

The fate of antibiotic resistance genes and Class 1 Integrons during two-phase anaerobic digestion

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Residue sewage sludge from wastewater treatment is becoming a major source of antimicrobial resistance, with large numbers of antibiotic resistance genes (ARGs) and bacterial pathogens. The behaviors of five classes of thirteen representative ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *tetG*, *tetO*, *tetW*, *tetX*, *ermB*, *ermF*, *dfrA1*, *dfrA12*, and *blaTEM-1*), as well as the class 1 integron genes (*intI1*) under different conditions in the lab-scale two-phase anaerobic digesters (Fig.1) have been investigated. The associated microbial communities and bacterial pathogens have been characterized by high-throughput sequencing.

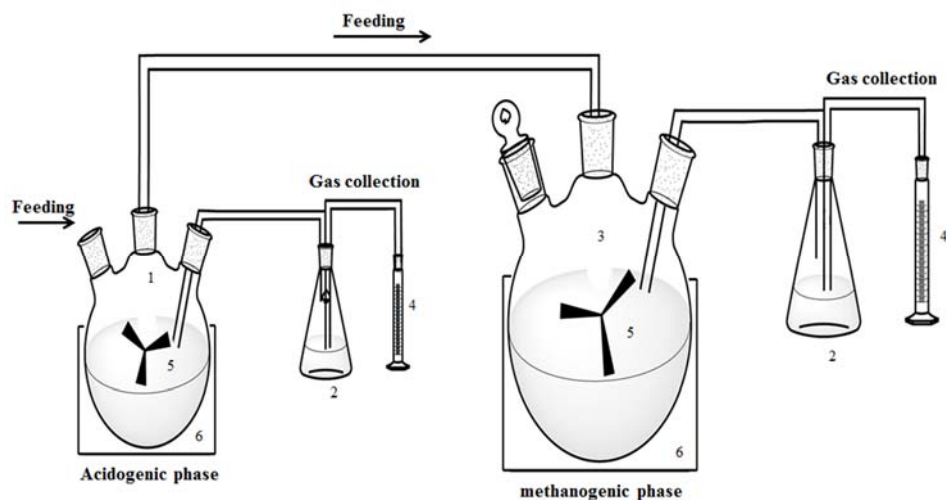


Figure 1 Schematic diagram of two-phase anaerobic digestion process. (1-Acidogenic reactor, 2-Effluent bottle, 3-methanogenic reactor, 4-Gas counter, 5-Mixer, 6-Water bath).

Results and Discussion. Thirteen selected ARGs (*tetA*, *tetG*, *tetO*, *tetW*, *tetX*, *sul1*, *sul2*, *sul3*, *ermB*, *ermF*, *blaTEM-1*, *dfrA1*, and *dfrA12*) were detected in all samples, and ranged from 10^5 ~ 10^9 copies/g in the feed sludge. Concentration changes of ARGs and *intI1* within the two-phase digesters were shown in Figure 2.

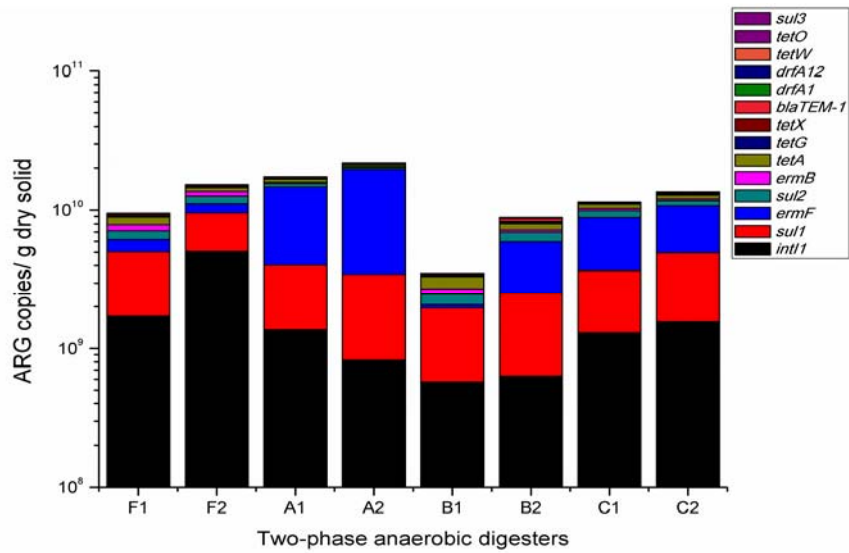


Figure 2 Total quantities of ARGs and *int11* in all samples. F1, F2 are feed sludge and feed sludge with ultrasonic pretreatment; A1, A2 are mesophilic acidogenic and methanogenic digesters; B1, B2 are thermophilic methanogenic digester; C1, C2 are mesophilic acidogenic and methanogenic digesters with ultrasonic pretreatment.

Specific ARGs demonstrated distinct behaviors within the acidogenic and methanogenic phases. Most ARGs decreased in thermophilic acidogenic digester. In particular, *tetX* and *ermF* significantly decreased by 1.07 and 1.00 order of magnitude, respectively ($p < 0.05$). It harbored the fewest families of bacteria, and its dominant bacterial Thermotogaceae was hardly related to any ARGs by redundancy analysis (Fig3). Archaeal families discovered in the methanogenic digesters were more diverse. This methanogenic diversity may lead to an increase of *ermF*, and *sul3* by 1.15, 1.72 order of magnitude in mesophilic and thermophilic methanogenic digesters, respectively ($p < 0.05$). In contrast, bacterial pathogen species decreased in two-phase anaerobic digesters. Therefore, environmental microorganisms, the presence of bacterial pathogens and the classes of ARGs carried could pose various risks to human health.

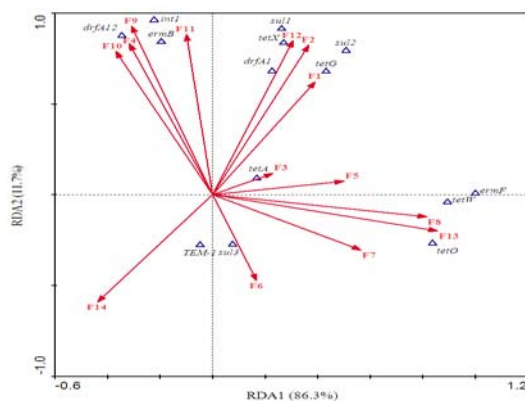


Figure 3. Redundancy analysis (RDA) identified the correlation of bacteria family and ARGs.