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Full Length Research Paper

Enhancement of anaerobic digestion of Nile perch fish processing wastewater

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In East Africa, Nile perch processing into fish fillets for export generates large proportions of both solid and liquid fish wastes. These wastes are improperly utilized and/or disposed off untreated leading to environmental pollution problems. On the other hand, Nile perch fish processing wastewater (FPW) contains high concentrations of lipids and proteins, which have high methane yield potential. However, anaerobic digestion (AD) of FPW for methane production is limited due to process inhibition by lipids and ammonia intoxication. To overcome these limitations, the effects of co-digestion, physical and biological pretreatments on extent of methane yield were investigated. At a loading ratio of 1:1 (inoculum to substrate) with raw FPW, a methane yield of 0.56 m^3/kgVS was obtained. Co-digestion of the residue with 10% gVS of brewery wastewater enhanced methane yield to a highest increment of 66%. Long chain fatty acids (LCFA) removal prior AD enhanced methane yield to an increment of 52% at LCFA removal of 8%. Pretreatment of FPW with aerobic microbial cultures isolated from a fish waste stabilization pond enhanced methane yield to an increment of 60% after 18 h, 68% after 15 h and 76.0% after 12 h of incubation, respectively, for strains CBR 11, BR 10 and a mixture of the two (CBR 11 + BR 10). The present study reports for the first time improvement of AD of Nile perch FPW by co-digestion, physical and biological pre-treatment methods and could be used as a basis for designing a pilot scale process.

Key words: Anaerobic digestion, co-digestion, LCFA removal, biological pre-treatment, Nile perch processing wastewater.

INTRODUCTION

Anaerobic digestion (AD) of organic wastes to produce methane would benefit society by providing a clean fuel from renewable feed tocks. This could substitute fossil fuel–derived energy and reduce environmental impacts including global warming and acid rain (Chynoweth et al., 2001; Tomei et al., 2008). Solid waste and wastewater from Nile perch processing represent a high potential energy resource if they can be properly and biologically converted to methane. Nevertheless, anaerobic digestion of lipid-rich wastes such as Nile perch fish processing wastewater (FPW) is limited by low bioavailability of lipids, process inhibition by long chain fatty acids (LCFA) and a delicate balance between syntrophic acetogenesis and syntrophic methanogenesis (Weng and Jeris, 1976; Petruy and Lettinga, 1997; Vidal et al., 2000; Cirne et al., 2007). Attempts to minimize these limitations have been reported. Studies on pretreatment of dairy wastewater with pure culture of *Penicillium restrictum* (Cammarota et al., 2001) and enzymatic pretreatment of slaughter house wastewater using commercial lipase from animal, microbial and vegetable sources did not significantly enhance the anaerobic digestion process (Masse et al., 2001; Masse et al., 2003). In addition, Masse et al. (2003) concluded that the use of commercial enzymes for direct enzymatic bioaugmentation makes the anaerobic digestion process quite expensive and thus not economically feasible.

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In the recent study by Cirne (2006), it has been reported that direct lipase enzyme addition and bioaugmentation of anaerobic digester with a lipolytic anaerobic microbial strain enhanced lipid hydrolysis but the intermediate products (LCFAs) inhibited the latter steps of biodegradation, lowering the overall efficiency of the process. It was also concluded that the bioaugmenting strain was unable to compete with the "native" biomass of the digester (Cirne, 2006).

Other studies on chemical pretreatment of lipid-rich wastewaters revealed that addition of alkaline chemicals such as NaOH, KOH and Ca $OH)_2$ to slaughterhouse wastewater and oil mill effluents improved hydrolysis of solid fatty residuals (Beccari et al., 1999; Masse et al., 2001; Mouneimne et al., 2003). Nevertheless, Masse and co-workers do not recommend pretreatment with an alkali because it results in an increase in pH in the bioreactor. Furthermore, results from pretreatment of lipid-rich wastewaters by addition of calcium chloride and bentonite clay indicated that these chemicals could reduce the inhibitory effects of LCFAs (Angelidaki et al., 1990; Broughton et al., 1998; Beccari et al., 1999). However, use of these chemicals was not recommended since they work by limiting the concentration of dissolved LCFA that subsequently led to reduced methane yields. Lefebre et al. (1998) suggested the removal of large proportions of lipids (fats and grease) from the lipid-rich wastewaters before treatment but the study revealed that dissolved and/or emulsified lipids can not be filtered off and gain access into treatment system (Cammarota et al., 2001). In addition, complete removal of oil and grease leads to loss of lipids that have very high methane production potential.

