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NILE PERCH FISH SCALES A NOVEL BIOFILM CARRIER IN THE ANAEROBIC DIGESTION OF BIOLOGICAL PRE-TREATED NILE PERCH FISH SOLID WASTE

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ABSTRACT

Improved stability and anaerobic digestion (AD) process in a packed bed bioreactor can be achieved if bacterial consortia are retained in the process through the use of biofilms carriers. Three methanogenic biofilms carriers for biomass retention were studied to evaluate the performance of methanogenesis AD of Nile Perch fish solid wastes pre-treated by bacterial culture coded (CBR-11). The carrier material evaluated consisted of sisal fibre waste, pumice stones and Nile perch fish scales. Process performance was investigated by increasing the organic loading rate (OLR) step-wise. The best results were obtained from the bioreactor packed with Nile perch fish scales. It had the lowest total volatile fat acids (TVFA) accumulated at OLR in the range of 1-12 g volatile solids (VS)/l/d. The degradation pattern showed that the TVFAs was limiting at higher OLRs. The pH profiles showed an increasing pattern with an increase in OLRs. The pH was low (8-8.3) at low OLRs (1-6) gVS/l/d and increased to a higher level (8.3-8.7) at higher OLR (9-12) gVS/l/d. Despite the high pH level reached, the bioreactor packed with fish scales had a good ability to withstand the changes in load and VFA concentrations shocks that can occur in packed bed anaerobic bioreactors. In conclusion Nile perch fish scales was demonstrated to be potential novel biofilms carrier that would work well in methanogenic biofilms bioreactors treating fish solid waste. Moreover, Nile perch solid fish wastes and fish scales are available within the vicinity, which could make AD scale-up at fish processing industry feasible and cost effective.

Keywords: n nile perch fish solid waste, packed bed biorector, fish scales, biofilm carriers, anaerobic digestion.

INTRODUCTION

Anaerobic digestion (AD) in the past has been largely confined to the stabilization of sewage sludge. However interest in the AD process has been well expanded as results of increasing demand for energy and the growing problems of pollution control. Renewable energy sources are often prioritized in efforts of mitigating the greenhouse effect and eventually achieve a completely sustainable energy supply [1].

Fish solid wastes from fish processing industries constitute a major source of renewable energy. Fish solid waste is rich in lipids which impedes the anaerobic treatment by several operational problems, such as inhibition, sludge floatation and washout [2]. These problems results mainly from the accumulation of long chain fatty acids (LCFA) on the microbial aggregates by mechanisms of adsorption, precipitation and entrapments [3, 4, 5]. If it is digested under anaerobic conditions, the energy recovery will be highly sustainable provided the methane produced is retained and is fully utilized, and the digestate produced is retained and is recycled back to the soil as fertilizer. Fish processing industries around Lake Victoria produces about 36,000 tons of fish solid waste annually [6]. Fish solid waste presents a potential problem in AD. This waste is rich in organic matter as fat and proteins, which tends to be toxic to the various life forms of the system, and pose a problem in digesting them anaerobically. Therefore pre-treatment of fish wastes streams prior to AD is very to important to achieve stable process.

In conventional anaerobic slurry digestion, the substrate needs to be homogenized and diluted down to a pumpable slurry containing only about 3- 8% TS [7]. This increases the handling costs of the end products [1]. Process disturbances inherent in the high water content of the slurry, such as crust and foam formation, often occurs as well, resulting in low maximum loading rates and poor decomposition [8]. Reduction of the amounts of process water that is need to be added, could help to avoid the problems that dilution creates, including the process problems just referred to [1].

The development of new reactor and process design for high-solids digestion of substrate such as fish solid waste could make it possible to utilize the large energy potential of the world's ample supply of fish waste substrate to a greater extent, even if only small-scale reactor were involved [1].

Success of high solids AD in developing countries such as Tanzania with low technology environment it is essential that the approach employed be as simple as much as possible, so as to minimize the investment needed and the operational costs. A hybrid approach one that combines the advantages of single and two stages AD has been proposed [8]. The emerging hybrid approach is in the form of a single-stage down-flow fixed bed bioreactor in which the bed is made up of plant biomass such as wheat straw (the bed act biofilm carrier) fed with fresh feedstock (substrate at the top) [8]. A modification to the model proposed above in which the plant biomass fixed bed reactor is replaced with biofilms carriers such as fish scales could be used in which the

