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ROLE OF VOLATILE FATTY ACIDS IN ACIDOGENIC AND METHANOGENIC REACTOR FOR TREATING PHARMACEUTICAL WASTEWATER

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Key words : *Acidogenesis Reactor (AR), Bi-phasic Methanogenesis (MR), Volatile fatty acid (VFA), pH, Pharmaceutical wastewater.*

Abstract – A laboratory scale of Bi-phasic digester with a working volume of 36.92 L was utilized to treat the actual time pharmaceutical wastewater with the bifurcation of acidogenic and methanogenic populations. The separation of acidogenic and methanogenic population in the individual reactor performed well with an effective volume of 6.15 L of acidogenic and 30.77 L of methanogenic reactor. In this study the acidogenic reactor was accomplished with suspended growth process and methanogenic reactor was utilized by attached growth process for improving the performance of the methanogenic bacteria. The reactor was operated under mesophilic conditions. The pH levels of the acidogenic and methanogenic reactors varied from 4.65 to 5.9 and 7.11 - 8.38. The maximum concentration of Volatile Fatty Acid in the acidogenic reactor was quantified as 6520 µg/L with an influent COD of 6786 mg/L at a HRT of 12 hours with an OLR of 6.268 kg COD/m^3 d and the minimum concentration was found 2128 mg/L at a HRT of 51 hours with an OLR of 3.578 kg COD/m^3 d. The result from this study visualized that the generation of VFA plays an important role for the generation of acidogenic microorganism in the acidogenic reactor and conversation of CH_4 and CO_2 in the methanogenic reactor for treating pharmaceutical wastewater.

INTRODUCTION

The pharmaceutical wastewater from the industry in worldwide were discharge under specific treatment approximately (Moore and Enick, 2007; Lang, 2006). Wastewater from pharmaceutical plants contains very higher concentration of organic and inorganic pollutants and also various salt concentrations (Halling-Sorensen, 1998; Schroder 1999; Sim *et al.*, 2010; Benitez *et al.*, 1995; Kilroy *et al.*, 1992). The anaerobic technology was a viable option to treat such a high strength wastewater with huge benefits in terms of biogas generation (Mahmoud 2008; Acharya *et al.*, 2008). The two stage anaerobic digestion process was utilized for kitchen waste by mixture of activated sludge (Zhang *et al.*, 2007); Waste Activated Sludge (Ghosh, 1991); Wine Distillery (Solera *et al.*, 2002); Distillery wastewater (Asha and Nehrukumar, 2007); vegetable and Fruit waste (Kasturi Dutta *et al.*, 2014). Anaerobic digestion requires a sequence of metabolic reactions that, as the main end product, sequentially reduce complex components in the feed to a mixture of carbon dioxide and methane. Those reactions are often simply referred to as fermentation of hydrolysis and methanogensis. Different physical–chemical conditions affect methane production, and inhibition of bacterial action by any substrate or product can be expected when their concentrations increase to limits. For example, the high concentrations of Volatile Fatty Acid (VFA) in the system cause methanogenesis inhibition (Marchaim, 1993; Masse, Droste, 2000; Van den Heuvel *et al.*, 1988; Fukuzaki *et al.*, 1990) difficult process involving hydrolysis, fermentative acidogenesis, acetogenesis and methanogenesis is digestion of organic wastes. Due to the high content of organic waste, the single phase anaerobic digestion of kitchen waste easily results in a subsequent accumulation of intermediate products resulted in a fall in pH, leading in unbalanced fermentation and a reduction in process stability (Jeyaseelan *et al.*,1995 and Ince, 1998). The two phase anaerobic digestion could optimize the condition for both the hydrolytic acidic group of bacteria and the acetogenic-methanogenic group and improve the stabilization of organics and gasification levels (Bhattacharya *et al.,* 1996; Bank and Wang, 1999; Yilmarzer and Yenign, 2002; Qi *et al*., 2003; Liu, *et al*., 2006) Several authors focused on this separately and splitting hydrolysis / acidgensis and methanogenesis to yield the maximum biogas for the mesophlilc and thermophilic condition overall reaction and process specific microorganisms present two-stage anaerobic digestion to expand the different biomass species to establish substrate conversion and enhance COD reduction and increase energy conservation percentage. Tong Zhang *et al.* (2015); Haiyan Wu *et al*. (2009); Zhou, *et al.* (2016) The initial pH value had obvious effect on the generation of methane and on the thermophilic anaerobic co-digestion process. Five different initial pH levels were measured with three different ratios of manure. After 35 days, all digesters in different initial pH displayed a diverse production of methane. The VFA/alkalinity ratio of the optimum reaction condition for methanogens operation was within a range of 0.1-0.3 and optimal condition that maximum total biogas production (146.32 mL/g Vs) was predicted at the 70% dung ratio and initial pH 6.81. The aim of this research article is the generation of VFA with respect to the impact on pH level in the acidogenic and methanogenic reactors with the support of suspended as well as attached growth biomasses.

