D.Hemusaiprakashreddy et al /J. Pharm. Sci. & Res. Vol. 12(4), 2020, 545-548

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evolution of microorganisms in thermophilic dry anaerobic digestion

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Abstract

Microbial population dynamics were studied during the start-up and stabilization periods in thermophilic- dry anaerobic digestion at lab-scale. The experimental protocol was defined to quantify Eubacteria and Archaea using Fluorescent in situ hybridization (FISH) in a continuously stirred tank reactor (CSTR), without recycling solids. The reactor was subjected to a program of steady-state operation over a range of the retention times from 35 to 25 days, with an organic loading rate between 4.44 and 7.8 of volatile solid/m3 /day. Changes in microbial concentrations were linked to traditional performance parameters such as biogas production and VS removal. The relations of Eubacteria: Archaea and H2-utilising methanogens: acetate-utilizing methanogens were 82:11 and 12:1, respectively, during the start-up stage. Hydrogenotrophic methanogens, although important in the initial phase of the reactor start-up, were displaced by acetoclastic methanogens at steady-state, thus their relation was 8:34. The methane yield coefficient, the methane content in the biogas and Vs Removal were stabilized around 0.40LCH4/gCOD, 52%, and 82%, respectively. Methanogenic population correlated well with performancemeasurements

INTRODUCTION

Anaerobic digestion has been widely used as a suitable treatment for organic waste, including the organic fraction of municipal solid waste (OFMSW) (Akao et al., 1992; Moorhead and Nordstedt, 1993). This process has many advantages and these include a low sludge generation, reduced energy consumption, and high methane production. The main disadvantage of anaerobic digestion is its slowness (Chanakya et al., 1992). Anaerobic processes operating under thermophilic (56 C) conditions have attracted a great deal of attention in recent years due to their apparent advantages, which include high pathogen destruction, enhanced hydrolysis of complex organic/biological materials, and foaming reduction (Hartmann and Ahring, 2005). Besides, with this technology two residual effluents are produced: biogas (mainly methane and carbon dioxide) which can be used as an energy source and a liquid effluent which could be used as a soil conditioner due to its physicochemical properties (Flotats et al., 1997).

Anaerobic digestion of the organic fraction of municipal solid waste (OFMSW), which is ultimately converted into methane and carbon dioxide, is carried out by the coordinated action of various groups of microorganisms and goes through several intermediate stages. The intermediary products are volatile fatty acids, acetic, propionic, and butyric acids. Two-thirds or more of the methane produced in anaerobic bioreactors is derived from acetate (Zinder, 1993). The conversion of acetate to methane by methanogenic populations becomes the rate-limiting step in biogas production, as methanogens are known for their slow growth, resulting in a relatively small population size (Zinder, 1993). The methanogens occupy the terminal position in the anaerobic food chain and are normally divided into two main groups based on their substrate conversion capabilities. Acetoclastic methanogens are capable of converting acetate to methane and carbon dioxide and are regarded as playing a dominant role in methane production since ca. 72% of the methane produced in disasters comes from acetate (Zinder, 1993). Hydrogenotrophic methanogens convert H2/CO2to methane. These species also play a key role in the overall process by maintaining the very low partial pressures of H2 (<10 Pa) necessary for the functioning of the intermediate trophic group, the syntrophic bacteria, which are responsible for the conversion of acids-organic and alcohol intermediates to direct methane precursors (Pauss 1990).

The parameters normally employed in the control of anaerobic digestion, such as the percentage of COD removal, the concentration of volatile fatty acids, and the amount and composition of biogas generated inthe process are not always representative of the composition and physiological state of biomass containedwithin the system. From a practical standpoint, given the importance of methanogens in anaerobic treatment processes, the ability to monitor methanogens and understand their ecology is essential to make effective controls of the start-up and operation of anaerobic bioreactors possible.