A study by Callaghan et al. (1999) on batch co-digestion of waste organic solids reported that co-digestion of cattle slurry with fish offal and brewery solids enhanced methane yield. Mshandete et al. (2004) also reported that anaerobic batch co-digestion of sisal pulp with fish solid waste (wet weight proportions; 33% fish waste: 67% sisal pulp) enhanced methane yield by 59-94% compared to sisal pulp and fish wastes alone. Furthermore, Ahring (2003) reported a two-fold increase in the yield of methane, from 25 to 50 m^3 methane/m^3 cattle waste when fish oil (total concentration 5%) was added to manure digester. Ahring (2003) recommended that adding lipids to anaerobic digester to enhance the production of methane is a promising approach that needs continued exploration. Similarly, Cirne (2006) recommended that lipid-containing waste has a very high methane production potential and therefore research on the limiting steps of the conversion of lipids to methane need to be continued. Therefore, this study aimed at investigating new alternative methods that can minimise and/or alleviate inhibition caused by lipids and LCFAs, and enhance the anaerobic digestion of Nile perch fish processing wastewater (FPW) generated along Lake Victoria. To the best of our know-ledge, this is the first report on enhancement of AD of FPW by co-digestion, physical and biological pre-treatment methods.

MATERIAL AND METHODS

Substrate and inoculum

The substrate investigated in this study was Nile perch fish processing wastewater (FPW) collected from a mixed stream at Vicfish limited in Mwanza City on the southern shore of Lake Victoria. The inoculum was collected from a stabilization pond receiving high strength FPW at the same industry where the substrate was collected. Brewery wastewater used in co-digestion experiment was sampled from a mixed stream at Tanzania breweries limited (TBL) in Mwanza City, Tanzania.

Bioreactor configuration and operation

Biogas production from FPW sample was investigated in 500 ml batch bioreactors consisting of wide mouth Erlenmeyer conical flasks with a working volume of 0.36 L. The bioreactors were designed and operated according to Mshandete et al. (2004). The methane content and biogas volume were measured after every 72 h. The biogas volume and methane content were determined according to Ergüder et al. (2001).

Pretreatment of FPW by addition of microbial cultures

The microbial strains used in this study were two bacterial strains: CBR 11 and BR10 isolated from a local stabilization pond treating high strength FPW at Vicfish industry in Mwanza city, Tanzania. The two strains exhibited lipolytic activity when cultured in Tributyrin Agar (TBA) and broth media with lipid as the sole carbon source. The experiment consisted of two set-up: addition of separate strain cultures and addition of a mixed culture of the two strains. The microbial cultures were added to the FPW according to Cirne (2006). The amount of the lipolytic strain added corresponded to 1.3% VS of the inoculum added for a 1:1 inoculum-to-substrate gVS loading ratio. For each set up, the experiment consisted of thirteen aerobic incubation periods: 0, 3, 6, 9, 12, 15, 18, 21, 24, 36, 48, 60 and 72 h. All batch bioreactors were incubated at 28^{o}C while shaking at 75 rpm. For addition of mixed cultures, 72-h broth cultures of the two bacterial strains were mixed in the ratio of 1:1. At the end of all the pretreatment periods, inoculum was added in a ratio of 1:1 inoculum-to-substrate gVS. The experiment was set up in triplicate and was run for 36 days when biogas production had ceased.

Co-digestion of FPW with brewery wastewater

The brewery wastewater was added to 160 ml (0.534 gVS) of FPW at increasing %VS concentrations of: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. Inoculum was added to each of the fractions including the control at the ratio of 1:1 (inoculum gVS-to-substrate (FPW) gVS). The investigation was carried out in triplicate and was run for 36 days when biogas production had ceased.