MATERIALS AND METHODS

The biphasic digester with a working volume of 36.92liters has been chosen to study the well-organized decrease of COD by acidogenic and methanogenic populations separately for different operating conditions of pharmaceutical wastewater in real time. The experimental model was made up of Plexiglass. The digesters phased for 1:5 ratio, which comprised of 6.15 L of fermentative acidogenic and 30.77 L liquid volume of methanogenic phase. Both the reactors are kept in series with individual gas collectors. The Acidogenic reactor (AR) was accomplished with suspended growth process and the Methanogenic reactor (MR) was established with attached growth process. Biphasic reactor was operated by means of a model PP30EX peristaltic pump with diluted pharmaceutical wastewater from the influent tank. The coconut coir fiber was used in the methanogenic reactor as a bio carrier and the bio carrier specification as shown in the Table 1. The photographic view of coconut coir fiber is shown Figure 1. The production of gas was continuously measured individually by means of the water displacement method. The experimental arrangement is shown in Figure 2 and the physical feature of the experimental arrangement is shown in Table.2.

Table 1. Specifications of Coconut Coir Fiber

SI. No.	Parameters	Specifications
a.	Length	5 to 20cm
b.	Moisture	Maximum 10 percentage
c.	Color	Yellow
d.	Impurity	d” 8 percentage
e.	Bale weight	180 to 20 metric tonnes
f.	Load ability	40 feet High Cube Container
g.	Packing	Plastic band

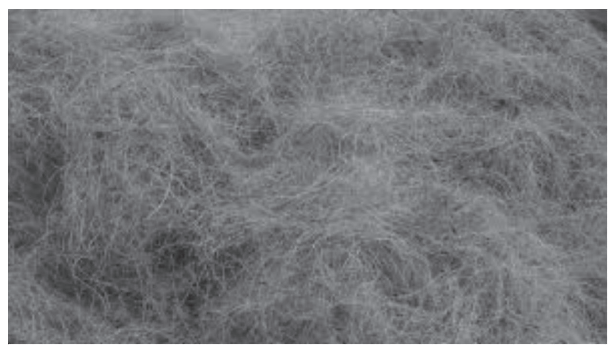

Fig. 1. Photographic view of the Coconut Coir Fiber

As per the (APHA 2017) method, the real time pharmaceutical wastewater collected from the M/S Life care Formulations, 91/5 Link Road Near, Mettupalayam Industrial Estate, Sonia Gandhi Nagar Extn, Mettupalayam, Puducherry 605009 was characterized the sample and analyzed as per procedures.

Inoculum

To stimulate the start process the active sludge collected from Annamalai university treatment unit and the active biomass plant of the pharmaceutical wastewater industries was utilized.