substrate fed at the top of the bioreactor will start to hydrolyze and acidify. The solutes will trickle down further into the inoculated bed which becomes more and more methanogenic in character and develop stratification that separate different steps of the anaerobic process in terms of their depth within the packed bed [9]. The performance of such high-solids stratified bed bioreactor depends to a considerable extent on the choice of the biofilms carriers to serve as the packed bed. The materials can be arranged in various confirmations, made out of different matter (plastics, granular activated carbon, sand reticulated form polymers, granite, quartz and stones) and can be packed in loose or modular configurations. The reactors could be operated in up-flow or down-flow mode [10, 11]. The optimal material at the bottom would be one acting both as a particulate filter and as support to facilitate biofilms accumulation forming micro-organisms [1, 8, 12], which will assure a shorter start-up period due to greater amount of retained inocula [13]. After digestion for a period of time, the starting bed materials would function as a primer for the growing bed, composed of the more easily degradable biomass being fed into the bioreactor. This primer material should be rigid structure and have a low biodegradability in order to maximize the period of continuous operation of the anaerobic bioreactor. For example, straw has been found to function well as a carrier material for biofilms processes in anoxic and anaerobic applications involving liquid feedstocks [14, 15, 16, 17] Likewise, [18] compared the performance of sisal fiber waste, pumice stones and glass beads packing materials on digesting sisal leaf leachate. From the results obtained, it was concluded that the performance of the packed-bed bioreactor containing sisal fiber waste as a biofilms carrier was superior to those of the other bioreactors investigated [18]. Therefore sisal fiber waste was proposed as appropriate biofilms carrier to use in methanogenic bioreactors digesting sisal leaf tissue waste leachate [18].

The aim of the present study was therefore to determine the performance of fish scales used as biofilms carriers in anaerobic packed bed bioreactor system for digesting fish solid waste containing high amount of fat and proteins. Operational conditions adopted were step wise increase of feed substrates concentration, constant hydraulic retention time and up-flow feeding mode. To the best of our knowledge, results on performance of fish scales as biofilms carrier for anaerobic digestion of fish solid waste is reported here for the first time.

MATERIALS AND METHODS

Substrate and inoculum preparation

Fish solid waste (a mixture of fish scales, viscera, fish scrap, fat solids, proteins and fish rejects) produced during fish processing was obtained from Tan-perch, Vic-fish and Mwanza Nile Perch fish processing factories in Mwanza, Tanzania. The FSW from different fish processing factories were mixed and stored at -20^{o}C until used. Before use in a frozen condition FSW was chopped to reduce particle sizes down to 20 mm using kitchen knife (Super Cut stainless steel, Germany). Thereafter, chopped FSW was shredded in a mechanical meat mincer to ensure particle size < 12 mm and homogeneity.

An active inoculums used in this experiment was obtained from anaerobic wastewater stabilization pond located at Vic-fish, fish processing factory in Mwanza. The inoculum was stored in 25-liter plastic containers with anaerobic headspace to ensure degradation of easily degradable organic matter still present in the inoculums.

Source of bacterial strain and commercial lipase

The bacterial strain (CBR-11), which have been found to express lipolytic activity without proteolysis was obtained from strain bank at the department of Molecular Biology and Biotechnology, University of Dar es Salaam, Tanzania. The CBR-11 bacterial strain is local isolate which has been primarily characterized and reported [6].

Microbial pre-treatment of nile perch fish solid waste

Prior to pre-treatment of FSW with bacterial strain, CBR-11 bacterial strain was first grown on tributyrin broth media (pH 7.0) containing 0.25% peptone meat, 0.25% peptone casein, 0.30% yeast extract and 1.0% tributyrin as the sole carbon source. Then the culture was incubated at 30^{o}C in a shaking incubator (Orbital Incubator S150, Stuart Scientific, UK) shaking at 120 rpm and grown to an optical density of 2.0, measured spectrophotometrically at 650 nm wavelength (Thermo Spectronic Helios Gamma, England). Then 3% (w/w) of CBR-11 bacteria strain was added to a plastic bottle containing Nile Perch FSW, mixed thoroughly and incubated for 12 hours at room temperature (27-33^{o}C). After pre-treatment, the pre-treated FSW (CBR-11-FSW) was analyzed for total solids (TS) and volatile solids (VS) which were used to calculate the amount of gVS of CBR-11-FSW to be used in evaluation processs of bioflm carriers. Specific data of fish solid waste (left of column) and inoculum (right of column) before bacterial pre-treatment are shown in Table-1.

Table-1. Characteristics of fish solid waste (FSW) used in packed bed bioreactors.

Parameter	FSW	Inoculum
pH	7.10±0.02	7.83±0.43
Total solids (%fresh)	37.4±0.03	0.64±0.07
Volatile solids (%TS)	82.37±0.28	39.03±0.02
Moisture content (%)	62.6 ±0.45	
Total organic carbon (%TS)	48.26±0.26	
Total organic matter (%TS)	86.87±0.47	
Total nitrogen (%fresh)	2.78±0.12	
NH4-N (mg/l)	8.86±0.25	
C:N	17.36	
Alkalinity ($gCaCO_3$/l)	5.23±0.3	
Volatile fatty acids (g/l)	121.06±0.21	
Soluble COD (SCOD) (gO_2/l)	31.19±6.15	
SCOD (gO_2)/gTS	83.39	
Total lipids (% fresh)	20.09±0.24	

