Digestion of wastewater pond microalgae and inhibition from ammonium and alum

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Abstract Algae are produced in considerable quantities in oxidation ponds, and may negatively affect receiving waters when discharged at high concentration Thus in some instances they requiring removal prior to effluent discharge using flocculants such as alum. Anaerobic digestion of this algae biomass can enable some energy recovery to off-set the costs of algae removal. Alum however has been reported to inhibit anaerobic digestion. Anaerobic digestion experiments with as little as 200 ppm alum in the flocculated algae caused some reduction in methane content, increasing to about 40% reduction at an alum concentration of 1600 ppm. Elevated ammonia levels (>261 g m^{-3} NH_4^+-N) also inhibited algal digestion using an inoculum of anaerobic bacteria from a municipal wastewater sludge digester. However, anaerobic bacteria from piggery anaerobic ponds (in which typical ammoniacal-N levels range between 200 and 2000 g m^{-3} NH_4^+-N) were unaffected by elevated digester ammonia levels and methane production actually increased slightly at higher ammonia concentrations. Thus, selecting anaerobic bacteria inocula that are already adapted to high ammoniacal-N levels, such as are present in anaerobic ponds treating piggery wastewater, may avoid ammonia inhibition of algal digestion.

Keywords Algae; alum; ammonia; digestion; inhibition; wastewater pond

INTRODUCTION

Algae are ubiquitous in aquatic environments wherever sunlight and even trace concentrations of nutrients are present. They invariably occur in wastewater ponds or water supply reservoirs. In the former they are generally beneficial to treatment processes, while in the later, they are more often a nuisance requiring some degree of removal. Indeed, even in the discharge of wastewater ponds they may be considered a nuisance when discharge at high effluent concentrations to small, low flow receiving waters, and often removal of algal biomass prior to discharge from wastewater ponds is required. Removal can be via a range of processes such as settling, filtration, centrifugation and dissolved air floatation, and often requires chemical flocculation with chemicals such as alum, ferric chloride or lime (Oswald and Golueke, 1960). Each of these processes results in a concentrated "algal soup", frequently ranging from 1-10% suspended solids. Where this removal has been undertaken, municipal authorities are then faced with the problem with what to do with the algae. Various uses have been suggested for this material, including fertiliser, animal feed supplement (Becker, 1988), or as an energy source via digestion (Chen, 1987; Oswald and Golueke, 1960). Often, the algal material is disposed of into landfills at considerable cost, or simply recirculated back into the ponds (with resulting increased sludge accumulation and nutrient release). Anaerobic digestion of algae biomass to methane gas has the potential to produce energy from the algae biomass prior to disposal or fertiliser use.

Anaerobic digestion is a two-step process, beginning with an "acid formation" step in which complex organic substrates (carbohydrates, fats and proteins) are converted into organic acids, carbon dioxide and water. This is followed by a "methane formation" step by cleavage of acetic acid into methane and carbon dioxide, and reduction of carbon dioxide in the presence of hydrogen gas into methane and water (USEPA, 1979). The acid forming microorganisms are fast growing (typical doubling time of hours) and are generally considered tolerant of a wide range of environmental conditions. The methane formers (methanogens), are much slower growing (typically requiring solids retention times of 4-10 days within a digester, USEPA, 1979), and are sensitive to changes in pH, temperature, substrate loading and the presence of various compounds. Investigations into the potential to anaerobically digest algae began in the late 1950s. However there is conflicting information in the literature regarding the inhibition of algal digestion due to the presence of alum flocculant. Golueke et al (1957) found that alum flocculant (at Al concentrations of 4% total solids) had no inhibitory effect on algal digester activity, but later research indicated that alum and other coagulants such as ferric chloride did inhibit algal anaerobic digestion to some degree (Dentel and Gossett, 1982; USEPA, 1979). Suggested causes of alum inhibition of anaerobic digestion include the locking of substrates or proteins (Rudolfs and Settler, 1931), or immobilisation of phosphate (Pfeffer and White, 1964). Anaerobic digestion of organic matter with a high nitrogen content (low C:N ratio) may be particularly susceptible to ammonia toxicity due to accumulation of high ammonia concentrations in the digesters as carbon becomes depleted (USEPA, 1979). In contrast, pig rearing operation wastes are commonly treated in anaerobic ponds where ammonium concentrations range between 200 and 2000 g m^{-3} NH_4^+-N (Sukias and Tanner, 2005), where vigorous release of biogas is generally apparent. The long hydraulic retention time of anaerobic ponds compared to anaerobic digesters may allow the bacterial population to adapt to the higher concentrations of ammonia to enable equivalent rates of digestion and methane production to those found in digesters with low ammonia levels.