Pretreatment of FPW by LCFA removal

The bacterial strain (CBR11) which showed lipolytic activity without proteolysis, was cultured in Tributyrin broth medium. The lipase enzyme was extracted according to the standard protocol described by Bezerra et al. (2006).The broth culture media were centrifuged

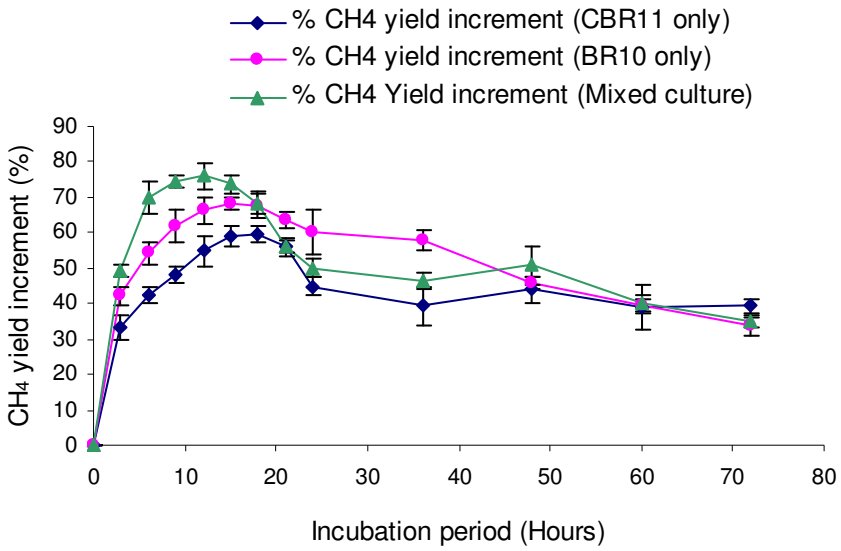

Figure 1. Comparison of microbial culture pretreatment with the extent of methane yield increment.

at 12,500 X g for 15 min in a refrigerated centrifuge. The supernatant containing crude enzyme was quickly pipetted off and stored at -4°C until needed. The lipase enzyme extract was added to the FPW at 1.0% (v/v). The mixture was incubated at 30°C for 24 h while shaking at 75 rpm. The LCFA were removed by urea adduct fractionation following standard protocol described by Wanasundara and Shahidi (1999) and Hsieh et al. (2005). The percentage LCFA removal was determined as the difference between the lipid content of the substrate before LCFA extraction and the lipid content of the substrate after the LCFA removal divided by the substrate lipid content before LCFA extraction. The substrate fractions at various LCFA removal percentages were transferred to batch bioreactors. The experiment consisted of ten wastewater fractions at LCFA removal percentages of: 0, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12. Each digester consisted of wastewater substrate and the inoculum in 1:1 gVS ratio. The investigation was carried out in triplicate bioreactors. The experiment consisted of 36 bioreactors and was terminated after forty-six days when biogas production had ceased.

RESULTS

Pretreatment of FPW with microbial cultures

Pretreatment with strain CBR 11 enhanced the AD process with maximum methane yield of 1.41 ± 0.01 m^3/KgVS (60% methane yield increment) after 18 h of incubating the substrate with the bacterial strain. On the other hand, incubation of FPW with strain BR 10 enhanced the AD process from 0.57 m^3/KgVS in untreated (control) to 1.80 m^3/KgVS in 15-h pretreated substrate, corresponding to methane yield increment of 68%. Pretreatment of the substrate with a mixed microbial culture optimally enhanced methane yield to 2.38 ± 0.01 m^3/KgVS corresponding to 76% yield increment, after 12 h of incubation as shown in Figure 1.

Co-digestion of FPW with brewery wastewater

Co-digestion of FPW with brewery wastewater enhanced the AD process to a maximum methane yield increment of 66 % when 10 % gVS brewery wastewater was added. Addition of more brewery wastewater led to a decrease in methane yield. The trend in process enhancement with increasing VS concentration of brewery wastewater is given in Figure 2.

Pretreatment of FPW by LCFA removal

The total methane production and the methane yield from the AD of LCFA-free fractions are given in Figure 3. The highest methane yield increment obtained by this pre-treatment was 52%, at LCFA removal of 8%. Further removal of LCFA after 8% caused slight increase in the methane yield.

