ENHANCED ANAEROBIC DIGESTION AS A SANITATION AND ENERGY RECOVERY TECHNOLOGY

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ABSTRACT

In this research, the potential of using an enhanced anaerobic digestion (AD) process as a sanitation and energy recovery technology for communities that lack access to basic sanitation is evaluated. For the enhanced AD system to generate a reliable supply of biogas, so that it can be adopted and self-sustained by the community, the use of algal biomass as a supplementary feedstock to the system is evaluated. In addition, the effects of operational parameters on waste mineralization and biogas production is investigated. The results show that the enhanced AD system has the potential to be developed into an effective waste treatment technology, and it has produced high energy yields and digested waste low in faecal bacteria and high in nutrients. Reductions of 42 to 51% in volatile solids (VS) and 29 to 45% in chemical oxygen demand (COD) were achieved at 35°C. On average, total coliform (TC) and faecal coliform (FC) concentrations of $7.6x10^5$ and $1.4x10^4$ CFU per gram of total solids (TS), respectively, were measured in the digested waste. Thus, the residual meets the EPA requirements for pathogen reduction (FC < $2x10^6$ CFU/g TS) and vector attraction reduction (> 38% reduction in VS) for land application. The total nitrogen and phosphorus content of the residual was determined to be in the range of 9-17% as N and 3-7% as P (7-16% as P_2O_5), respectively. The biogas yields varied in the range of 470-570 L per kg of VS digested.

Key words: ANAEROBIC DIGESTION, ALGAE, RESOURCE RECOVERY, SANITATION, WASTE TREATMENT

INTRODUCTION

2.6 billion people in developing countries – 42% of the world's population – lack access to basic sanitation facilities (WHO and UNICEF 2005). In these countries, human waste is released into the environment without treatment, polluting potable water supplies. As a consequence, 2.2 million people, mostly children, die every year from diseases contracted through direct and indirect contact with pathogenic microorganisms found in human excreta (WHO and UNICEF 2000). Even for those who have access, mostly urban populations, small bores, septic tanks, pour-flushes, latrines and simple pits sanitation systems are the main methods of capturing, containing and "treating" human excreta. 63, 51, 45 and 68% of the urban population in Africa, Asia, Latin America & Caribbean and Oceania, respectively, are served by these types sanitation systems (WHO and UNICEF 2000). These sanitation systems fail to kill pathogenic microorganisms (NSW Government 1998), contaminate drinking water supplies (Dzwairo et al. 2006), serve as breeding grounds for insects (Owusu 2010), and generate noxious odours. Moreover, resource recovery – biogas and biosolids – is very difficult, if not impossible, with these types of sanitation systems.

Technologies that can be used to treat waste reliably, inexpensively and sustainably must be developed to address these challenges. The technologies should be built from locally available resources, operated and managed by individuals with minimal training, and not require complex monitoring equipment. In this research, we propose a novel enhancement to an existing technology to contribute to the solution of sanitation in

developing countries. The idea is to enhance and adapt an anaerobic digestion (AD) system that will treat waste and generate a reliable supply of biogas from the co-digestion of algal biomass and waste, providing an incentive for a community to properly operate and maintain the enhanced AD system. An additional benefit is the recovery of biosolids, residual solids formed after co-digestion, as fertilizer.

AD has been used for decades in developed countries for treatment of waste sludge and high-strength industrial wastewaters. The process is operated under controlled conditions, such as temperature, sludge loading rate, solids retention time, alkalinity, and pH. It achieves a high level of waste mineralization, eliminates odours and other nuisances, and meets the requirement for faecal coliform levels of less than $2x10^6$ CFU/ g of total solids for land application of biosolids (USEPA 1999a). It can also be used to recover energy, in the form of biogas, from sludge at large wastewater treatment plants (WWTPs) where large quantities of sludge are produced. At small WWTPs, the amount of biogas is not high enough to make energy recovery practical.

The conventional AD system is impractical in developing countries because of the capital and operating & maintenance costs and the lack of skilled personnel to operate the system. Moreover, the low recoverable energy content of human excreta will not provide enough incentive for a community to adopt and self-sustain the system. The COD, a measure of the organic content, of excreta for people in developing countries is about 90 g/capita/day (USEPA 1999b). On the basis of this value, the excreta of one person could produce about 50 L of biogas/day (0.30 kWh/day), if anaerobically digested at 25°C. This represents about 5% of the cooking energy demand for a household of 5 to 6 people, which is estimated at 1000 L of biogas/day (GTZ 2010).

In this research, we propose a novel enhancement of AD that addresses these limitations. First, the potential of algal biomass as a supplementary feedstock to generate a reliable supply of biogas is evaluated. Second, the effects of operational parameters for the enhanced AD system pertaining to developing countries are investigated. Operational parameters are critical to the performance of the system and can significantly influence the rates of waste mineralization, pathogenic inactivation and the quantity of biogas generated.