Consequently, and in order to acquire more detailed information in respect of this biomass, other parameters have also been used in the characterization of the microorganisms responsible for the anaerobic processes. Direct count procedures by microscopic methods yield the highest estimates of members of microorganisms and are occasionally used for indirect calculation of biomass. Epifluorescence microscopy with fluorometric stains are widely used for direct counting of microorganisms, since it does not require culturing (Kepner and Pratt, 1994).

There are, however, several drawbacks to direct observational methods, including the inability to distinguish living from dead microorganisms and the inability to perform further studies on the observed microorganisms. Because of this, an important avenue of

research has been the development and utilization of molecular techniques. Molecular techniques have successfully been applied for the direct detection and identification in situ of individual microbial cells and have therefore been used to monitor the spatial distribution of microorganisms in environmental samples and treat systems. However, it must be kept in mind that the physiological state (living or non-living) is operationally defined and based on the general properties of a particular stain, and dormant or extremely slow-growing cells cannot be detected (Williams et al., 1998). Whole-cell fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled phylogenetic oligonucleotide probes to detect specific whole cells/organisms in biological samples. DeLong et al. (1989) first demonstrated its use with bacteria. It can be a valuable tool for the study of microbial dynamics in natural environments (Hugenholtz et al., 2001; Davenport and Curtis, 2004). For instance, it is possible to carry out a hierarchical phylogenetic analysis on a particular environment to identify the dominant groups of microorganisms present, after which temporal and spatial changes in the diversity and abundance of specific microbial population can be monitored in relation to environmental effects (Amann et al., 2001; Head et al., 1998; Zheng et al., 2006). Two good examples can be found in Sekiguchi et al. (1999), who used FISH to study the morphology of the flock in a UASB reactor, and Santegoeds et al. (1999), who studied the morphology of aggregates present in three UASB lab-scale reactors. Raskin et al. (1994a, b) have used to identify and quantify species and genus of methanogens present in anaerobic reactors. The microorganisms in anaerobic reactors belong to three domains: Bacteria, Archaea, and Eucarya. Bacteria are the majority of the microorganisms in the reactors, Archaea are present in smaller amounts and Eucarya is present at very low levels (below 2% in most cases) which it indicates that anaerobic protozoa likely is not abundant in anaerobic digesters (Griffin et al., 1998).

Microbial characterization of the inoculum

Since the methanogenesis is critically important during start-up, we determined methanogens levels in the inoculum using oligonucleotide hybridization probes . The inoculum contained a percentage of Archaea, approximately 24%. This value is higher than those obtained by Griffin et al. (1998) (12.1%) and McMahon et al. (2001, 2004) (5.52%). Therefore, high-content methanogens in our inoculum could be due to its previous acclimation and the utilization of anaerobic sludge and leachate as a source of inoculum. In fact, the protocol used allows a reduction in the time necessary for the start-up and stabilization stages of a thermophilic anaerobic reactor operating with a high solid concentration (30% TS). This procedure uses SEBAC (Sequencing Batch Anaerobic Composting) technology to adapt an inoculum to the solid waste and the operational conditions prior to seeding the reactor.

The relation of main methanogenic group (H2-utilising methanogens and acetoclastic methanogens) present in the inoculum was 15:8, respectively. Previous work (Griffin et al., 1998) showed levels of H_2-utilising methanogens lower than obtained in our case. The high percentage of H_2-utilising methanogens in the inoculum used could explain the very fast start-up of our disaster due to the key role played for this microbial group asH_2 Consumers during hydrolytic/acidogenic stage.

Digester performance and microbial population dynamics

The reactor was started under thermophilic conditions (60°C) and four organic loading rates (OLR0) were assayed in order to study the dynamics of microbial population during the start-up and stabilization phase of anaerobic process. The organic loading rate added to the system was modified, but a constant organic loading rate (expressed as aV_S/M^3 /day) was maintained in eachperiod.