DISCUSSION

Effect of Pretreatment with microbial cultures

Addition of microbial strains to the substrate enhanced the AD process and hence methane yield. This was in agreement with the report by Björnsson (2000) and Cirne (2006) that addition of pre-adapted microbial strains can reduce the inhibition due to LCFA and high ammonia concentration (Breure et al., 1986). Since these microbes were naturally living in a stabilization pond receiving high strength lipid-rich wastewater, they were perhaps adapted to degrade lipids at high concentrations. Furthermore,

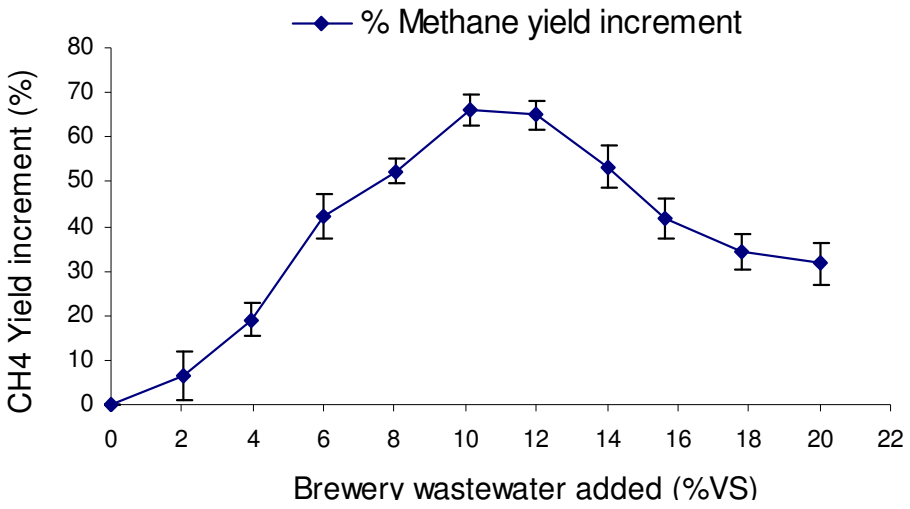

Figure 2. Extent of methane yield increment with addition of various amounts (VS) of brewery wastewater.

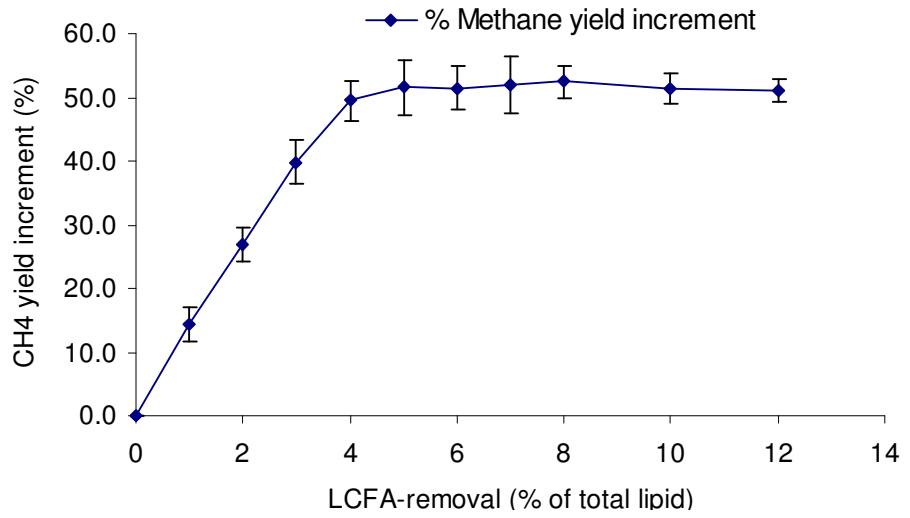

Figure 3. The trend in percentage methane increment with percentage LCFA removal

the addition of mixed culture in which one strain was both proteolytic and lipolytic (strain BR 10) could have hydrolysed proteins that readily provided the system with nitrogen supply in form of amino acids and sugars from amino acid bioconversions. These released nutrients together with simultaneous enhanced lipolysis might have had a positive effect on establishment of syntrophic association between β-oxidation and syntrophic methanogenesis that counteracts the inhibition of LCFAs. This explains the higher methane yields obtained in substrate pretreatment with mixed microbial cultures. These findings are in line with Kuang et al. (2002) who reported a decrease in inhibition of LCFAs (Oleate) on methanogenic activity and an improvement on granulation when sugar (glucose) and amino acid (Cystein) were digested together with the LCFAs. It was also observed that the highest methane yields were obtained when the microbial cultures were incubated with the substrate for less than 20 h (Figure 1). Substrate pretreatment for more than 18, 15 and 12 h, respectively with strains; CBR 11, BR 10 and a mixture of the two (CBR 11 + BR 10) led to a decrease in methane yield, which is due to nutrient loss through aerobic oxidation. This concurs with the report by Mshandete et al. (2005), which revealed that prolonged aerobic pre-treatment period causes degradation of significant amounts of organic material leading to lower methane yields.