In this study we examined the influence of elevated alum and ammonium concentrations on anaerobic digestion of algal biomass in a series of separate experiments.

METHODS

Algal biomass was digested in un-mixed laboratory-scale anaerobic digesters (1 or 2 L glass vessels) that were kept in a constant temperature room at 20°C (psychrophillic temperature range). In each experiment, the digesters were operated in "batch mode", with addition of algae and anaerobic bacteria inoculum at the start of the experiment.

Algae used in the experiments were collected from two sources: an experimental High Rate Algal Pond (HRAP) (Ruakura Research Centre, Hamilton, New Zealand) and Waihi Municipal Sewage Treatment Ponds (Waihi, New Zealand), both of which received domestic sewage with little or no industrial inputs. Algae from the Ruakura HRAP were collected from gravity settling cones (settled algae) (see Tanner et al., 2005 for a detailed description). Algae from the Waihi oxidation ponds were collected from the foam discharged from an induced air floatation system using alum (6 g m^{-3}) as a flocculant (alum flocculated algae), resulting in a sludge with an Al concentration of 130 g m^{-3}.

The algal digesters were inoculated with anaerobic bacteria from a mesophilic anaerobic digester at the Hamilton municipal sewage treatment plant (mesophillic anaerobic bacteria inoculum), except in the ammonia inhibition experiment when anaerobic bacteria from the discharge of an anaerobic pond treating wastewater from a commercial pig production facility was used in half of the digesters (piggery anaerobic bacteria inoculum).

The organic matter in the influent (algae and bacterial inoculums) of the digesters was measured as total and volatile solids by standard methods (APHA, 1998, methods 2540 B&E). Where the efficacy of different bacterial inoculums was compared, the total solids content of the inoculums was equalised (by diluting the inoculum with higher solids content with deionised water which had been deoxygenated by bubbling with nitrogen gas) prior to addition to the digesters.

The volume of biogas produced within each digester was monitored using inverted measuring cylinders in a jar filled with high salinity water (close to saturation) of a similar depth to the cylinder. Biogas produced in the digesters was transferred through silicone tubing to the (now inverted) base of the cylinder, enabling both gas collection and measurement of the gas volume. Biogas samples were withdrawn from the cylinder through a rubber septum in the base of the cylinder for gas composition analysis using a portable gas analyser (GA2000 plus, Geotechnical Instruments (UK) Ltd, Leamington Spa, England). The gas analyser was calibrated weekly against a certified standard methane/CO_2 gas. Gas production and composition was monitored daily at the beginning of each experiment, when gas production was high. After the first 2 weeks, gas production was monitored less frequently, depending on the production rate. Each experiment was halted when production levels had reduced to an extent that continued analysis was impractical (around 20 mL per day).

Digestion of alum flocculated algae

This experiment was designed to determine the optimal ratio of alum flocculated algae to bacterial inoculum required for efficient digestion. Six two-litre digesters were filled with alum flocculated algae and mesophyllic anaerobic bacteria inoculum in varying amounts to give a total volume added of two litres (Table 1). Prior to combining the two liquids, some deoxygenated deionised water was added to the inoculum so that it had the same concentration of total solids as the algal solution, however as the VS content of the bacteria inoculum was higher than that of the alum flocculated algae, the six digesters still showed some difference in organic (VS) loadings (Table 1).

Table 1. Loading rates to each digester.

Digester Algae: Inoculum Ratio	1 0:100	2 20:80	3 40:60	4 60:40	5 80:20	6 100:0
Volume of alum flocculated algae (L)	0	0.4	0.8	1.2	1.6	2.0
Volume of inoculum (L)	2.0	1.6	1.2	0.8	0.4	0
Organic Loading (kg VS_A m^{-3})*	6.56	6.38	5.44	5.30	5.27	5.00
Al concentration (g m^{-3})	0	26	52	78	104	130

VS_A = volatile solids added.