The microbial community structure was evaluated in combination with physicochemical parameters to assess digester performance during start-up and stabilization periods. A selection of rRNA-oligonucluotide probes was used to determine the concentrations of the main domains (Eubacteria and Archaea) and H_2- utilising and acetoclastics methanogens in samples collected from the reactor during the course of the experiment.

Performance and operating parameters for the control of the anaerobic process are shown in (Ferna´ndez- Gu¨elfo et al., 2005). Results of microorganisms obtained by FISH 3. All the results shown are the average values for each OLR assayed. The dynamics of microbial populations are represented in. The sum of the relative amounts of Eubacteria and Archaea was estimated as 100% because the main anaerobic groups in the anaerobic reactors are contained within these two domains (Griffin et al., 1998).

In the first stage, the OLR0 imposed was relatively slow (5.42 kg volatile solids/m3 /day) in order to check if the system evolved appropriately. This value reported in the literature. Bolzonella et al. (2003) carried out a start-up phase in the mesophilic range with an extremely low organic loading rate – less than 1 kg volatile solids/M^3 /day – for approximately 42 days, and continuing subsequently with an increase in the OLR and temperature. As consequence, the start-up period described in the literature are around 250 days (Bolzonella et al., 2003; Sebastien et al., 2002), whereas the start-up of the reactor studied was reached to 15 days approximately as described in Section 2.3. This can be explained by considering that, in our case, the start- up phase was carried out using inoculum adapted to the waste and operational conditions: i.e., thermophilic range and dry condition, with a high content of H_2-utilising methanogens.

During the hydrolysis phase complex molecules are transforming into other's simpler products, without methane production. In the first days, fermenters can acclimate more quickly to new conditions because of

their relatively high growth rates, while methanogens grow much slower. Hydrogen, carbon dioxide, and butyrate were the main products of the fermentation pathways of hydrolytic and fermentative bacteria. Because of the metabolic capacity of methanogens was initially not sufficient to balance the increasing activity of the fermenters, acetate, and hydrogen were not consumed at the same rate at which they are produced. In this sense, even though significant levels of methanogens were present in the disaster, they were apparently not able to adjust within 1 day to operation conditions, as demonstrate by the low methane levels in the bios on the first stage. Under these conditions, the electron flux through reduced intermediates (butyrate) increased In fact, Eubacteria were the main group in the reactor (88%) while Archaea were maintained constant 14%, except on the first day. Thus, the gas production rate was low and its composition was usual for hydrolytic phase:H_2(20%) and Co_2 (80%).

The total methanogen concentrations remained relatively constant during the first few days of operation, because the loss in acetate-utilizing methanogens was compensated by an increase in the H_2-utilising methanogens levels. These microorganisms are the most important H_2- consumer in the acidogenic phase. The latter apparently served as the main hydrogen scavengers during this period of rapidly increasing activity, reflected by rapid increases in the gas production rate and the level of methane in the biogas during the next stage. When the levels of H_2-utilising methanogens increased, butyrate levels also decreased after 8 days of operation in the digester. However, acetate levels increased continuously during all studied period. The relation between H_2-utilising methanogens and acetate-utilizing methanogens was 21:2 during start-up stage.

The results obtained in the first 14 days were favorable and therefore the organic loading rate was increased to 5.05 of v_s/m^3 /day. During this stage, the methane content in the biomass had increased until reaching 43% at 30 days. This can be explained by the VFA biodegradation by acetogens and subsequent methane generation by acetate-utilizing methanogens. In fact, during this period, the decrease of the hydrogen content was accompanied by an increase of acetate-utilizing methanogens until these became a 23% of Archaea. After a short start-up period (30 days) stable performance was observed with high organic removal efficiency (88% VS), high gas production rates (1.94 v_s /m^3 / day) and substantial levels of methane in the biogas (45%). The increase of Archaea was higher than Eubacteria, thus the relation between them was 32:62, respectively. The rise of stable performance was paralleled by an increase in acetoclastic methanogens.