Start-up Process

The wastewater was collected from the treatment

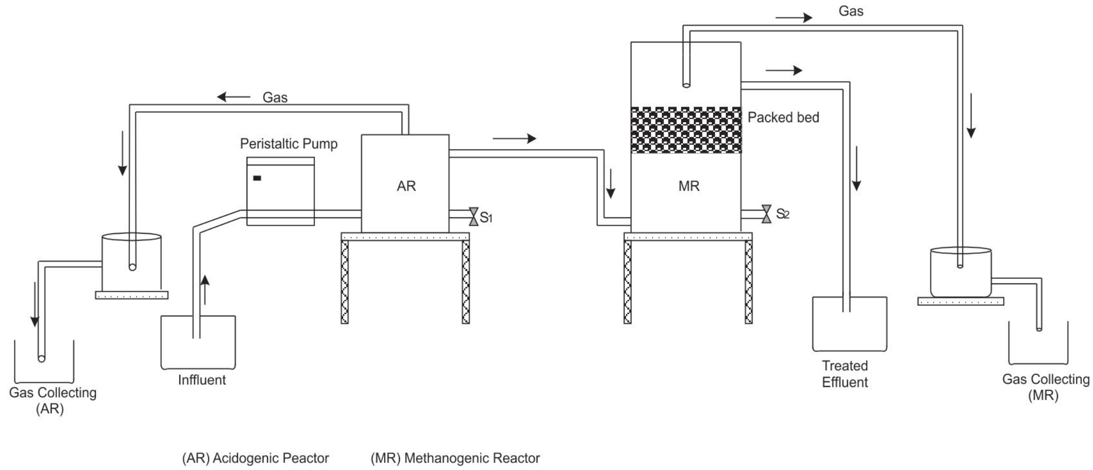

Fig. 2. Schematic Diagram of Experimental Set up

facility located at Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu for start- up of the reactor. During start up the digested slurry from the pharmaceutical waste was mixed for rapid acceleration. The experimental study was conducted in the Advanced Environmental Laboratory, Department of Civil Engineering, Annamalai University with a mesophilic range of temperature. The digester achieved at steady state conditions from the period of 18^{th} to 21^{st} day with a maximum removal efficiency of COD 92% during start-up (Figure 3 and 4) in the methanogenic reactor. As per Bala *et al.*, 2001, It is difficult to maintain the effective number of useful microorganisms in the reactor. But in our study we achieved steady state with short time span of 18 to 21 days by incorporating the suspended as well as attached growth process in the acidogenic and methanogenic reactors.

Table 2. Biphasic Anaerobic Bioreactor of Physical Features

Description	Measurements
Radius of the Acidogenic reactor, cm	7
Radius of the Methanogenic reactor , cm	15
Radius of inlet pipe & outlet pipe, cm	0.5
Effective volume of acidogenic reactor	6.15
Effective volume of methanogenic reactor	30.77
Total working volume of the digester, liters	36.92
Peristaltic pump	PP-30(EX)

The start-up stage of the process was conducted by continuous feeding of wastewater from Annamalai University wastewater collection unit with the pharmaceutical slurry to the reactor with

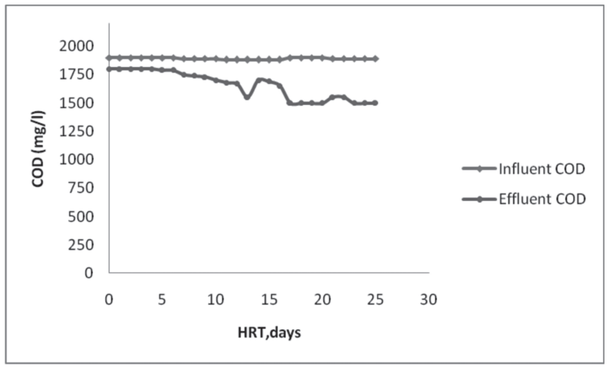

Fig. 3. Profiles of COD, mg/L in the Acidogenic Reactor during start-up process

an average influent COD concentration of 1890 mg/ L. The maximum COD removal efficiency were recovered and reached about 31% in acidogenic reactor and 93% in methanogenic Reactor 22 and 25days at the end of the start-up process.