METHODS

Experimental Approach

To validate the concept, lab-scale anaerobic digesters were set-up using 250 mL glass bottles. Thickened waste activated sludge (TWAS) was used as a representative "human waste" while *Chlorella vulgaris* (*C. vulgaris*) was used as a representative microalgae. During a typical experiment, digester bottles were washed with phosphate-free detergent and allowed to dry. A mixture of algae, TWAS, and DI water was added to each digester and the headspace was purged with compressed N_2 gas in order to create an anaerobic environment. The inoculum (seed bacteria) was added to the digesters and the digesters were purged again with N_2 gas. Then digesters were placed inside an incubator/shaker at 150 rpm. Two incubators, New Brunswick Innova 42R Incubator Shaker (Edison, NJ) and VWR 1575R Incubator Shaker (Cornelius, OR), were utilized for the research.

Preliminary studies were conducted to determine the ideal substrate, i.e., *C. vulgaris* and TWAS, loading to inoculum ratio such that the biogas production would not be limited by substrate. Three substrate loading rate to inoculum ratios, on the basis of VS, were tested: 0.5, 1.0 and 1.5. Digesters were set-up in triplicate following the approach outlined above and were incubated at 35°C for 30 days. The results showed the best substrate to inoculum ratio to be 1. Additionally, a second preliminary test was conducted to determine the appropriate VS loading for the digesters. Substrate to inoculum ratio was kept at 1 for both low VS loading (400 mg per digester or 2 mg/L) and high VS loading (1500 mg per digester or 7.5 mg/L) sets. Again, digesters were prepared in triplicate and incubated at 35°C for seven days. It was observed that a low VS loading produced an easily measurable amount of biogas, and therefore, it was used for the remainder of the tests.

Several experiments were carried out to evaluate the influence of operational parameters, such as algal biomass loading, temperature, and alkalinity on the quantity of biogas gas generated, see Table 1. The first set of experiments, exp. run 1 through 5, was designed to evaluate the potential of the microalgae, *C. vulgaris*, as a supplementary feedstock. In these experiments, the contribution of the VS from *C. vulgaris* was 0, 30, 56, 80, and 100% of the total substrate VS. The second set of experiments, exp. run 7 and 8, was conducted to determine the effect of temperature on waste mineralization and biogas production. For these experiments, 50% of the substrate VS was from *C. vulgaris*. The effect of alkalinity was investigated in the final set of experiments, exp. run 11, 12 and 13. Alkalinity was adjusted using sodium bicarbonate ($NaHCO_3$) and three alkalinity levels were tested: 70 (alkalinity level without addition of $NaHCO_3$), 1600, and 3200 mg/L as $CaCO_3$. Control experiments, exp. run 6, 9, 10, 14, 15 and 16, were conducted for all sets of experiments. In controls, only inoculum and DI water were added, as well as $NaHCO_3$ in exp. runs 15 and 16. All experiments were conducted in triplicate. The volume of the mixture of algae, TWAS and inoculum was 200 mL for all the experiments, resulting in a 50 mL headspace.

Biogas was sampled on a schedule determined by the amount of biogas that was being produced. TS, VS, COD, nutrient content (total nitrogen and phosphorous), and microbial density, were determined at the start and end of each experiment. In addition to at the start and end of each experiment, pH and alkalinity were measured during the course of the experiment by sacrificing some digesters.

Table 1: Experimental Set-up Parameters

Exp. Run	Detention time (days)	Temp. (°C)	% of substrate VS from *C. vulgaris*	Alkalinity (mg/L as CaCO$_3$)	COD loading (mg)	COD from *C. vulgaris* (mg)	COD from TWAS (mg)	COD from Inoculum (mg)	VS loading (mg)	VS from *C. vulgaris* (mg)	VS from TWAS (mg)	VS from Inoculum (mg)	Ratio of VS from substrate to VS from inoculum
1	30	35	0	70	417	0	197	220	250	0	108	142	0.76
2	30	35	30	70	425	55	150	220	260	36	82	142	0.82
3	30	35	56	70	432	110	102	220	268	71	55	142	0.89
4	30	35	80	70	433	165	48	220	275	107	26	142	0.93
5	30	35	100	70	440	220	0	220	284	142	0	142	1
6	30	35	0	70	220	0	0	220	142	0	0	142	--
7	30	10	50	70	465	117	105	243	296	74	74	148	1
8	30	20	50	70	465	117	105	243	296	74	74	148	1
9	30	10	0	70	243	0	0	243	148	0	0	148	--
10	30	20	0	70	243	0	0	243	148	0	0	148	--
11	30	35	50	70	731	185	177	369	440	110	110	220	1
12	30	35	50	1600	731	185	177	369	440	110	110	220	1
13	30	35	50	3200	731	185	177	369	440	110	110	220	1
14	30	35	0	70	369	0	0	369	220	0	0	220	--
15	30	35	0	1600	369	0	0	369	220	0	0	220	--
16	30	35	0	3200	369	0	0	369	220	0	0	220	--

<u>Analytical Methods</u>
The volume of biogas produced was measured using a clean, dry 20 mL glass syringe (Perfektum Micro-Mate, Popper & Sons 5037, New Hyde Park, NY) purchased from Fisher Scientific. The amount of methane (CH_4) and carbon dioxide (CO_2) in the biogas was determined using an Agilent 6890 gas chromatography (GC) equipped with thermal conductivity detector (TCD). The column utilized was an Alltech Chromosorb 106 80/100 (6'x1/8" x 0.085"). The GC oven was held at 40°C for four minutes, then ramped up by 50°C each minute for three minutes, and held at 190°C for two minutes before being allowed to cool back down to 40°C where it was held for one minute before another sample could be injected. The TCD oven was set at 250°C, the nitrogen gas reference flow at 25 mL/min, and the nitrogen gas make-up flow at 5 mL/min. A computer with Agilent ChemStation software was connected to the GC and used to view and export results.