Effect of co-digestion with brewery wastewater

Co-digestion of the FPW with 10% gVS of brewery

Table 1. The extent of methane yield by different pretreatment methods

Pre-treatment method	Maximum total methane produced (ml)	Methane yield (m^3/kgVS)	% CH_4 yield increment
LCFA removal	641.1 ± 4.5	1.20	52
Co-digestion	903.1 ± 6.1	1.69	66
Addition of strain CBR11	754.3 ± 4.1	1.41	60
Addition of strain BR10	961.0 ± 3.2	1.80	68
Addition of Mixed culture (CBR11 + BR10)	1268.7 ± 6.5	2.38	76

wastewater enhanced methane yield to a highest increment of 66%. Further addition of brewery wastewater caused no more increment in methane yield due to lowering of pH as excess brewery wastewater was added, since the latter was acidic with a pH of 4.9. Nevertheless, it was observed that addition of brewery wastewater increased the surface tension thus enhancing the stability and sedimentation of biomass granules. This indicated that co-digestion with brewery wastewater reduces the mass transfer limitations (Pereira et al., 2004) and biomass degranulation due to LCFAs (Rinzema et al., 1994; Hwu et al., 1996; Kuang et al., 2002; Kim et al., 2004). More over, brewery wastewater is a rich source of Vitamin B_{12} (Cyanocobalamin) which is an essential co-enzyme required for biodegradation of propionate formed from β-oxidation of odd-number carbon LCFAs (De Muylder et al., 1989; Lehninger et al., 1993). Cyanocobalamin harnesses the β-oxidation process and consequently reduces the concentration and the inhibition of LCFAs. The high sugar content in brewery wastewater might have contributed to the relief of toxicity by LCFAs. This concurs with the study by Kuang et al. (2002) which revealed that co-digestion of LCFAs with sugar (glucose), causes higher methane production than co-digestion with pure amino acid (Cystein) or a combination of amino acid and glucose.

LCFA removal

The enhancement of AD caused by LCFA removal could be attributed to the reduction in oils and generally lipid content, and the increased liquefaction of proteins caused by urea addition (Archer, 2001). At 8% LCFA removal prior AD, maximum methane yield was obtained corresponding to an increment of 52% as shown in Figure 3. LCFA removal reduces the concentration of lipids particularly unsaturated LCFAs that are more inhibitory to methanogens and the AD process than the saturated ones (Rinzema, 1988; Beccari et al., 1996). Hence, LCFA removal might have restored the optimal aqueous-lipid interface required for activation of lipase enzyme (Sanders, 2002; Saxena et al., 1999). The use of urea helps to liquefy most of the suspended solids including solid fatty particles and proteins thus enhancing liquefaction step (Sayed et al., 1988; Archer, 2001). However, high rate of protein liquefaction elevates the pH alkalinity of the bioreactor content causing ammonia intoxication and this might have contributed to the low methane yield increment when compared to other pretreatment methods investigated in this study (Table 1). In addition, the reduction of LCFA lowered the concentration of lipids that have very high biogas potential.

Conclusion

Addition of microbial mixed cultures enhanced the AD process highest with an increment in methane yield of 76% after 12 h of incubation. Co-digestion of the wastewater with 10% gVS of brewery wastewater optimally enhanced methane yield to an increment of 66% and was the simplest enhancement method. Long chain fatty acid (LCFA) removal prior AD caused the lowest enhancement with methane yield increment of 52%, at LCFA removal of 8%. Further investigations on the effect of co-digestion of the wastewater previously pretreated with mixed microbial culture needs to be pursued. Additional studies on these pretreatment methods at scaled-up level such as continuous stirred tank reactor (CSTR) need to be conducted before pilot scale operations.

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