Inhibition of digestion with separate addition of alum

Based on the results of the digester experiments using alum flocculated algae, this experiment was designed to determine the influence of alum concentration on algal digestion. Five two litre digesters were filled with settled algae and mesophyllic anaerobic bacteria inoculum in the optimal ratio for rapid digestion determined by the previous experiment (40% algae to 60% inoculum) to give a total volume added of two litres. Total VSA was 10.3 kg m^{-3} to each digester due to higher algal solids collected within the settling cones,. Each digester received a dose of alum

($Al_2(SO_4)_3.16H_2O$) to give a final concentration of 0, 200, 400, 800 and 1600 ppm of alum (0, 16, 32, 65 and 130 g m^{-3} Al). A sixth digester only contained mesophyllic anaerobic bacteria inoculum without alum and with de-oxygenated water in place of the algae, giving a VS_A of 6.5 kg m^{-3} for this control treatment.

Inhibition of digestion with addition of ammonium

This experiment was designed to determine the influence of ammonia concentration on digestion of algae using different anaerobic bacteria inocula. Eight one litre digesters were filled with 900 mL of settled algae and 100 mL of anaerobic bacteria inoculum from either a mesophyllic anaerobic digester (4 digesters, loading 2.51 kg VS m^{-3}) or a piggery anaerobic pond (loading 2.43 kg VS m^{-3}). Ammonium chloride was added to give a final concentration of 0, 0.5, 1.0 and 3.0 g L^{-1} (0, 131, 261, and 785 g m^{-3} as NH_4^+-N). This experiment was undertaken 3 times.

RESULTS AND DISCUSSION

Digestion of alum flocculated algae

Results from the digestion of alum flocculated algae are given in Table 2. Anaerobic digestion of alum flocculated algae occurred even without addition of a bacterial inoculum. Methane production rates (0.224 – 0.399 m^3 CH_4 kg^{-1} VS_A) increased in association with an increasing proportion of algal solids added (Table 1). A decrease in production was apparent where no bacterial inoculum was added (100% algae). These results indicate that the concentration of alum in alum-flocculated algae does not cause complete inhibition of algal digestion and VS may not reliably predict digestibility as seen with the bacterial inoculum.

Table 2. Effect of varying ratios of sewage inoculum and alum-settled algae.

Digester	1	2	3	4	5	6
Algae: Inoculum Ratio	0:100	20:80	40:60	60:40	80:20	100:0
Average % Methane (± 1 s.d.)	72.1 ± 9.6%	80.6 ± 3.9 %	81.1 ± 4.2 %	79.3 ± 3.4 %	75.8 ± 5.9 %	69.7 ± 10.0 %
% Carbon Dioxide (± 1 s.d.)	9.4 ± 2.1 %	14.1 ± 3.7 %	14.1 ± 2.2 %	15.5 ± 2.2 %	19.3 ± 2.3 %	18.6 ± 3.6 %
Methane production per VS Added (m^3 CH_4 kg^{-1} VS_A)	0.125	0.224	0.288	0.328	0.399	0.306

Cumulative methane production of each of the digesters is shown in Figure 1. For algal:inoculum ratios up to 40:60, cumulative methane production and initial methane production rates both increased with increasing proportion of algae added. At algal:inoculum ratios of 60:40 and above, cumulative methane production continued to increase, probably as a result of higher algae VS loading, however, there was an increasing time lag before maximum methane production rates were achieved. This time lag could be either due to alum inhibition of anaerobic digestion, or to the time required for an effective methanogenic bacteria population to establish in the digester. The effect is most extreme in the 100% algae treatment, which took nearly 140 days to “catch-up” to the cumulative methane production of the 80% algae treatment.