Biofilm carrier materials

Nile Perch fish scales (NPFS) were collected from fish processing industries in Mwanza, Tanzania. The NPFS are usually associated with liquid waste from fish processing industries. From a fish wastewater pond, a sieve was immersed to fish-out the scales from the pond bottom. The NPFS were rinsed with tap water to remove mud and soil sediments. The cleaned NPFS were was sun dried for 7 days, placed in a plastic bag and transported to the Molecular Biology and Biotechnology laboratory at the University of Dar es Salaam, where were stored at room temperature until use. Prior to use, the fish scales were first soaked in tap water for two days. Thereafter the NPFS were washed to remove any debris and finally were rinsed two times in clean tap water. The NPFS was sun dried for seven days ready for packing in the bioreactor. The NPFS are light, collagenous, grey in colour with a size of 10 -30 mm and porosity of 90%. The sisal fiber and pumice stones were obtained from the Department of Molecular Biology and Biotechnology laboratory, University of Dar es Salaam, Tanzania. The characteristics of sisal fibers (SF) and pumice stones (PU) have been previously described by [18]. The specific experimental conditions involved are shown in Table-2.

Table-2. Summary of the experimental condition in packed bed bioreactors.

Experimental designation	Bioreactor		
	NPFS	SF	PU
Packing density initial (g/l)	200	145	271
Bed volume (l)	1.5	1.5	1.5
Carrier state	Dry and fresh	Dry and fresh	Dry
Start-up time (day)	140	140	140
Total experimental time (days)	290	290	290
Organic loading rate (gVS/l/d)	1-12	1-12	1-9
Temperature (^oC)	25-30	25-30	25-30

Bioreactor design

The experimental set-up was done according to [18] with minor modifications as seen in Figure-1. The set-up consisted of three methanogenic bioreactor configurations of identical size (volume 2 l, height 420 mm and an internal diameter of 60 mm). On the bottom of each bioreactor a perforated plastic with a diameter of 58 mm and a thickness of 10 mm was placed to fit down of each bioreactor and covered with a plastic mesh with pore size of 2 mm before packing. Then the bioreactors were

packed with different biofilm carriers and were operated under identical conditions and run in parallel. The sisal fibre and pumice stone bioreactors were packed to a density of 145 and 271 g/l. respectively, according to [18]. The NPFS bioreactor was packed to a density of 200 g/l, the packing was done after soaking fish scales in the inoculum to ensure uniform packing. After packing a perforated polyvinyl rubber with diameter of 58 mm were placed on top of the packed bed to avoid floating of the packed-bed during running of the experiment. To each bioreactor a total of 800 ml of pre-digested inoculum were added from the top and recirculated through the packed-bed bioreactor with a constant downflow recirculation of 10 ml/min using a peristatic pumps (Watson Marlow 100 series, Alitea AB, Solkraftsvagen 35, S-13570 Stockholm, Sweden). The study was carried out at room temperature range of 27-35^{o}C. During the start-up period of 140 days all the bioreactors were run by re-circulating the pre-digested inoculum in order to allow the establishment of biofilms and to degrade the biodegradable part contained in carrier materials which would otherwise wrongly contribute to the methane production of that process [1, 13]. During this period, 100 ml of the effluent from the bioreactors were withdrawn for analysis and feeding the same volume of pre-digested inoculum to each bioreactor once every week. The effluent were analysed for VFA, COD, alkalinity, pH, methane and biogas production until steady state values were obtained. During this period the feeding of the pre-digested inoculum were done in a semi-continous mode by pumping through a 50 ml syringe barrel placed on top of Polyvinyl rubber stopper to the upper part of the biofilm carrier and closed immeadiately to ensure anaerobic condition by leaving the plunger intact. The whole exercise was done in less than five minutes for each bioreactor to minimize the chance of oxygen getting inside the bioreactors. The effluent were discharged through the outlet when the liquid move from top of the bioreactor through the bed and back to the top. The gas separated from the liquid and was collected in a gas-tight alluminium bag placed on top of the bioreactor.

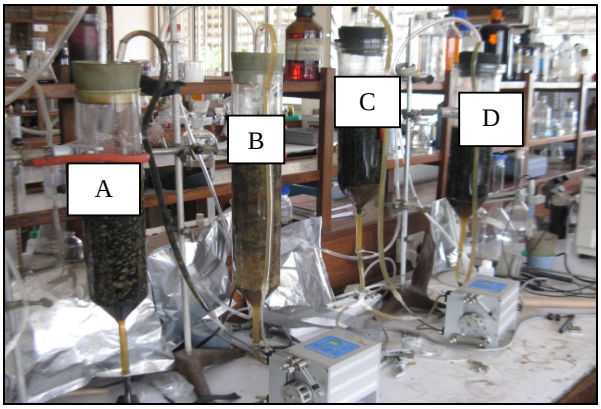

Figure-1. Anaerobic bioreactor packed with different biofilms carriers; pumice (A); Sisal fiber waste (B); Nile perch fish scales (C and D).

