improved the hydrolysis of proteins during anaerobic digestion of vegetable and flower waste.

Soluble protein concentration profiles for the batch reactor tests with diluted sludge show similar trends as the tests with raw sludge (Fig. 6). During the first 13 days of reactor operation, protein concentrations of the diluted sludge increased from 1,300 to 11,200 mg/L in the control reactors, and 17,600 mg/L in the micro-aeration reactors. Similar to the tests with raw sludge, micro-aeration resulted in a greater increase in the soluble protein concentrations during the first 13 days of reactor operation and maintained between 17 and 43 % higher protein concentrations from day 13 to the end of the batch reactor experiments.

Effect of micro-aeration on carbohydrate concentrations

Carbohydrates are food for the microorganisms present in the sludge. They exist in simplest form as sugars and in complex forms such as starch and cellulose. A measurement of the carbohydrates concentration helps to assess the microbial conditions and the stage of digestion.

Figure 7 shows the profiles of the carbohydrate concentrations during batch reactor tests with raw and diluted sludge samples. Micro-aeration of raw sludge did not show any significant differences in the consumption of carbohydrates relative to the control reactors ($p > 0.05$). Within the first 2 weeks of reactor operation, the carbohydrate concentrations of all reactors with raw sludge decreased to a baseline concentration between 2,000 and 2,500 mg/L. The micro-aeration reactors maintained slightly higher carbohydrate concentrations than the control reactors, although the difference was quite minimal.

Batch reactor experiments with diluted sludge show almost identical trends as the samples with raw sludge, with no significant difference among the reactors. The baseline carbohydrate concentrations of the diluted sludge samples were between 700 and 1,100 mg/L, which is roughly half the baseline concentration of the raw sludge reactors.

Effect of micro-aeration on ammonia concentrations

Ammonia measurements on the sludge samples were used to evaluate the degradation of nitrogen containing compounds, such as proteins and urea, in the sludge samples, and also to monitor the anaerobic acclimatisation period.

Micro-aeration helped improve the degradation of nitrogen containing compounds and the generation of
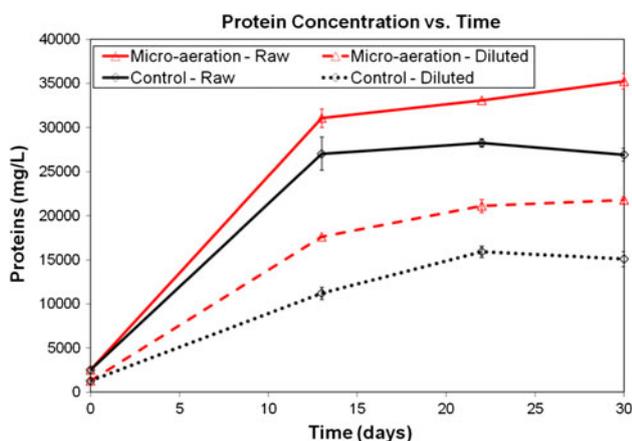

Fig. 6 Profiles of the protein concentrations during the batch reactor tests with raw and diluted primary sludge treated with micro-aeration (raw: $p = 0.00000845$, diluted: $p = 0.0000983$)
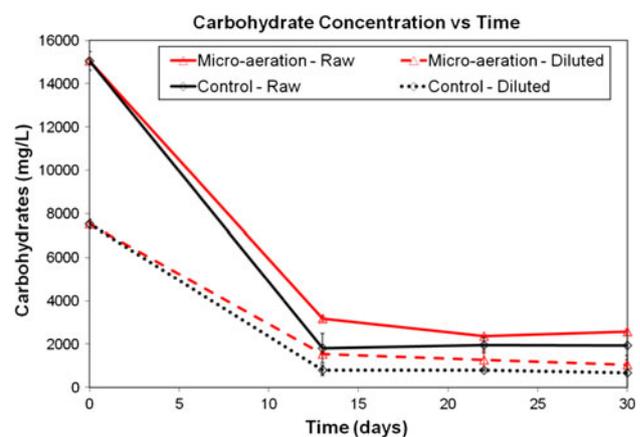