During the start-up process, acidogenic phase, biogas production was gradually increases from

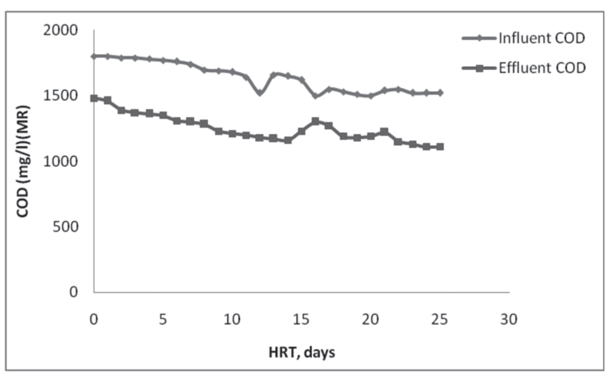

Fig. 4. Profiles of COD, mg/L in the Methanogenic Reactor during start-up process

0.002 to 0.004 m^3 of gas /kg COD removed Figure 6. Biogas rapidly increases in the methanogenic phase ranging from 0.02 to 0.024 m^3 /kg COD removed Figure 6 at an Organic Loading Rate of 0.520 kg COD/m^3day in acidogenic reactor and 0.418 kg COD/m^3 day in methanogenic reactor. After a steady state, pharmaceutical wastewater was gradually allowed in the 20%, 40%, 60%, 80% and100% ratio to the reactor.

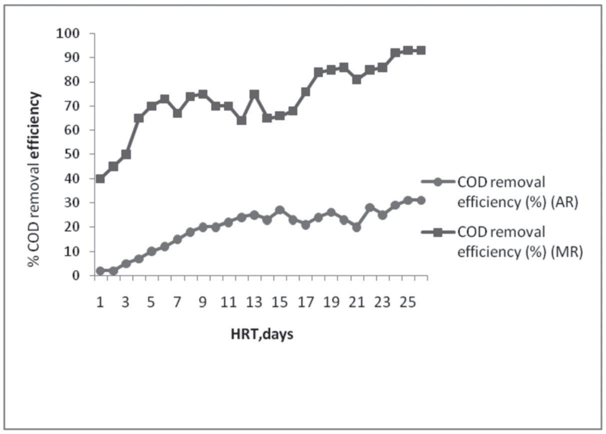

Fig. 5. COD removal efficiency profile in acidogenic and methanogenic reactor during start-up process with respect to hydraulic retention time.

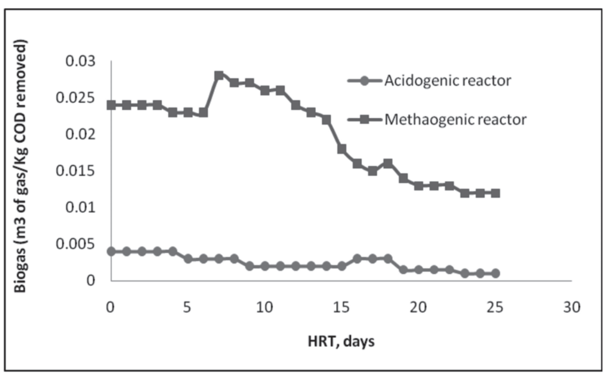

Fig. 6. Profile of Biogas production and HRT, days in acidogenic and methanogenic reactor during start-up.

RESULTS AND DISCUSSION

The diluted pharmaceutical wastewater of varying average influent COD concentration utilized to the acidogenic reactor were 1858, 2864, 4051, 5180, and 6786 mg/L. The supernatant from the acidogenic reactor was used through the peristaltic pump PP 30EX as an influent to the methanogenic reactor with an average COD of 1412, 2198, 3174, 4291, and 5526 mg/L. The influent flow rate was from 2.59, 3.456, 4.32, 6.048, 7.776, 11.23, 15.12, 19.44 l/day with the HRT of 5, 7, 12, 19, 24, 31, 48, 51 hours for acidogenic and 24, 48, 72, 96, 120, 168, 192, 288 hours in the methanogenic reactor. The reactor was operated at the temperature of mesophilic range varied from 26ºC – 37ºC during this study. By recording the room temperature on daily basis, the reactor performances were studied at different OLR ranging from 1.448 kg COD/m^3 .d to 30.800 kg COD/m^3.d in the acidogenic reactor and methanogenic reactor, the OLR was ranging from 0.364 kg COD/m^3.d to 9.435 kg COD/m^3.d.