COD was determined using HACH method 8000 in the range of 20-1500 mg/L, based on the Standard Method 5220 D (APHA 1998). TS and VS were determined based on Standard Methods 2540-B, 2540-E and 2540-G (APHA 1998). Alkalinity was determined by titration according Standard Method 2320 B (APHA 1998). Total nitrogen was measured using the HACH Persulfate Digestion Method 10072, which is similar to Method 4500-N C of Standard Methods for Water and Wastewater (APHA 1998). Total phosphorous was determined by the HACH Molybdovanadate Method with Acid Persulfate Digestion, Method 10127, which is adapted from Method 4500-B C of Standard Methods (APHA 1998).

TC and FC counts were determined using the IDEXX Colisure® method, which is approved by the EPA for coliform quantification in drinking water (Olstadt et al. 2007).

<u>Materials</u>
Primary effluent, TWAS and inoculum used in the research were collected from a local WWTP. *C. vulgaris* culture was purchased from Carolina Biological (Burlington, NC). The culture was re-suspended and grown in 10 L glass bottles using primary effluent from the WWTP as the source of nutrients and water. The culture was mixed by a magnetic stirrer on a stir plate (Fisher Scientific 11-600-100SH, Bubuque, Iowa). A Lithonia fluorescent lighting fixture (Conyers, GA) and two F40T 12/CW 40 watt bulbs (Amsterdam, Netherlands) were used to provide light to the culture. CO_2 was supplied to the culture using an air stream containing 2.5% CO_2.

RESULTS AND DISCUSSION

<u>Potential of Algae as a Supplementary Feedstock</u>
The potential of algal biomass as a supplementary feedstock to anaerobic digesters, to generate a reliable supply of biogas, was evaluated by varying the contribution of *C. vulgaris* to the substrate VS. Experiments were conducted at 0, 30, 56, 80 and 100% of the total substrate VS contribution from *C. vulgaris*. The results are presented in Figure 1. The error bars presented in the figure span one standard deviation above and below the mean and were obtained from triplicate experiments. Analysis of variance (ANOVA) performed on the data using an α value of 0.05 resulted in an F value of 0.43 which is less than the F_{crt} of 2.53. This shows that there is no significant difference between the cumulative gas productions from the various ratios. It can be inferred that algal biomass has a comparable biogas yield to TWAS, making it a potential candidate as a supplementary feedstock for situations where reliable biogas supply is needed.
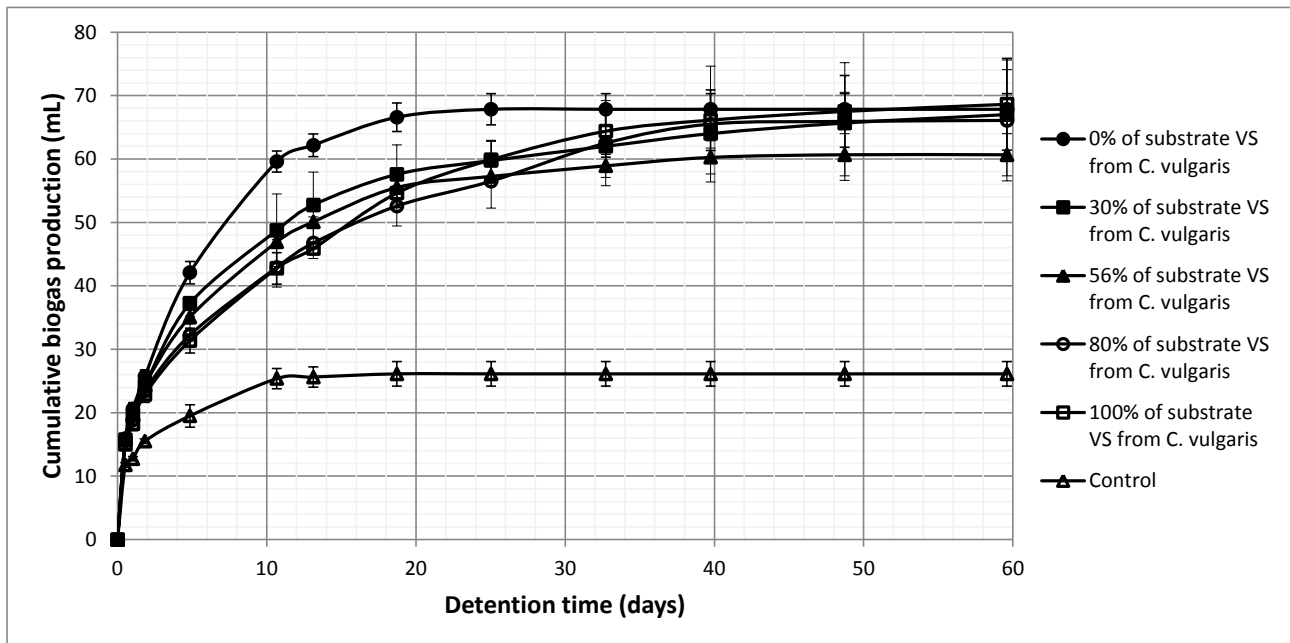
Figure 1: Cumulative volume of biogas production for varied proportions of *C. vulgaris* to TWAS over time.