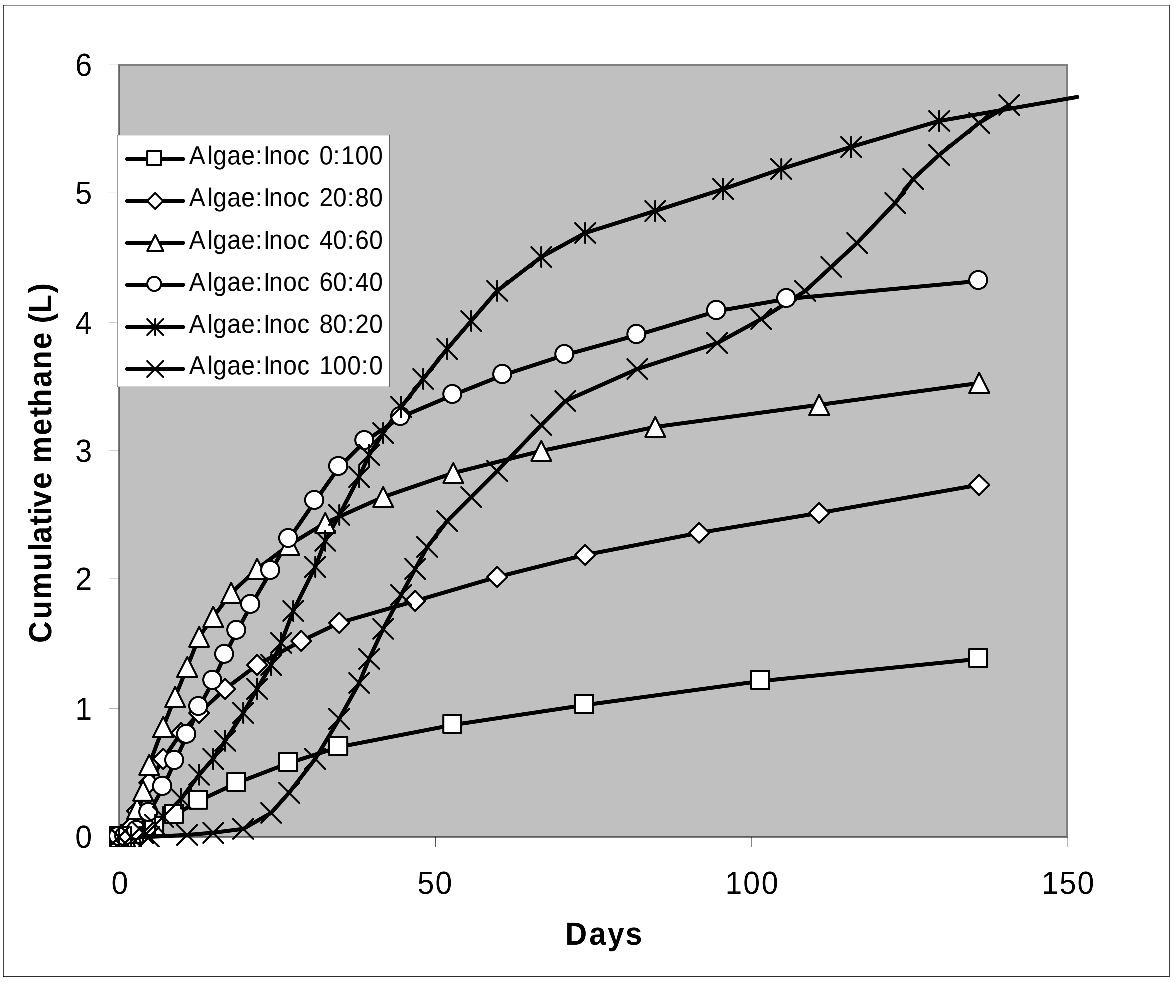

Figure 1. Cumulative methane production in relation to the ratio of alum-settled algae to mesophyllic bacteria inoculum added to the digesters.

Inhibition of digestion with separate addition of alum

This experiment was designed to determine the influence of alum concentration on algal digestion while eliminating the binding associated with alum flocculated algae. Physical enmeshment within alum flocs was considered an important factor limiting floc digestion by Dentel and Gossett (1982) which could otherwise have resulted in higher overall inhibition than that observed here. Additionally, we maintained the biomass of algae/inoculum constant across all treatments so effects of different VS_A are eliminated. Results are given in Table 3 and show that even low alum concentrations (e.g. 200 ppm) had some inhibitory effect on methane production per VS_A as well as the proportion of methane in the biogas produced compared to the untreated control. Total production was, however somewhat lower than in the previous experiment, where 0.288 m^3 CH_4 kg^{-1} VS_A was produced in the 40:60 treatment (with equivalent ratios to used here). This however is likely to be partly due to the reduced time that this experiment had been operating (101 days at the time of writing). However, even at an alum concentration of 1600 ppm (130 g m^{-3} Al), methanogenesis continued, with production per VS_A inhibited to 58% of that without alum addition.