Experimental procedures

After the start-up period elapsed, the pre-treated FSW substrate was added to each bioreactor and all three bioreactors were operated continuosly for 150 days. In order to determine the maximum efficiency of each packed-bed bioreactor, the organic loading rate (OLR) was increased step-wise. The performance of the processes was monitored by analyzing the volume and the content of the gas, concentration of VFAs, COD, total alkalinity, and pH of the effluents. The OLRs in the range of 1-12 gVS/l/d were investigated, and each OLR was maintained for at least 30 days in order to attain a steady state. The evaluation of reactor performance was based on the fact that during anaerobic degradation of COD, VFAs are formed as intermediates. As the conversion of these acids to methane and carbon dioxide is the rate limiting step in the anaerobic process, at too high an organic loading loading rate, VFA will accumulate in the system [19].

Scanning electron microscopy

Microbial cell immobilization on Nile perch fish scales carrier was visualized using scanning microscopy (SEM). The fish scales before and after stabilization (start-up) were scanned. Preparation of fish scales for SEM was done according to [20]. It involved fixation of Nile perch fish scales in 2.5% glutaraldehyde in phosphate buffer saline (PBS) (pH 7.4) for 10 minutes, aspirated and washed in PBS. After washing the samples were dehydrated in (50, 70, 90 and 100%) series of ethanol solution followed by critical point drying (Quorum Technologies models E3100, Guelph, Ontario Canada). After drying the fish scales were then sputtered with Gold using a SEM coating system (SPI sputter coater 11430 West Chester, PA, USA). Morphological analysis was undertaken using scanning electron microscopy (JEOL, JSM-5610, Tokyo, Japan).

Analytical methods

Measurement of biogas volume was performed using 100 ml gas-tight glass syringe with a gas lock (Fortuna®, Poulten and Graf GmbH, 97877 Wertheim, Germany). The methane gas composition was estimated by KOH concentrated absorption method [21]. In this method only methane was determined while other biogas components such as CO_2 and H_2S are dissolved in the KOH solution. Total alkalinity, total volatile fatty acids (TVFAs) and pH were measured as previously described [22]. Total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and total nitrogen were measured as described in Standard Methods [23].

RESULTS AND DISCUSSIONS

Properties of Nile perch fish scales as biofilm carriers before and after microbial colonisation

Nature has increasingly serves as a model and inspiration to scientist and engineers, and biometrics has the potential to lead to novel engineering materials and systems with new combinations of properties, multi-

functionalities, adaptability and environmental sustainability [24]. Fish scales displays interesting combinations of flexibility, strength, resistance to penetration, light-weight and transparency, fish scales exhibit large variations in shape, size and arrangements. Some fish scales are so tough that they cannot be easily fractured even after immersions in liquid nitrogen [25]. The variation in size, shape, hydrophobicity, surface to volume ratio of the matrix, surface charge, porosity and roughness of the carrier materials have been reported to influence bacterial colonization on the support media [18].