The volume of biogas produced per mass of VS digested and COD oxidized is presented in Figure 2 with the error bars representing again one standard deviation above and below the mean and were obtained from triplicate experiments. The results show that the volume of biogas produced varied in the range of 0.47 to 0.57 mL per mg of VS digested (0.23 to 0.27 mL per mg of VS added). This agrees with the results reported in the literature. Golueke et al. (1957) found biogas production from a mix of *Chlorella* spp. and *Scenedesmus* spp. to be 0.45 mL per mg of VS digested and from TWAS to be 0.47 mL per mg of VS digested. Samson and Leduy (1983, 1986) determined a yield of 0.26-0.34 mL per mg VS added from anaerobic digestion of the blue-green algae, *Spirulina maxima*. Yuan et al. (2011) also studied a blue algae but only obtained a yield of 0.19 mL of methane gas per mg VS added. Cecchi et al. (1996) found that anaerobic co-digestion of the macroalgae *Ulva rigida* and *Gracilaria confervoides* from a lagoon with wastewater sludge produced a maximum of 0.31 mL of methane gas per gram of VS added. Ras et al. (2011) performed an experiment that coupled the algae production and digestion process using *Chlorella vulgaris* and found a methane production of 0.24 mL per mg of VS added for a 28 day retention time.

Similarly, the volume of biogas produced varied in the range of 0.35 to 0.53 mL per mg of COD oxidized. Biogas composition was found to be 72 percent methane (CH_4) by GC analysis. Therefore, CH_4 produced was in the range of 0.25-0.38 mL per mg of COD oxidized. These are comparable values to the theoretical CH_4 yield of 0.395 mL per mg of COD (Ras et al. 2011).
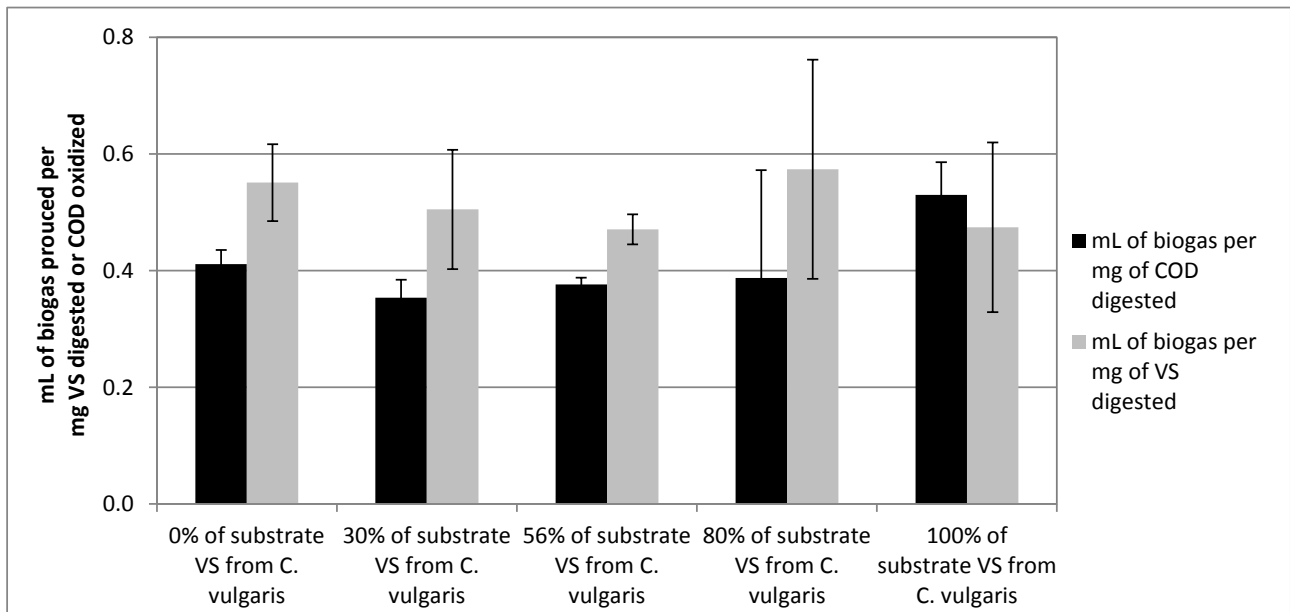
Figure 2: Volume of biogas produced per mass of VS digested and COD oxidized.