Fig. 7 Profiles of the carbohydrate concentrations during the batch reactor tests with raw and diluted primary sludge treated with micro-aeration (raw: $p = 0.1465$, diluted: $p = 0.4137$)
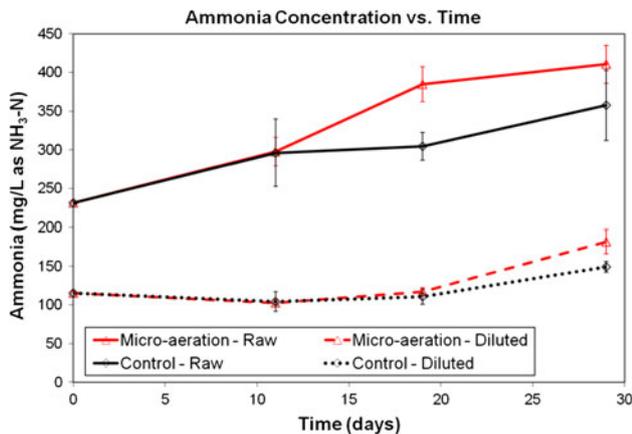
Fig. 8 Profiles of the ammonia concentrations during the batch reactor tests with raw and diluted primary sludge treated with micro-aeration (raw: $p = 0.0986$, diluted: $p = 0.0192$)

ammonia in the raw and the diluted sludge samples; however, micro-aeration did not increase the initial generation of ammonia within the first 13 days (Fig. 8). The ammonia concentration in all reactors with raw sludge increased from 230 to 300 mg/L, regardless of the micro-aeration treatments. The increase in ammonia during the early stages of reactor operation coincided with the initial solubilisation period whereby the TS and VS concentrations decreased and the sCOD and protein concentrations increased. Similar trends were observed in the experiments by Park et al. [4], as discussed earlier. Although micro-aeration did not improve the ammonia generation during the first 13 days, by day 19, the micro-aerobic reactors with raw sludge had 25 % higher ammonia concentrations than the control reactors. On day 29, however, the ammonia concentration of the micro-aerobic reactors with raw sludge was 410 mg/L and the ammonia concentration of the control reactors was 360 mg/L, with no significant difference among the reactors ($p > 0.05$).

During batch reactor tests with diluted sludge samples, the ammonia concentrations of the micro-aerobic and the control reactors stayed between 100 and 120 mg/L from day 0 until day 19, with no significant difference among the reactors. This suggests that a lower initial TS and VS concentration delayed the increase in ammonia that was seen in the early stages of batch reactor operation with raw sludge samples. The ammonia concentration of micro-aerobic reactors with diluted sludge were almost identical to the control reactors up to day 19, however, from day 19 to 29, the ammonia concentrations increased from 110 to 150 mg/L in the control reactors, and from 120 to 180 mg/L in the micro-aerobic reactors. This suggests that micro-aeration ultimately improved the degradation of nitrogen containing compounds, causing a greater increase in ammonia ($p < 0.05$).

Conclusions

The purpose of this study was to investigate and compare the effects of micro-aeration on anaerobic digestion of primary sludge under septic tank conditions. The research intended to see if micro-aeration increases solubilisation of organics and enhances digestion rates that eventually would maximise the pump-out intervals using a relatively simple technology that does not require overly expensive or complex modifications to septic tanks.

Micro-aeration rates as low as 0.00156 vvm effectively solubilised the COD of the system and subsequently improved the degradation of COD. Micro-aeration also increased the generation of soluble proteins and ammonia, but did not have an effect on the degradation of carbohydrates or the reductions in TS and VS, which occurred predominantly within the first 8 days of reactor operation. Batch reactor experiments with diluted sludge samples, representing sludge with a lower initial solids concentration, generally followed the same trends observed from tests with raw sludge samples.

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