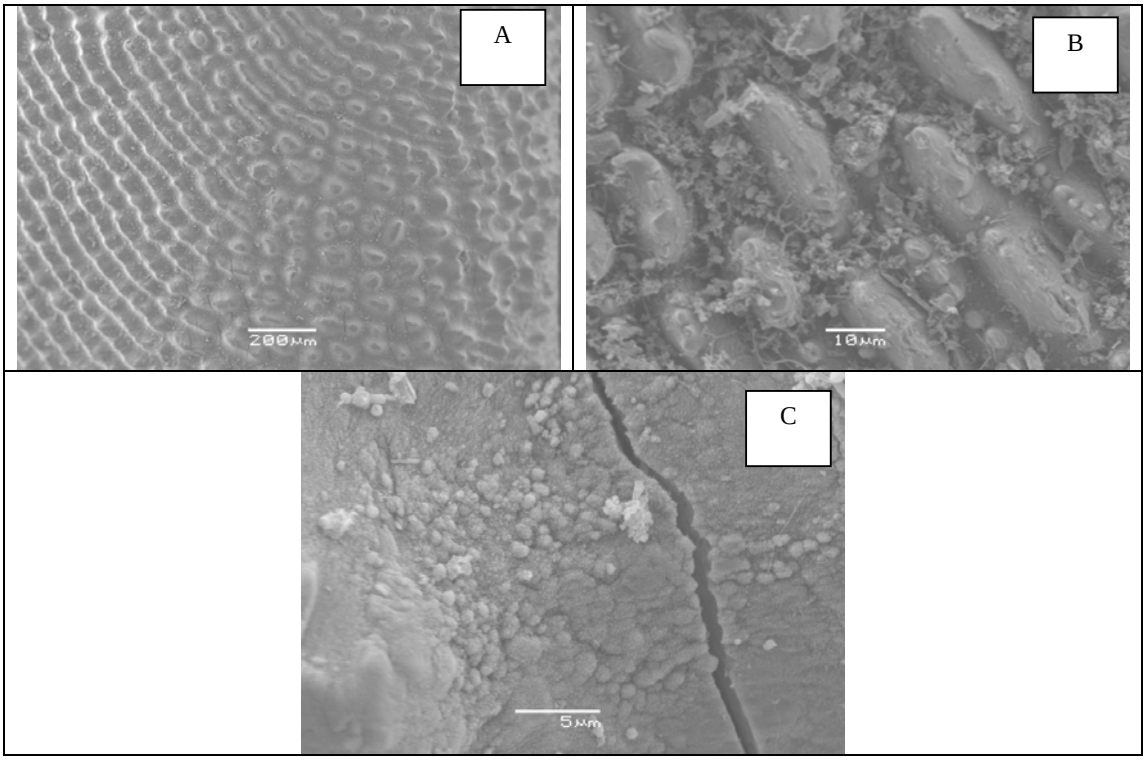

Figure-2. Scanning electron micrographs of Nile perch fish scales biofilms carriers at different stages of anaerobic digestion. (A) Before microbial colonization (mag. X 600). (B) After 140 days of start-up/microbial colonization (mag. X 3000) and (C) mag. X 5000).

Observation of micrograph picture (Figure-2A) showed that, the Nile perch fish scale before microbial colonization showed rough, uneven surface with crevices and microscopic ridges. Micrograph picture of Nile perch fish scales after feeding and stabilization showed good attachment and entrapment of microbes (Figure-2 B and C). In this study, micrograph picture of sisal fibers and pumice stones biofilms carriers before and after colonization of microbes were not taken since they have been described in details by [18]. The sisal fibers and pumice stones had rough surface, high porosity, pores, variable macrostructures as well microscopic ridges, which allowed microbial attachment to the two carriers [18]. Based on Nile perch fish scales micrograph observation (Figure-2 B and C) and those micrograph of sisal fibers and pumice stones reported by [18] it evident that Nile perch fish scales displayed good properties as carriers for biofilms attachment. It has been shown that in anaerobic filters, the organic removal contribution from entrapped biomass is relatively significant [26, 27]. Hence the amounts of suspended biomass retained by sisal fiber waste and Nile perch fish scale waste may have played an important role in the observed differences in process performance [18]. Comparison of biodegradation of biofilms carriers after nine months (270 days) of the entire experimental period showed that around 40% of sisal fiber waste and about 10% of the Nile perch fish scale were degraded. Although, clogging of the tube problems was encountered in Nile perch fish scales biofilms carriers but could not stop the process since it was amenable. This finding is important and it illustrates that Nile perch fish scale waste is a novel biofilms carrier which could be successfully applied in anaerobic biotechnology systems treating fish solid waste and probably other similar wastes for long time without serious technical operational problems.

Total volatile fatty acid profile degradation

It is well documented that high TVFA concentration in the anaerobic processes cause the inhibition of methanogenesis. Under conditions of overloading and in the presence of inhibitors, methanogenic activity cannot remove hydrogen and volatile organic acids as quickly as they are produced. The

result is the accumulation of acids and the depression of pH to levels that also inhibit the hydrolysis or acidogenesis phase [28, 29]. It has also been shown that even when process pH is optimal, the accumulation of TVFAs may contribute to a reduced rate of hydrolysis of the solid organic substrate [20, 28]. Organic acids such as acetic, propionic, butyric and isobutyric are usually taken as central to evaluating the performance of anaerobic digestion [1, 30]. The TVFA concentration of the three anaerobic packed-bed bioreactor is shown in (Figure-3). During the start-up period TVFA concentration for bioreactor packed with pumice stone was less than 2, 000 mg/l within the first 20 days and stabilized to < 100 mg/l for the rest of start-up period. For the bioreactor packed with sisal fiber waste TVFA concentration decreased from 12,000 mg/l to 2, 000 mg/l for the first 20 days, and remained at 2, 000 mg/l for another 20 days before stabilizing to < 100 mg/l for the rest of the period. For bioreactor packed with Nile perch fish scales TVFA concentration decreased from 30, 000 mg/l to 4000 mg/l for the first 40 days and remained at 4000 mg/l for 25 days before stabilizing to < 100 mg/l for the rest of the experimental period (Figure-3 A). The quick stabilization of bioreactor packed with pumice stones to TVFA level < 100 mg/l in the first 20 days of the operation showed that the little available easily degradable organic materials were quickly metabolized by microbes in the inoculum consequently decreased the TVFAs to low concentration. However the situation was different for bioreactor packed with sisal fiber waste and Nile perch fish scales, where the easily degradable organic materials concentration was higher as shown by TVFA and took longer time for microbes the metabolize the organic matter (Figure-3 A). During the start-up period no substrate feeding was made but feeding of 100 ml of pre-digested inoculum were done once every week to allow monitoring of anaerobic digestion parameters. The start-up period allowed microbial and structural stabilization to occur and TVFA concentration to decrease significantly. In both bioreactors, the feeding was initiated on day 140 at a background TVFA < 100 mg/l. The first feeding was done at OLR of 1gVS/l/d with HRT of 30 days (Table-3). The TVFA concentration for pumice bioreactor was raised to 3000 mg/l for the first week before stabilizing to 2000 mg/l. Bioreactor packed with sisal fiber waste and Nile perch fish scales TVFA concentration quickly metabolized and remained below 2, 000 mg/l, which was stable for the methanogenesis to occur. The TVFA concentration in all bioreactor remained at 2, 000 mg/l with an increase in OLR up to 6 gVS/l/d. However, the TVFA concentration increased to 3, 000 mg/l when the OLR was increased to 9 gVS/l/d for sisal fiber waste and pumice stones packed bioreactors. On the other hand, for Nile perch fish scales packed bioreactor TVFA concentration was well below 2, 000 mg/l at 9 gVS/l/d (Figure-3B).