Effect of operational parameters on the performance of enhanced AD

To be implemented in rural communities and developing countries, the AD system must be cost-effective and easily maintained. In addition, due to the lack of resources, the operational parameters cannot be controlled, adjusted or monitored. Therefore, understanding the effects of operational parameters on the performance of AD is critical. In this study, we evaluate the effect of two critical operational parameters, temperature and alkalinity.

To evaluate the effect of temperature, experiments were conducted at 10, 20 and 35°C for 30 days, see Figure 3. For the experiments conducted at 10 and 20°C, the contribution of VS from *C. vulgaris* was 50% of the total VS, while for the experiment conducted at 35°C the contribution was 56%. The volumes of biogas measured at 10 and 20°C have been adjusted for temperature and the values in the figure represent equivalent volumes at 35°C. As expected, the cumulative biogas production decreased with a decrease in temperature. Though the gas production was lower at 20°C than at 35°C, substantial production did still occur with a steady increase in biogas, almost matching the production at 35°C by day 30. The biogas production at 10°C was nearly 80% less than that at 20°C, but it did still show increasing biogas production through the duration of the digestion.
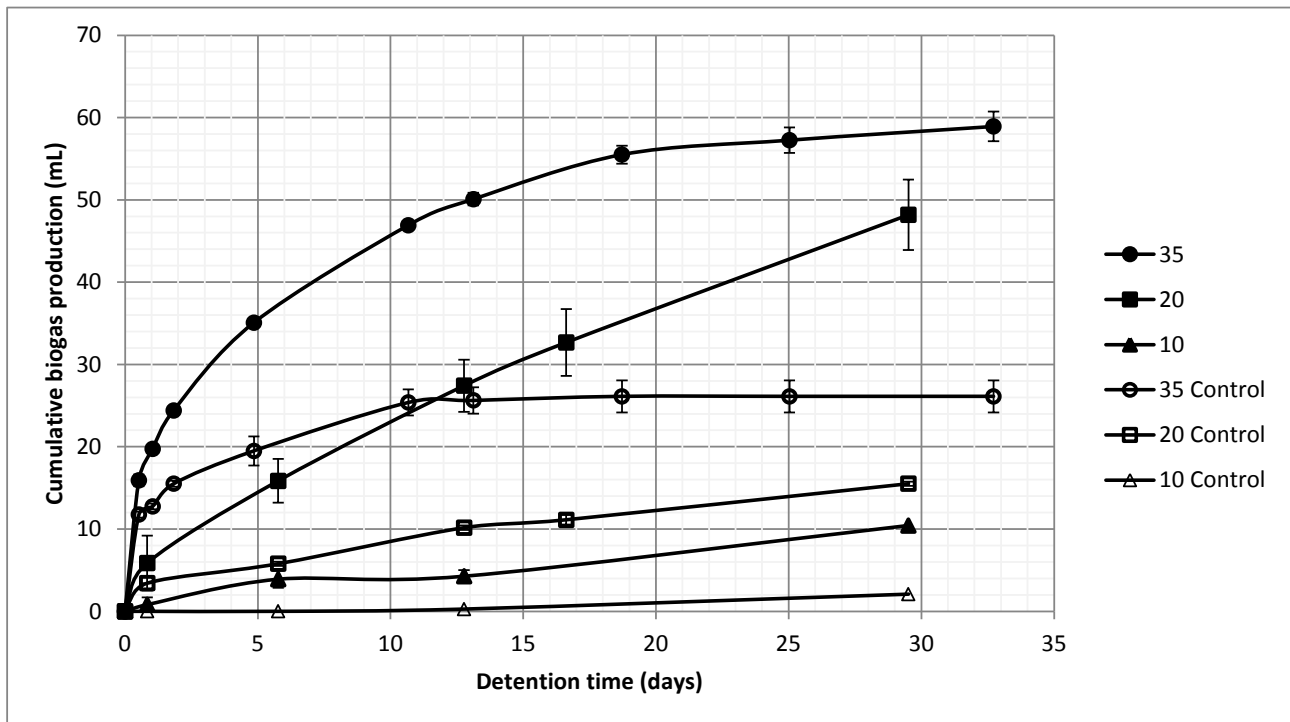
Figure 3: Cumulative volume of biogas production at 10, 20 and 35°C for a 1-to-1 ratio of *C. vulgaris* to TWAS over time.

Alkalinity plays an important role by providing a buffering capacity for change in pH. In anaerobic digesters, two mutually beneficial groups of bacteria – acidogens and methanogens – carry out the degradation of waste sequentially and have different growth rates. For instance, if the strength of the feed waste abruptly increases above normal operating levels, the acidogens – which grow relatively rapidly – convert the hydrolyzed waste to organic acids. However, the methanogens – which grow more slowly – do not utilize the acids fast enough. This will result in the accumulation of the acids in the AD reactor and a reduction in pH, which can cause a process upset.