In the OLR0 5.72 of V_S/m^3 /day period, after a slight decrease in the Archaea, these was recovered, no accumulation of great amount of VFA because of control of pH by controller on/off connected system. Nevertheless, the volume of biogas generated and methane yield coefficient decreased from 1.94 to 1.16 m3 /m3 /day and from 0.42 to 0.34 LCH4/gCODdegraded, respectively. This was because most of the initial residue which the reactor was loaded had been degraded. However, the methane content in the biomass increased from 25% to 48% . At the end of this stage, the composition of biogas was stabilized with values of co2and CH4 at around 50%, which indicated that the balance between the different microbial populations involved in the digestion was reached. The relative abundance between Eubacteria and Archaea was 61:39, while acetoclastic constituted 33% of those all of methanogens.

In the last period, the microorganisms concentrations increased since there was an increased of OLR0 (7.5 kg V_S/m3 /day). The relation Eubacteria and Archaea were 60:40, with 32% of acetoclastic methanogens. These values were practically similar to those obtained in the previous stage and according to the physicochemical parameters. Thus, the organic removal efficiency and methane yield coefficient were maintained constant around 80% VTS and 0.30 LCH4/gCODdegraded, respectively.

The gas production rate increased to 1.36 m^3 /day due to the higher organic loading rate. This result was compared favorably to those obtained in other studies where gas production rates varied between 1.6 and 1.7 m^3 /day (Stroot et al., 2001). The VS removal obtained to our study (88%) was better compared to data provided in the literature (67–68%) (Stroot et al., 2001). However, an increase of organic loading rate is accompanied by an increased of acetate concentration. Thus, acetatoclastic methanogens were nor able to consume acetate quickly in the digester to prevent acid accumulation. A history of high acetate concentrations appears to select for a population of methanogens capable of more rapid acetate turnover. This effect was observed previously in a similar system (Griffin et al., 1998; McMahon et al., 2001), confirming previous hypothesis (Zinder, 1993): Methanosarcina (generalist with high growth rates at elevated acetate concentrations) should be favored in systems with significant acetate accumulation, while Methanosaeta (specialist with a higher affinity for acetate) should have a competitive advantage in much more stable habitats, in which acetate levels are low. In fact, it had been shown that dieters that started up successfully contained high levels Methanosaeta (McHugh et al., 2003; McMahon et al., 2004; Pender et al., 2004).

Since the propionate level persisted at relatively low (between 110 and 150 mg/L) during all assay, we suggest that propionate-degrading syntrophs (e.g., Syntropherwolinii) could be present in high numbers in our inoculum. These syntrophs only can use a very limited range of substrate (Schink, 1992) and have very low specific growth rates, so they need an extensive amount of time to reduce propionate concentrations. On the other hand, butyratedegradingsyntrophs (e.g., Syntrophomonaswolfei) could be not present in high

numbers in our inoculum. Thus, while propionate was consumed rapid in the digester, the accumulated butyrate was removed very slowly by washout and/or conversion to acetate by butyrate-degrading syntrophs. To further investigate this hypothesis, our studies of population dynamics in digester systems need to be complemented with studies of population dynamics of propionate-degrading syntrophs and others syntrophic fatty acid-oxidizing bacteria.

CONCLUSIONS:

It has been shown that the application of 88% inoculum from thermophilic SEBAC with 23% of methanogens (15% H_2-utilising methanogens: 8% acetoclastic methanogens) has been successful to reach rapid start-up of the reactor. The development of a stable microbial community, Eubacteria, and Archaea, during start-up of the reactor has been shown with a ratio 88:12, respectively. It was clearly indicated that hydrogenotrophic methanogens, although important in the initial phase of the reactor start-up (11% of total methanogens), were displaced by acetoclastic methanogens at steady-state (32% of total methanogens). In the stable conditions, the percentages were maintained at 60:40 for Eubacteria and Archaea. We demonstrated links between digester operating conditions, physical and chemical performance parameters, and microbial population dynamics. The results have clearly indicated that the relative abundance of Archaea and acetoclastic methanogens was directly correlated with the organic loading rate, volatile solids removals, and methane production by anaerobic reactor.