Table-3. Biological pre-treated FSW feeding regimes of packed bed anaerobic bioreactors at fixed hydraulic retention time.

Day	**OLR** (gVS/l/d)	**HRT** (d)
140-170	1	30
171-200	3	30
201-230	6	30
231-260	9	30
261-290	12	30

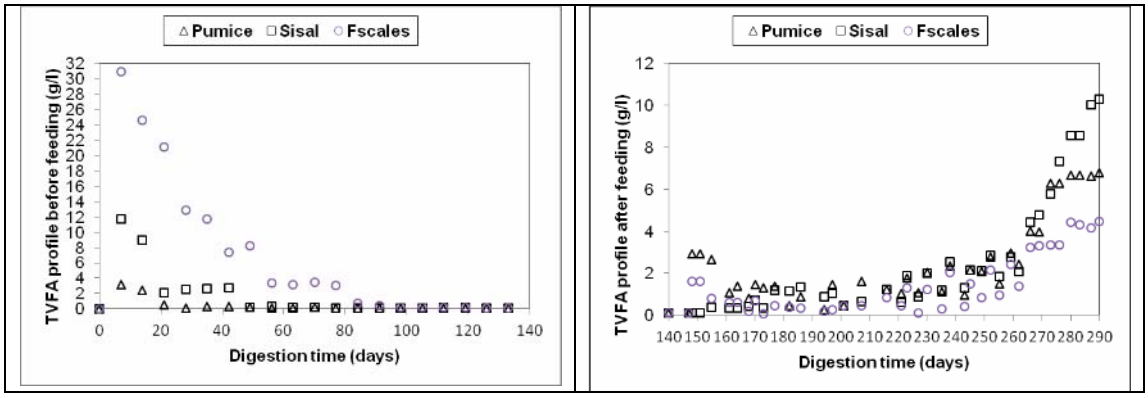

Figure-3. Total VFA profile before (A) and after (B) feeding the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

For pumice packed bioreactor, when TVFA concentration started to increase, the bioreactor started foaming (Figure-4). As a measure feeding was stopped to allow the bioreactor to recovery. However the TVFA concentration continued to increase and reached 6, 000 mg/l. Despite an increase in TVFA concentration, the pH of all bioreactors ranged from 8- 8.3 at OLR of 1-3 (gVS/l/d) and pH 8.5-8.7 at OLR of 6-12 (gVS/l/d (Figure-5). It has been observed previously that an accumulation of VFAs results in a decrease in pH and finally lead to failure of the methanogenic stage and the whole degradation process [31]. pH has been considered an unreliable monitoring parameter depending on variations in buffering capacity [32]. The use of pH for monitoring

process failure can only be recommended in systems where the buffering capacity is low and also in systems without biofilms carriers (support materials). The pH has been found to be higher in reactors with growth-support material than in reactors without support materials [19]. Slight change in substrate composition may affect the buffering capacity of the system, and a process imbalance causing considerable accumulation of VFAs, which can be masked by buffering effect [32]. Results from this experiment showed that despite the increase in TVFA the bioreactors packed with Nile perch fish scale was stable enough without leading to failure in degradation process. The increase in TVFA was a problem to pumice stones packed bioreactor and resulted in methanogenic and degradation process failure, which demonstrate that the microbial activity in the bioreactor was inhibited at a high OLR [18].

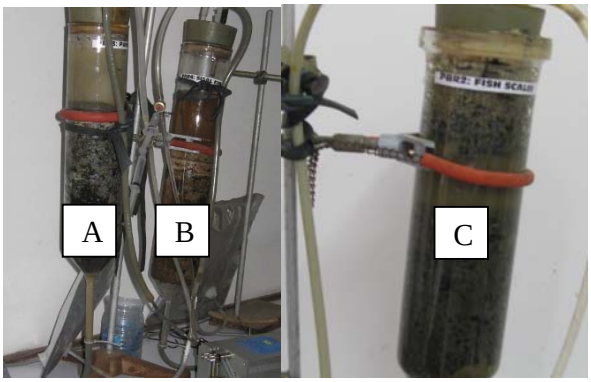

Figure-4. Pumice stones packed bed anaerobic bioreactor showing foaming (A) after 200 days of feeding. Sisal fiber waste (B) and Nile perch fish scales (C) packed bed anaerobic bioreactors did not show any foaming after 200 days of feeding.

