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ASSESSMENT OF BACTERIAL AND ARCHAEAL COMMUNITY STRUCTURE IN SWINE WASTEWATER TREATMENT PROCESSES

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SUMMARY: Microbial communities from two field scale swine wastewater treatment plants (WWTPs) were assessed by pyrosequencing analyses of bacterial and archaeal 16S rDNA fragments. Effluent samples from secondary [anaerobic covered lagoons and upflow anaerobic sludge blanket (UASB)] and tertiary treatment systems (open pound natural attenuation lagoon and air-sparged nitrification-denitrification tank followed by alkaline phosphorus precipitation process) were analyzed. A total of 56,807 and 48,859 high quality reads were obtained from bacterial and archaeal libraries, respectively. Dominant bacterial communities were associated with the phylum Firmicutes, Bacteroidetes, Proteobacteria or Actinobacteria. Bacteria and archaea diversity were highest in UASB effluent sample. Among the archaeal communities, 80 % of the reads was related to hydrogenotrophic methanogens *Methanospirillum*. Enrichment of hydrogenotrophic methanogens detected in effluent samples from the anaerobic covered lagoons and UASB suggested that CO_2 reduction with H_2 was the dominant methanogenic pathway in these systems. Overall, the results served to improve our current understanding of major microbial communities' changes downgradient from the pen and throughout swine WWTP as result of different treatment processes.
Keywords: Archaeal, bacterial, pyrosequencing, swine wastewater.

INTRODUCTION

Brazil is the sixth largest swine meat consumer (15 kg yr^{-1} $person^{-1}$) and the fourth largest exporter (0.52 Mt yr^{-1}) in the world (ABIPECS, 2011). The increase in swine agribusiness activities is accompanied by larger volumes of wastewater that requires adequate treatment prior to land application and/ or discharge into water bodies. Among the swine wastewater treatment processes known, anaerobic biodigesters followed by open pound storage lagoons are widely used (Viancelli et al., 2013).

Molecular biology tools have been used to characterize microbial communities present in swine wastewater lagoons, pit storage and anaerobic biodigesters (Kim et al., 2010). These, studies contribute to valuable information about WWTPs biological system performance and efficiency. Moreover, these studies can elucidate the effects that each particular treatment may exert on microorganism's selective pressure and growth of specific strains which could be detrimental to water quality and biosafety (Jeong et al., 2011). The latter is especially important when considering the significantly high water demands employed (ranging from 0.63 to 3.63 gallons pig $space^{-1}$ day^{-1}) and the emerging needs for water reuse (Viancelli et al., 2013). Nonetheless, little is known about the microbial population shifts that are likely to occur downgradient from the swine pens throughout the different treatment processes.

Therefore, the objective of this work was to investigate the microbial communities present in the effluent samples from two independent field-scale swine WWTPs. The structure of both bacterial and archaeal communities encountered on the pen floor sediment and effluents from anaerobic covered lagoons, open pond storage lagoon, UASB and air-sparging denitrification system followed by phosphorus alkaline

precipitation were assessed by high-throughput pyrosequencing analyses of 16S rDNA fragments.

MATERIAL AND METHODS

Bacterial and archaeal communities were sampled from different swine wastewater treatment plants: WWTP1 and WWTP2. Samples from WWTP1 were collected from two anaerobic covered lagoons connected in series (designated as bio1 and 2, respectively) and the final effluent. Samples from WWTP2 were collected from the pen, the UASB reactor and the final effluent. Genomic DNA was extracted using MoBio UltraClean® kit for the liquid or PowerSoil® kit (Carlsbad, CA, US) for sediment samples according to the manufacturer's protocol. Pyrosequencing of the 16S rDNA region was performed with primers for bacterial 16S and for archaeal 16S according to Nossa et al. (2010). All 16S rDNA pyrosequencing reads were analyzed using the original standard flowgram format (SFF) output file from the sequencer in Mothur, version 1.32.1 following the 454 standard operating process (Schloss et al., 2009). Sequence alignment was performed in Mothur using SILVA database (release 111) clustered at 97% similarity as reference (Caporaso et al. 2010).