The pH level in the acidogenic reactor was identified from 4.65 to 5.9 (Figure, 7) and 7.11to 8.38 in the methanogenic reactor (Figure 8). The pH level at 5hours HRT was identified as 5.66 and further the pH level was incremental up to 24th hours of the continuous study. Again the pH level was detrimental up to 51 hours, which showed that the healthy population in the acidogenic reactor with an average influent COD of 1858 mg/L. The pH level at 5hours HRT was identified as 5.39 and further the pH level was raise up to 19 hours of the continuous study. Again the pH level was fall up to 48 hours, which showed that the healthy microorganism in the acidogenic reactor with an average influent COD of 2864 mg/L. The level of pH at 5 hours hydraulic

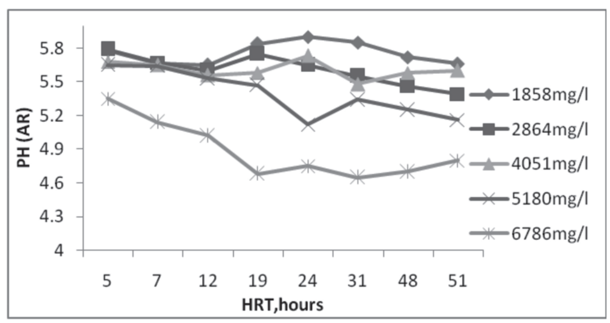

Fig. 7. Profiles on Hydraulic retention time in hours on the pH in Acidogenic reactor

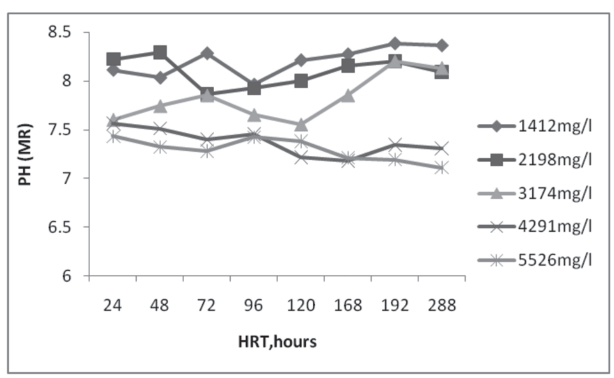

Fig. 8. Profiles on Hydraulic retention time in hours on the pH in Methanogenic reactor

retention time was identified as 5.6 and further the level of pH was raise up to 19 hours of the continuous study. Again the pH level was dropped upto 48 hours, which showed that the healthy microorganism in the acidogenic reactor with an average influent COD of 4051 mg/L. The pH level at 5 hours HRT was found as 5.16 and further the pH level was fall in 19 hours of the continuous study. Again the pH level was raise up to 51 hours, which showed that the well micro-organism in the acidogenic reactor with an average influent COD of 5180mg/L.

The pH level at 5 hours HRT was identified as 4.8 and further the pH level was incremental up to 19th hours of the continuous study. Again the pH level was detrimental up to 51 hours, which showed that the healthy population in the acidogenic reactor with an average influent COD of 6786 mg/L.