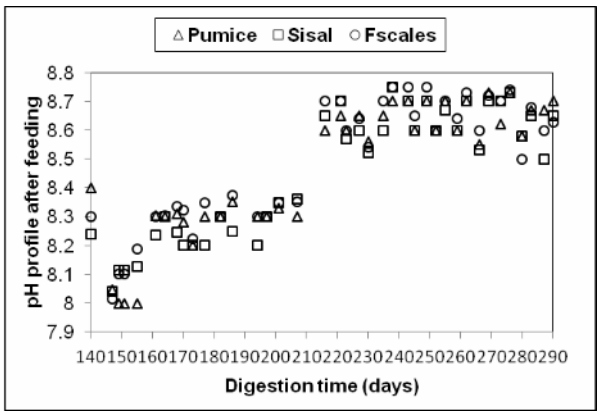

Figure-5. pH profile after feeding the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

Measuring alkalinity provides a picture of how safe the bioreactor is in relation to buffering acidic loadings. During feeding process, the buffering capacity of bioreactor started above 5000 mg CaCO3/l and slowly increased to 20, 000 mg/l for bioreactor packed with Nile perch fish scales and about 22, 000 mg/l for pumice stones and sisal fiber wastes packed bioreactors (Figure-6). Despite the higher buffering capacity of 22, 000 mg/l recorded for pumice packed bioreactors the process failed and stopped working at OLR 9 gVS/d/l probably due to ammonia inhibition on acidogenic methanogens [33, 34, 35, 36]. There are two different mechanisms attributed to ammonia inhibition of methanogens. In the first mechanism activities of methane synthesizing enzymes are directly inhibited by free ammonia. In the second one, hydrophilic free ammonia molecules diffuse passively into the cell and are rapidly converted to ammonium ion (NH_4^+) owing to the intercellular pH conditions Ammonium accumulate inside the cell and become toxic by altering the intracellular pH [37, 38, 39, 40, 41. 42]. The ammonia fraction in the bioreactors have been found to increases with an increase in temperature and pH, at high pH (see Figure-5) the free ammonia concentration will be up to ten times higher than the free ammonia concentrations reported as inhibitory and the effect is more pronounced in propionate degrading Acetogenic bacteria than in methanogenic Archaea [39, 40].

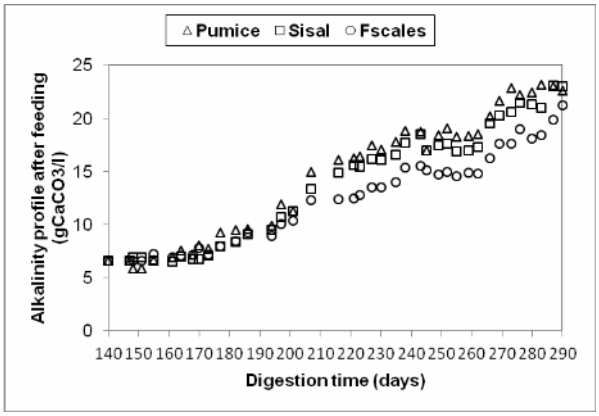

Figure-6. Total alkalinity profile after feeding the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

COD removal

Results in (Figure-7) showed temporal changes in the total soluble COD of the anaerobic packed bed bioreactors. At OLR of 1gVS/d/l the soluble COD reduction was relatively low around 22 - 40% (7-11gO2/l effluent COD) from 140 to 160 days of operation. The low COD removal observed was probably due to lag phase caused by microbial acclimatization to the fish solid waste. [12] Observed similar behavior when analyzing pharmaceutical wastewater using packed bed bioreactor system. From day 180 -240 of operations, the COD removal was around 80-90% (0.6 -1.5 gO2/l effluent COD) when OLR was increased up to 6 gVS/d/l. However when the OLR was increased to 9-12 gVS/l/d the COD

removal efficiency decreased gradually up to the range of 60-70%. Soluble COD removal illustrated that as OLR was increased the increasing load of TVFA probably affected the methanogenesis. Fish solid waste contains high proportions of long chain fatty acids (LCFA) that needs or require long hydrolytic retention time for efficient treatment [33]. At higher OLR, the COD removal efficiency was not good for pumice packed bioreactor since at that OLR the bioreactor performance was very poor. This finding is in consistent with observations reported previous by other researchers [37]. Using UASB to treat pharmaceutical wastewater it was found that the COD removal efficiency was 90% at an OLR of 1.5 kg COD/m^3/d at HRT of 11 days. However by increasing OLR to 2 kg COD/m^3/d with HRT of 7 days, the COD removal efficiency dropped dramatically to 70%. Other researchers have also demonstrated that substrate removal efficiency increases with an increase in HRT when herbal based pharmaceutical wastewater was treated anaerobically using fixed bed bioreactor [35].