RESULTS AND DISCUSSION

A total of 56,807 and 48,859 high quality sequence reads were obtained from bacterial and archaeal 16S rDNA, respectively. Among the samples evaluated, the effluent sample from the UASB at WWTP 2, showed the highest number of bacteria OTUs (2,015 at 97% similarity, or 660 if normalized sequences). However, the highest number of archaea OTUs (56 or 31 if normalized sequences) was observed in the effluent sample from bio1 at the WWTP 1. Bacterial diversity from highest to lowest was UASB > final effluent from WWTP 2 > pen floor > effluent from bio1 > final effluent from WWTP 1 > effluent from bio2. The increased bacterial diversity observed in the UASB effluent sample could be explained by the reactor intrinsic characteristic that stimulates the growth of different microorganism's communities living in syntrophic association in suspended cell granules as biofilms (Chong et al., 2012). Bacterial communities were mostly associated with phylum Firmicutes, Bacteroidetes, Proteobacteria or Actinobacteria. Negligible bacteria phylogenetic distances ($p < 0.01$) were verified among the pen floor (at WWTP 2) and all effluent samples analyzed from WWTP 1. These results served as circumstantial evidence to support that both WWTPs had similar bacteria diversity entering in the systems as inoculum. Significant discrepancy in bacteria phylogenetic distances ($p < 0.01$) was only verified for the sample collected at the UASB effluent at WWTP 2.

The methanogenic enrichment was related to (% of the total 16S rDNA relative abundance) *Methanospirillum*, *Methanogenium*, *Methanobrevibacter*, *Methanocorpusculum*, *Methanosarcina*, and *Methanoculleus* (Figure 2). *Methanosarcina* population was higher in UASB effluent sample as compared to anaerobic covered lagoons (bio1 and bio2) effluent samples. Members of this genus are metabolically very versatile and capable of producing methane from all three known pathways i.e., hydrogenotrhophic, acetoclastic and methylotrophic. Methane production in the anaerobic biodigesters from both WWTPs was predominantly associated with the reduction of CO2 with H2 by hydrogenotrophic methanogens.

CONCLUSIONS

Bacterial and archaeal 16S rDNA pyrosequencing analyses performed in effluent samples from two independent and full scale swine wastewater treatment plants served to improve our current understanding on microbiology at these systems. Bacteria and archaea diversities were significantly higher in UASB effluent sample. Among the identified archaea, hydrogenothrophic *Methanoculleus* and *Methanocorpusculum* were only observed in UASB effluent sample. The relative abundance of *Methanosarcina* was also notably much higher in the UASB effluent as compared to the anaerobic covered lagoons. The increased abundance of hydrogenotrophic methanogens (>80% of the 16S rDNA relative abundance) provides fundamental information to support that methane production in swine anaerobic biodigesters is largely dependent on CO_2 reduction with H_2 in these anaerobic systems.

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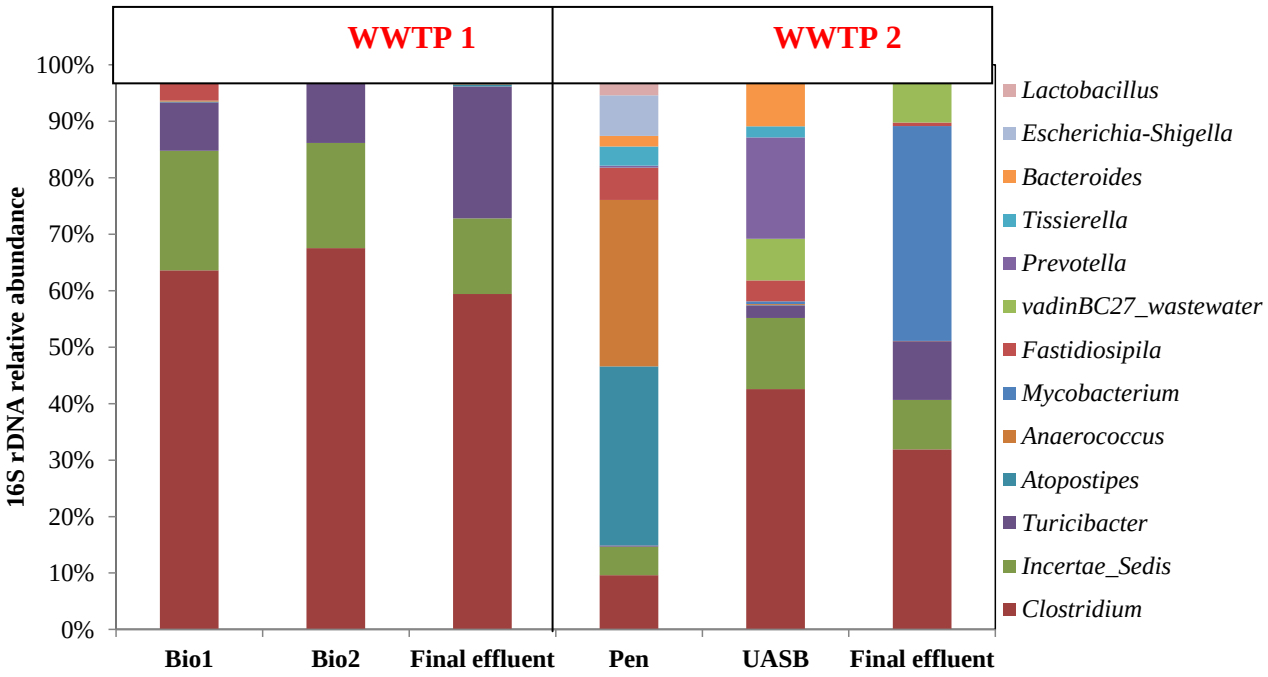