Three levels of initial alkalinity were tested, 70, 1600, and 3200 mg/L as $CaCO_3$. Figure 4 shows the effect of alkalinity on biogas production over 30 days at 35°C for digesters consisting of 50% substrate VS from *C. vulgaris*. The ANOVA for the data, using an α value of 0.05, resulted in an F value of 0.23 and F_{crt} of 3.28, indicating that no significant difference in biogas production is seen between the varied initial alkalinity levels. After 30 days, the alkalinity levels in the digesters had increased to 670, 2100, and 3450 mg/L as $CaCO_3$, respectively, while pH remained approximately 7.5. The increase in alkalinity could be due to the ammonification, where organic nitrogen is transformed to ammonium nitrogen and bicarbonate. Since *C. vulgaris* is composed of 50-60% proteins (Becker 2004), it therefore, may be the source of the organic nitrogen in the anaerobic digesters (Abril and Frankignoulle 2001). Thus, adding algae as a supplementary feedstock to the AD system may also serve as a source of alkalinity. It should also be noted that the presence of too much protein in anaerobic digesters may pose an adverse effect since ammonia produced during the ammonification of proteins is known to inhibit microorganisms responsible for degrading organics in anaerobic digesters (Parkin and Owen 1986).
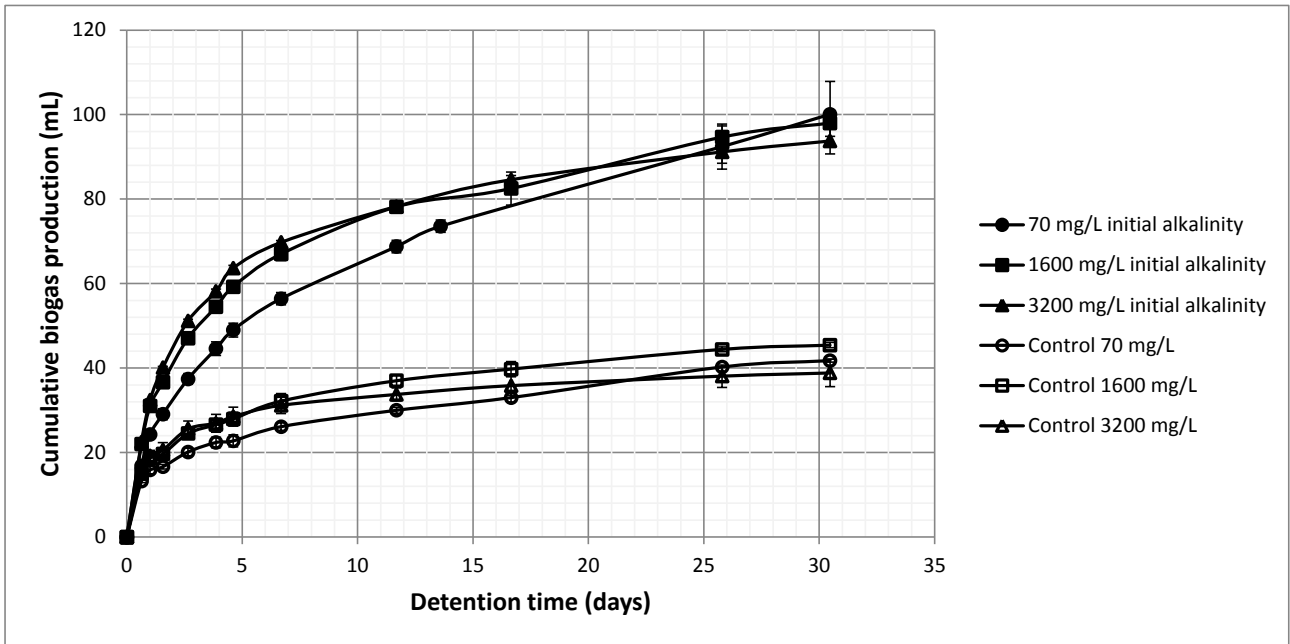
Figure 4: Cumulative volume of biogas production at various initial alkalinity levels for a 1-to-1 ratio of *C. vulgaris* to TWAS over time.