The maximum Volatile Fatty Acid was identified as 6520 mg/L in the acidogenic reactor with an influent COD of 6786 mg/L. At this stage the pH was quantified as 4.65 with an OLR of 6.268 kg COD/m^3.d. The Volatile Fatty Acid (VFA) levels in all five stages in acidogenic and methanogenic phase were computed with respect to HRT is shown in (Figure 11 and 12). The VFA concentration was initially increased with the increase of OLR and a minimum interruption was identified with respect to the decline level of pH in the acidogenic reactor. The digestion path way is strongly depending on the levels of pH only. In this study the dropping of pH showed that the health population of acidogenic in the acidogenic reactor. The production of Volatile Fatty Acid was dominated in acidogenic reactor. An obvious effect on biogas production was also identified in acidogenic reactor due to the decreased levels of pH. The maximum pH level in the methanogenic reactor was identified as 8.38 with a HRT of 48 hours at an OLR of 6.683 kg COD/m^3.d (Figure 8). With the support of incremental levels of pH in the methanogeic reactor, the conversion of Volatile Fatty Acid (VFA) was taken place and attained a maximum production of biogas in the methanogenic reactor and the VFA level in the methanogenic reactor was estimated from 450 to 884 mg/L and 2128 to 6520 mg/L in the acidogenic reactor. The biogas conversion in the acidogenic reactor with respect to the VFA generation was ranging from 0.009 to 0.053m^3 of gas/kg COD removed (Fig. 9) and the biogas production due to the conversion of VFA in the methanogenic reactor was estimated from 0.047 to 0.09 m^3 of gas/kg COD removed (Fig. 10).

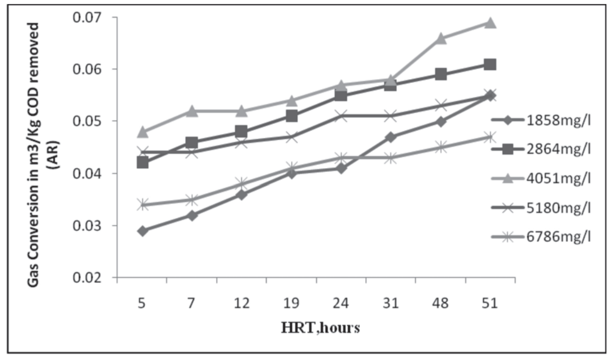

Fig. 9. Profiles on Hydraulic retention time, hours in the Gas conversation in m^3/kg COD removed (AR)

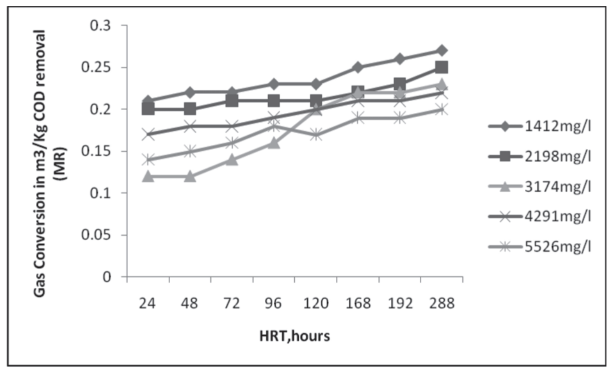

Fig. 10. Profiles on Hydraulic retention time, hours in the Gas conversation in m^3/kg COD removed (MR)

SEM Image of the Acidogenisis effluent

In a scanning Electron microscopy the sample was observed under different magnifications. Most of the research focused on the distribution of microbial population in the ABR, and the finding revealed slight variations in the distribution of microbial population under various experimental conditions. (Uyanik and Sallis, 2003). In this study, the sludge was taken for SEM examination.

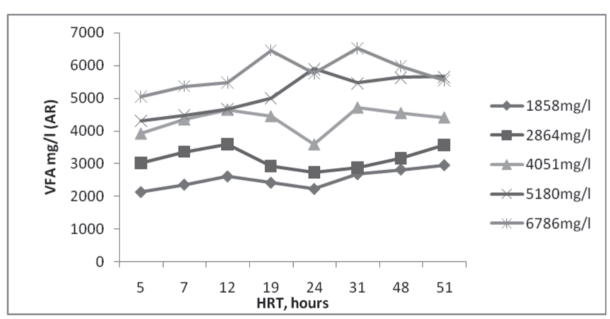

Fig. 11. Profiles on Hydraulic retention time in hours on the VFA mg/L in Acidogenic reactor