Figure 1. The relative abundance of bacterial 16S rDNA genes found in sediment from the pen floor and effluent samples from two independent WWTPs.

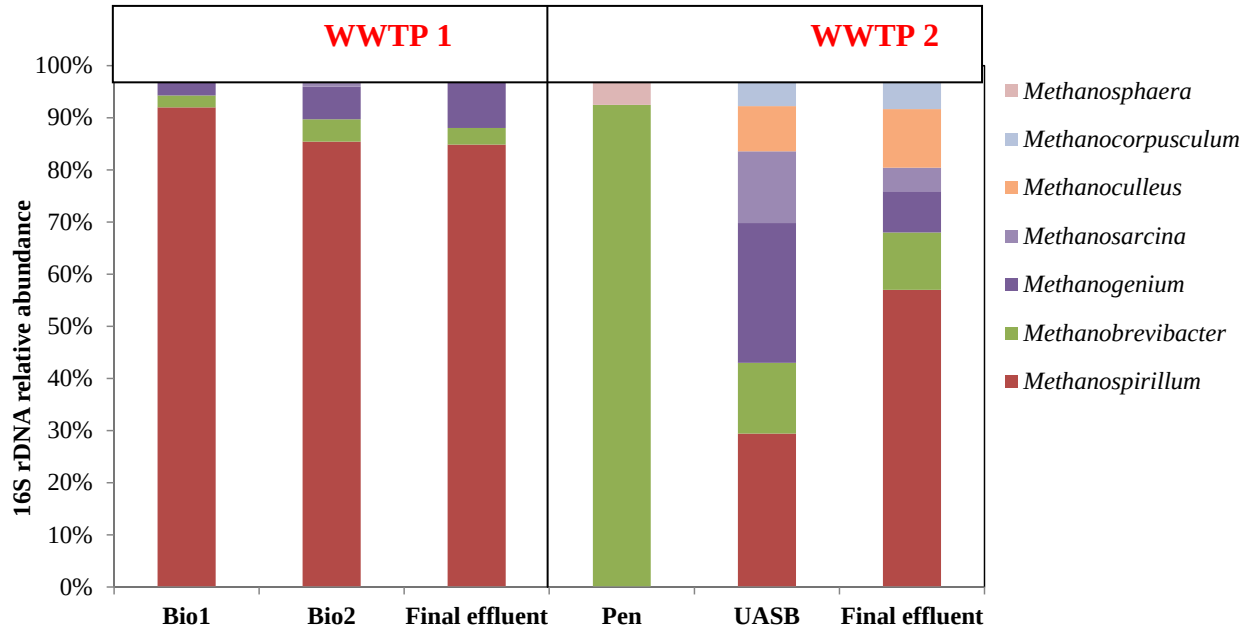

Figure 2. The relative abundance of archaeal 16S rDNA genes found in sediment from the pen floor and effluent samples from two independent WWTPs.