Composition of Residuals

To evaluate the usefulness of the residuals as a fertilizer, total nitrogen and phosphorous were determined. Figure 5 shows the percent of nitrogen and phosphorus on weight basis, *i.e.*, g of total nitrogen as N or g of total phosphorus as P per g of TS. The results show that total nitrogen content of the residuals varied in the range of 9-17% as N while for total phosphorus the range was 3-7% as P (7-16% as P_2O_5). Commercial fertilizers contain a wide range of nutrient levels, from 0-82% for N and 0-48% for P_2O_5; therefore, the residuals could be utilized as a fertilizer.
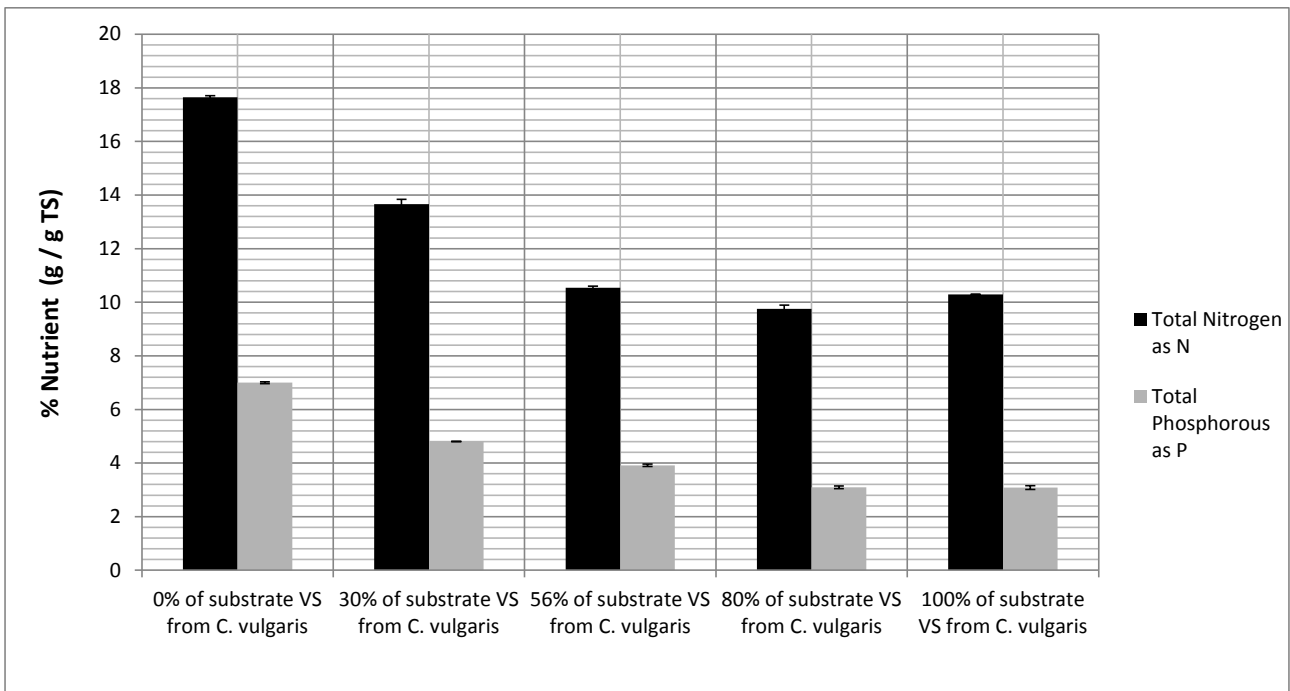

Figure 5: Percent total nitrogen and phosphorous in the digestate post-digestion for each experimental run.

The percent of TS, VS and COD reduced are presented in Table 2. The results show reductions of 25 to 42% for TS, 42 to 51% for VS, and 29 to 45% for COD for experiments conducted at 35°C. A VS reduction of 38%

or higher was achieved, and therefore, the residuals meet vector attraction reduction requirements for land application (USEPA 1999a).

Table 2: Reductions in total and volatile solids and COD

Exp. Run	0% of substrate VS from C. vulgaris	30% of substrate VS from C. vulgaris	56% of substrate VS from C. vulgaris	80% of substrate VS from C. vulgaris	100% of substrate VS from C. vulgaris
Initial TS (mg/L)	1700	1900	2100	2300	2400
Initial VS (mg/L)	1200	1300	1300	1400	1400
Initial COD (mg/L)	2100	2100	2200	2200	2200
% TS reduction	42	33	30	25	31
% VS reduction	49	51	48	42	51
% COD reduction	40	45	37	39	29

An important aspect of AD is that it reduces harmful pathogens, viruses and bacteria. To demonstrate this reduction, TC and FC were measured pre- and post-digestion. Table 3 presents the initial TC and FC and the reduction in both. FC were reduced at least 77% in all experimental runs at 35 ^{o}C. The residuals meet the EPA requirements for pathogen reduction (FC < $2x10^6$ CFU/g) for land application (USEPA 1999a).

Table 3: Reductions in Total and Faecal Coliforms

Exp. Run	0% of substrate VS from C. vulgaris	30% of substrate VS from C. vulgaris	56% of substrate VS from C. vulgaris	80% of substrate VS from C. vulgaris	100% of substrate VS from C. vulgaris
Initial TC (CFU/g TS)	$7.3x10^6$	$5.6x10^6$	$4.1x10^6$	$2.8x10^6$	$1.8x10^6$
Initial FC (CFU/g TS)	$2.4x10^6$	$1.7x10^6$	$1.1x10^6$	$5.8x10^5$	$1.7x10^5$
Final TC (CFU/g TS)	$2.8x10^6$	$5.0x10^5$	$7.9x10^4$	$1.2x10^5$	$2.5x10^5$
Final FC (CFU/g TS)	$3.2x10^3$	3.2x104	$1.8x10^4$	$5.1x10^3$	$1.3x10^4$
% TC reduced	67.61	69.84	73.12	79.26	90.17
% FC reduced	99.89	93.58	76.90	95.82	94.97

CONCLUSIONS

The results show that the enhanced AD system is an effective waste treatment technology, and it has produced high energy yields and digested waste low in faecal bacteria and high in nutrients. Reductions of 42 to 51% in VS and 29 to 45% in COD were achieved at 35°C. On average, TC and FC concentrations of $7.6x10^5$ and $1.4x10^4$ CFU per gram of TS, respectively, were measured in the digested waste. Thus, the residual meets the EPA requirements for pathogen reduction (FC < $2x10^6$ CFU/g TS) and vector attraction reduction (> 38% reduction in VS) for land application. The total nitrogen and phosphorus content of the residuals were determined to be in the range of 9-17% as N and 3-7% as P (7-16% as P_2O_5), respectively. The biogas yields varied in the range of 470-570 L per kg of VS digested.