Table 3. Effect of alum concentration upon the digestion of settled algae.

Alum concentration	0 ppm	200 ppm	400 ppm	800 ppm	1600 ppm	0 ppm No Algae added
Average % Methane (± 1 s.d.)	62.6 ± 6.4 %	60.1 ± 7.2 %	55.8 ± 11.6 %	52.9 ± 9.4 %	49.6 ± 7.8 %	35.5 ± 14.3 %
% Carbon Dioxide (± 1 s.d.)	25.7 ± 4.4 %	28.0 ± 4.3 %	23.1 ± 3.1 %	26.8 ± 4.0 %	33.4 ± 2.8 %	9.5 ± 3.7 %
Methane production per VS added (m^3 CH_4 kg^{-1} VS_A)	0.191	0.167	0.159	0.138	0.111	0.057

Inhibition with ammonium

The results of the experiment to determine the influence of ammonia concentration on digestion of algae using different anaerobic bacteria inocula are shown in Table 4. The anaerobic digesters using a bacterial inoculum from a piggery anaerobic pond (ammonia adapted inoculum) had higher methane production per kg of VS_A at higher ammonia concentrations (1.0 g/L and 3.0 g/L) than those digesters with the sewage mesophyllic digester inoculum. The higher methane production per kg of VS_A at lower ammonia concentrations (0 g/L and 0.5 g/L) in the digesters with the sewage mesophyllic digester inoculum indicates the bacteria were operating close to their optimum. Lower production and % methane in the biogas of the treatments with high doses of ammonium are both indicators that methanogenesis was being inhibited within these digesters.

These results indicate that the piggery bacterial inoculum was more adapted to high ammonia concentrations than the mesophyllic bacterial inoculum, and potentially could be used as seed material where high ammonia concentrations are expected within a digester.

Table 4. Effect of added ammonium upon methane production in anaerobic digesters inoculated from piggery or sewage anaerobic digestion systems.

Treatment	Piggery				Sewage			
NH_4Cl added	0 g m^{-3}	500 g m^{-3}	1000 g m^{-3}	3000 g m^{-3}	0 g m^{-3}	500 g m^{-3}	1000 g m^{-3}	3000 g m^{-3}
Average % Methane	79.8%	78.7%	82.1%	80.9%	82.2%	84.3%	70.4%	72.7%
% Methane (SD)	3.5%	3.2%	2.6%	2.9%	6.0%	4.5%	9.9%	9.9%
% Carbon Dioxide	7.4%	6.7%	8.6%	9.9%	9.0%	10.3%	11.3%	8.0%
% Carbon Dioxide (SD)	1.7%	1.6%	1.6%	1.9%	1.6%	2.8%	3.5%	1.2%
Methane production per VS Added (m^3 CH_4 kg^{-1} VS_A)	0.540	0.552	0.582	0.575	0.655	0.650	0.539	0.435

CONCLUSIONS

Alum flocculated algae was able to be anaerobically digested, and total methane production increased with the amount of VS_A. The greater lag in methane production in digesters with a high initial algae:inoculum ratio could be either due to alum inhibition or the time required for an effective methanogenic bacteria population to develop.

Adding alum directly to the digesters with settled algae and bacterial inoculum showed that methane production declined with increasing alum concentrations, with up to 40% reduction found at rates present in an alum flocculated algae. This appeared to be due to a direct inhibitory effect from the aluminium.

Algal digestion using a piggery bacterial inoculum was less inhibited at high ammonia concentrations (1.0 g/L and 3.0 g/L) than digestion using sewage mesophyllic digester inoculum, suggesting that anaerobic bacteria from piggery ponds are more adapted to elevated ammonia levels and could be a more suitable inoculum for algal digestion.

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