REFERENCES:

1. Tour JM, Kittrell C, Colvin VL. Green carbon as a bridge to renewable energy. Nature materials. 2010; 9 (11):871–4. Epub 2010/10/23.
2. Weiland P. Production and energetic use of biomass from energy crops and wastes in Germany. Applied Biochemistry and Biotechnology. 2003; 109
3. Sreekrishnan T, Kohli S, Rana V. Enhancement of biogas production from solid substrates using differ- ent techniques—a review. Bioresource technology. 2004; 95(1):1–10.
4. Bo ¨rjesson P, Mattiasson B. Biogas as a resource-efficient vehicle fuel. Trends in biotechnology. 2008; 26(1):7–13.
5. Shih JC. Development of Anaerobic Digestion of Animal Waste: From Laboratory, Research, and Com- mercial Farms to a Value-Added New Product. Anaerobic Biotechnology: Environmental Protection and Resource Recovery: World Scientific; 2015. p. 339–52.
6. Demirel B, Scherer P. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Reviews in Environmental Science and Bio/Technology. 2008
7. McHugh S, Carton M, Collins G, O'FlahertyV. Reactor performance and microbial community dynamics during anaerobic biological treatment of wastewaters at 16–37 C. FEMS microbiology ecology. 2004; 48(3):369–78.
8. Leclerc M, Delgènes JP, Godon JJ. Diversity of the archaeal community in 44 anaerobic digestor as determined by single-strand conformation polymorphism analysis and 16S rDNA sequencing. Environ- mental Microbiology. 2004; 6(8):809–19.
9. Klocke M, Ma ¨hnert P, Mundt K, Souidi K, Linke B. Microbial community analysis of a biogas- producing completely stirred tank reactor fed continuously with fodder beet silage as mono-substrate. Systematic and applied microbiology. 2007; 30(2):139–51.
10. We've ´n L, Eriksson AR, Schnu¨rer A. Effect of process temperature on bacterial and archaeal communi- ties in two methanogenic bioreactors treating organic household waste. FEMS microbiology ecology. 2007; 59(3):683–93.
11. Gause G. The struggle for existence, 163 pp. Williams and Wilkins, Baltimore. 1934.
12. Harcombe W. Novel cooperation experimentally evolved between species. Evolution; international jour- nal of organic evolution. 2010; 64(7):2166–72. Epub 2010/01/27.
13. Proulx SR, Promislow DEL, Phillips PC. Network thinking in ecology and evolution. Trends in Ecology
14. & Evolution. 2005; 20(6):345–53.
15. Newman MEJ. The structure and function of complex networks. Siam Rev. 2003; 45(2):167–256.
16. Faust K, Raes J. Microbial interactions: from networks to models. Nature reviews Microbiology. 2012; 10(8):538–50. Epub 2012/07/17.
17. Zhou J, Deng Y, Luo F, He Z, Yang Y. Phylogenetic molecular ecological network of soil microbial com- munities in response to elevated CO2. MBio. 2011; 2(4).
18. Deng Y, Jiang YH, Yang Y, He Z, Luo F, Zhou J. Molecular ecological network analyses. BMC bioinfor- matics. 2012; 13:113.
19. Sun Y, Cai Y, Mai V, Farmerie W, Yu F, Li J, et al. Advanced computational algorithms for microbial community analysis using massive 16S rRNA sequence data. Nucleic acids research. 2010; 38(22): e205.
20. Zhou J, Deng Y, Luo F, He Z, Tu Q, Phi X. Functional molecular ecological networks. MBio. 2010; 1(4).
21. Duran-Pinedo AE, Paster B, Teles A, Frias-Lopez J. Correlation network analysis applied to complex biofilm communities. PloS one. 2011; 6(12): e28438