The enhanced AD process can be designed to collect, contain and treat waste in the same reactor, making it suitable for rural and urban communities with no sewer connections. It can be built from locally available materials. Unlike conventional AD systems, the enhanced AD system can be operated and managed by individuals with minimal training and does not require complex monitoring equipment. Additionally, it is versatile and the design can be modified to fit for communities of all income levels. For instance, the system could be equipped with a toilet seat made from locally available inexpensive materials (*e.g.* wood) for low-income communities or high-end materials (*e.g.* ceramic) for the affluent. Furthermore, it can be scaled to treat waste at any size facility, from a single household to an entire city block.

In summary, enhanced AD has the potential to be developed into a reliable, inexpensive, and sustainable waste treatment system with several benefits such as: an increase in access to improved sanitation facilities, a reduction in the release of untreated waste to the environment, reduction in deaths from diseases contracted from food and water tainted with faecal matter, and the recovery of valuable resources – biogas and biosolids.

ACKNOWLEDGEMENTS

REFERENCES

WHO and UNICEF 2005 *Water for Life : Making it Happen*, World Health Organization and United Nations Children's Fund, Geneva, Switzerland.

WHO and UNICEF 2000 *Global water supply and sanitation assessment*, World Health Organization and United Nations Children's Fund, Geneva, Switzerland.

NSW Government 1998 *On-site Sewage Management for Single Households - Environment & Health Protection Guidelines*, Department of Local Government, Department of Health, Department of Urban Affairs & Planning, Environment Protection Authority, Department of Land & Water Conservation, Sydney, Australia.

Dzwairo, B., Hoko, Z., Love, D. and Guzha, E. 2006 Assessment of the impacts of pit latrines on groundwater quality in rural areas: A case study from Marondera district, Zimbabwe. Physics and Chemistry of the Earth, Parts A/B/C 31(15-16), 779-788.

Owusu, G. 2010 Social effects of poor sanitation and waste management on poor urban communities: a neighborhood-specific study of Sabon Zongo, Accra. Journal of Urbanism: International Research on Placemaking and Urban Sustainability 3(2), 145-160.

USEPA 1999a *Biosolids Generation, Use, and Disposal in the United States*, United States Environmental Protection Agency, Washington, DC.

USEPA 1999b *Quantification of Methane Emission and Discussion of Nitrous Oxid, and Ammonia Emissions from Septic Tanks, Latrines, and Stagnant Open Sewers in the World*, United States Environmental Protection Agency, Washington, DC.

GTZ 2010 *Technology review Biogas sanitation*, Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany.

APHA 1998 Standard methods for the examination of water and wastewater 20th edition, American Public Health Association, American Water Works Association and Water Environment Federation, Washington, D.C.

Olstadt, J., Schauer, J.J., Standridge, J. and Kluender, S. 2007 A comparison of ten USEPA approved total coliform/E. coli tests. J Water Health 5(2), 267-282.

Golueke, C.G., Oswald, W.J. and Gotaas, H.B. 1957 Anaerobic Digestion of Algae. Applied Microbiology 5(1), 47-55.

Samson, R. and Leduy, A. 1983 Improved Performance of Anaerobic-Digestion of Spirulina-Maxima Algal Biomass by Addition of Carbon-Rich Wastes. Biotechnology Letters 5(10), 677-682.

Samson, R. and Leduy, A. 1986 Detailed Study of Anaerobic-Digestion of Spirulina-Maxima Algal Biomass. Biotechnology and Bioengineering 28(7), 1014-1023.

Yuan, X.Z., Shi, X.S., Zhang, D.L., Qiu, Y.L., Guo, R.B. and Wang, L.S. 2011 Biogas production and microcystin biodegradation in anaerobic digestion of blue algae. Energy & Environmental Science 4(4), 1511-1515.

Cecchi, F., Pavan, P. and MataAlvarez, J. 1996 Anaerobic co-digestion of sewage sludge: Application to the macroalgae from the Venice lagoon. Resources Conservation and Recycling 17(1), 57-66.

Ras, M., Lardon, L., Bruno, S., Bernet, N. and Steyer, J.P. 2011 Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. Bioresource Technology 102(1), 200-206.

Becker, W. 2004 Handbook of microalgal culture. Richmond, A. (ed), pp. 312-351, Blackwell Publishing, Oxford.

Abril, G.l. and Frankignoulle, M. 2001 Nitrogen-alkalinity interactions in the highly polluted scheldt basin (belgium). Water Research 35(3), 844-850.

Parkin, G.F. and Owen, W.F. 1986 Fundamentals of Anaerobic Digestion of Wastewater Sludges. Journal of Environmental Engineering 112(5), 867-920.