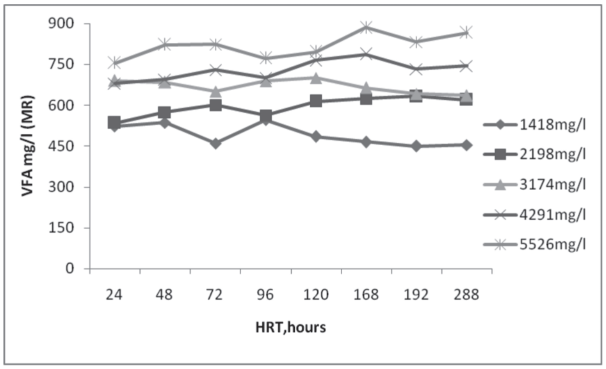

Fig. 12. Profiles on HRT in hours on the VFA mg/L in Methanogenic reactor.

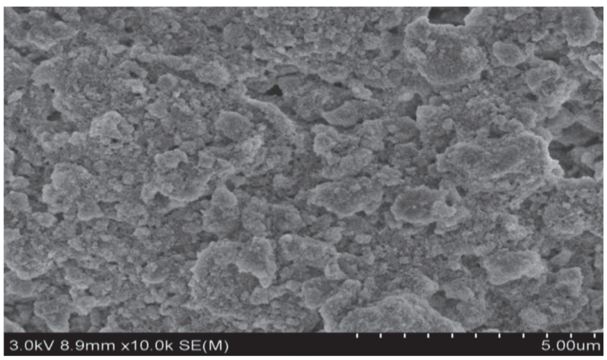

Fig. 13. SEM Image of the Acidogenic effluent

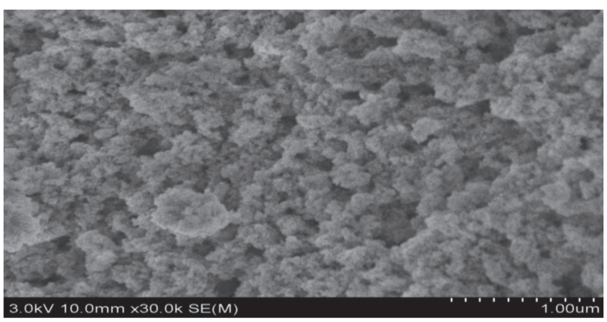

Fig. 14. SEM Image of the methanogenic effluent

SEM Image of the Methonogenic effluent

In an Electron Microscopy scanning the sample was observed under different magnification. Most of the studies concentrated on the microbial population in the ABR, and the result showed partial differences in the distribution of microbial population under different experiment conditions. (Uyanik and Sallis, 2003). In this study, the sludge was taken for SEM examination

CONCLUSION

A suitable and stable pH was maintained in both the Acidoogenic and Methanogenic reactor for the generation and conversion of VFA. The diluted pharmaceutical wastewater utilized in this study was ranging from 1456 mg/L to 7000 mg/L. The level of pH in the acidogenic reactor was from 5.02 to 5.9 and 7.0 to 8.4 in the methanogenic reactor. The minimum volatile fatty acid generation in the acidogenic reactor was 2128 mg/L at an OLR of 3.578 kg COD/m^3.d with a HRT of 51 hours and the maximum VFA generation with respect to HRT was 6520 mg/L at an OLR of 6.268 kg COD/m^3 d with a hydraulic retention time of 12 hours. The results showed that pH range in the reactor made favorable condition for bacterial growth in the digester and produced better biogas yield. The Volatile Fatty Acid level in Acidogenic Reactor was from 3152 to 6254 mg/L and in the case of Methanogenic Reactor from 555 to 1000 mg/L. The maximum pH in the methanogenic reactor was 8.38 with a HRT of 48 hours and minimum was 7.11 at a HRT of 24 hours. The maximum volatile fatty acid converted in the Methanogenic Reactor was 884 mg/L at an OLR of 1.728 kg COD/m^3 d and minimum was estimated at 450 mg/L at an OLR of 6,683 kg COD/m^3 d with a HRT of 48 hours. The maximum Biogas was quantified in the overall reactor was 0.31 m^3 of gas/ kg COD removed.

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