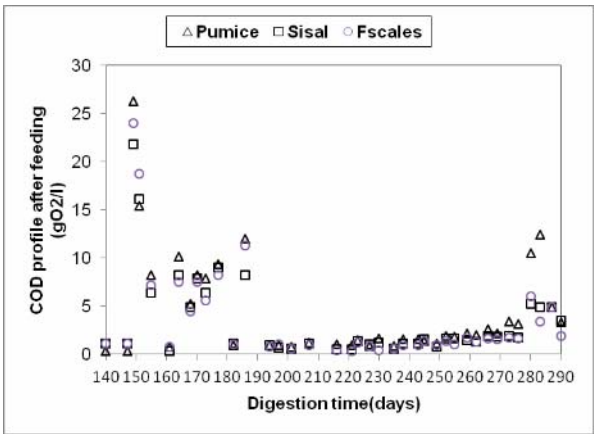

Figure-7. Total soluble COD profile after feeding the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

Biogas composition and methane yield

Biogas composition was monitored in order to assess the performance the three anaerobic packed bed bioreactors in gas phase. Results in (Figure-8) showed that all the three anaerobic packed bed bioreactors had relatively higher levels of methane concentration (around 70-80%) at OLR range of 1to 6 gVSl/d. On the other hand when the OLR was increased to 9-12 gVS/l/d methane content was reduced to (60-70%) for sisal fiber waste and Nile perch fish scale anaerobic packed bed bioreactors while for pumice stones anaerobic packed bed bioreactor methane concentration was further reduced to 50%. Considering the changes in VFA concentration that occurred with an increase in OLR, (Figure-3B) it is likely that this was adversely affected by physic-chemical condition created by the acidogens at higher level of OLR.

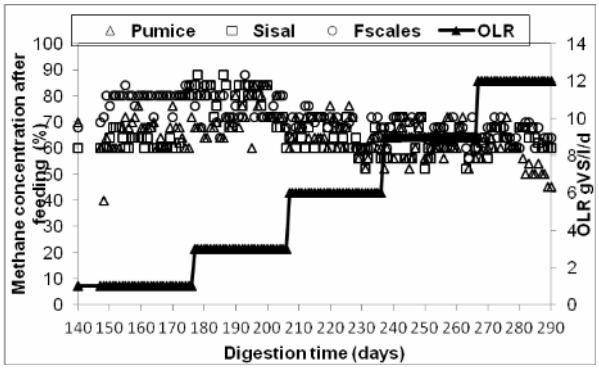

Figure-8. The methane concentration (%) profile after feeding the the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

Figure-9 showed the methane yield which increased with an increase in OLR. However, after each feeding, the methane yield dropped before increasing again to a higher level. Methane yield from bioreactors packed with NPFS and Sisal fibers were significantly higher (1.52 and 1.57 l CH4/gVS added) respectively compared to pumice packed bioreactors (1.34 l CH4/gVSadded). Similar kind of behaviour was obtained by [43] when studying energy recovery from fruit and vegetable solid wastes.

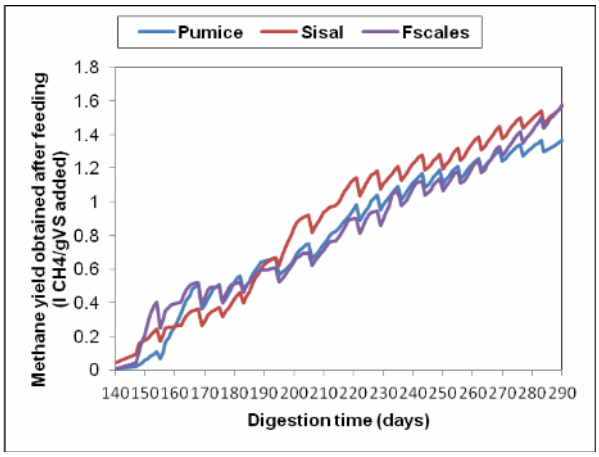

Figure-9. The methane yield profile after feeding the sisal fiber wastes, pumice stones and Nile perch fish scales anaerobic packed bed bioreactors with biological pre-treated FSW at different organic loading rates.

CONCLUSIONS

This is the first study report on the utilization of Nile perch fish scales waste as a biofilms carrier in anaerobic digestion of biological pre-treated fish solid waste. From the results obtained it can be concluded that the performance of the packed bed bioreactor containing Nile perch fish scales waste as a biofilms carrier worked similar to sisal fiber waste biofilm carrier but superior to

pumice stones as biofilm carrier. Similar trend was obtained in methane yield, where Nile perch fish scale and sisal fiber bioreactors produced significantly higher methane yield compared to pumice packed bioreactor. Nile perch fish scale waste is an appropriate biofilms carrier to use in methanogenic bioreactors digesting fish solid waste. Higher COD removal and degradation efficiency of VFA as well as an effluent with high pH and high alkalinity were observed. Furthermore, Nile perch fish scale carrier did not suffer from clogging although some degradation of fish scales occurred. More benefit comes from the fact that both fish scales and fish solid waste are wastes produced in Nile perch fish processing factories, which makes scale-up at fish processing factory level cost-effective in terms of handling and transport. However, Nile perch fish scale bed increased in size with each OLR increase need further study to gain more understanding of seems self granulation typical of UASB. Furthermore, feasibility of pilot scale up remains to be investigated in Tanzanian context.

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