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Can sludge dewatering reactivate microorganisms in mesophilically digested anaerobic sludge? Case of belt filter versus centrifuge

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ABSTRACT

The anaerobic digestion process that successfully reduces the organic content of sludge is one of the most common alternatives to meet pathogen reduction requirements for particular classes of biosolids. However, recently it was reported that, much higher densities of indicator bacteria were measured in dewatered cake samples compared to samples collected after anaerobic digestion. Additionally, this increase was commonly observed after centrifugation but not after belt filter dewatering. Several hypotheses were tested to explain this occurrence; however, much of the attention was given to the reactivation of the indicator bacteria which might enter a viable but non-culturable state (VBNC) during digestion. The objective of this research is to examine sludge samples from 5 different full-scale treatment plants in order to observe the effect of dewatering processes on the reactivation potential of indicator bacteria. The bacterial enumerations were performed by both Standard Culturing Methods (SCM) and quantitative polymerase chain (qPCR) on samples collected after digestion and dewatering. Results obtained by SCM indicated that in two investigated treatment plants operating belt filter dewatering, an average 0.6 log decrease was observed after the dewatering process. However, 0.7–1.4 log increases were observed immediately after centrifuge dewatering for the other three treatment plants. On the other hand, qPCR results gave 0.1–1.9 log higher numbers compared to SCM. Comparative evaluation of results obtained by two analytical methods for five treatment plants indicates that the differences observed might be originating from both reactivation of VBNC bacteria and amplification of DNA from dead cells found in the sludge.

1. Introduction

Sustainable approaches in sewage sludge management aim to promote beneficial reuse of sludge by taking necessary precautions to protect the environment and human health. Sewage sludge contains high levels of organic matter and nutrients as well as a variety of microorganisms (Kinney et al., 2006). Typical wastewater treatment processes reduce the number of microorganisms in wastewater but these microorganisms are concentrated in sludge (Gerba and Smith, 2005). Therefore, one of the most vital issues to be considered about the land application of sewage sludge is the investigation and quantification of pathogenic microorganisms in sludge.

The presence of pathogens in sewage sludge has been determined by indicator microorganisms for years and limit values of corresponding regulations have been set mostly for

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these microorganisms. In the United States for example, biosolids are divided into two classes as Class A and Class B, based on the pathogenic and indicator microorganism content of sludge (U.S. EPA, 1993). Class A is defined as the sludge which has a lower concentration than 1000 MPN/g TS for Fecal Coliforms or 3 MPN/4 g TS for Salmonella immediately after the stabilization processes. The criteria for Class B biosolids require the concentration of Fecal Coliforms to be less than 2,000,000 MPN/g TS in stabilized sludge. The Sewage Sludge Directive (86/278/EEC) applicable in EU countries has no limits related to pathogens. On the other hand, member states are allowed to set more stringent levels or include additional parameters in their national regulations. Because of this, member countries including France, Italy and Luxembourg have pathogen limits set in their own regulations.

Among all the stabilization alternatives involved in the aforementioned regulations, anaerobic digestion has been widely adopted by many wastewater treatment facilities all over the world. Biological activity is reduced along with the organic matter content of sludge during anaerobic digestion. However, it has recently been reported that significantly higher densities of Fecal Coliform and/or *Escherichia coli* have been measured in dewatered cake samples compared to the samples collected after anaerobic digestion (Hendrickson et al., 2004; Iranpour et al., 2003; Cheung et al., 2003; Monteleone et al., 2004; Qi et al., 2004). This finding is more pronounced in the centrifuge dewatered sludge cakes rather than in belt filter dewatered cakes (Cheung et al., 2003; Erdal et al., 2003; Hendrickson et al., 2004; Higgins et al., 2007; Qi et al., 2007). Moreover, densities of indicator bacteria have been observed to further increase for stored sludge cake samples (Erdal et al., 2003; Qi et al., 2007; Higgins et al., 2007). The increases in bacterial content were observed in both plants operating thermophilic digesters as well as mesophilic digesters. The observed increases were more pronounced in thermophilically digested sludge (Iranpour et al., 2003; Higgins et al., 2007; WERF, 2008).

Several suggestions have been made to explain this occurrence including false positive results associated with enumeration techniques (Qi et al., 2004), contamination from the centrifuge (Hendrickson et al., 2004), effect of floc break up on improved culturability (Cheung et al., 2003), removal of growth inhibitors during dewatering (Qi et al., 2007) and effect of day-light throughout the storage of biosolids (Gözen and Örmeci, 2010). Recently, Chen et al. (2011a) have suggested that both odor production and regrowth are signals of microbial activity which is induced upon the increase in shear and oxygen levels during centrifuge dewatering. However, most of the attention in the literature related to regrowth after dewatering is directed to a special microbial state that bacteria display. Since some stress conditions are known to induce pathogenic and non-pathogenic organisms to enter dormancy state, under the unfavorable conditions of anaerobic digestion the indicator bacteria are suggested to enter a viable but non-culturable (VBNC) state (Higgins et al., 2007; Chen et al., 2011b). The presence of non-culturable bacteria results in a low number of indicator bacteria determined after anaerobic digestion process by standard culturing methods, giving an impression that they are dead even though they are technically viable.

Several reasons such as deficiency of nutrients (Cook and Bolster, 2007), altered temperatures (Besnard et al., 2002), oxidative and osmotic stresses (Asakura et al., 2008), UV irradiation, toxic or inhibitory substances or extreme conditions of pH may cause bacteria to enter the VBNC state (Grey and Steck, 2001; Trevors, 2011). However, a critical point about the VBNC concept is that, even though the cells enter the VBNC state, they are still considered viable and carry their infectious properties in vivo (Cappelier et al., 2007). In addition, those bacteria do not persist in the VBNC state indefinitely. Provided that growth promoters and enrichments are available, cells typically continue to grow on media which is called "resuscitation" or "reactivation" (Lleò et al., 2001; Reissbrodt et al., 2002). In this context, Higgins et al. (2007) suggested that, anaerobic digestion may be the reason for indicator bacteria to enter VBNC state owing to stressful conditions developed during the process including low substrate and nutrient levels.

The purpose of this research is to examine the selected full-scale wastewater treatment plants that operate mesophilic anaerobic digesters in Turkey in order to investigate whether similar increases in the number of indicator bacteria exist after the implementation of dewatering. For this reason, samples taken from full-scale treatment plants operating both belt filter and centrifuge dewatering were examined for their indicator bacteria content by Standard Culture Methods (SCM) as well as a culture independent method, quantitative polymerase chain reaction (qPCR).

2. Material and methods

2.1. Selection of the wastewater treatment plants

Five municipal wastewater treatment plants are selected to be evaluated during the study. All the plants are operating mesophilic digesters since thermophilic anaerobic digestion is not applied in wastewater treatment plants currently operated in Turkey. In order to designate the effect of different dewatering processes, 3 treatment plants using centrifuge and 2 treatment plants using belt filter as dewatering processes were selected. Five different wastewater treatment plants which were coded as A, B, C, D and E were sampled throughout the study. All of the treatment plants were selected from metropolitan municipalities of Turkey which implement preliminary treatment, primary treatment and activated sludge or an equivalent biological system for the treatment of wastewater and operate thickener and mesophilic anaerobic digester followed by a dewatering system for the treatment of sludge. Treatment Plants A and B operate belt filter, while Treatment Plants C, D and E use centrifuge dewatering on the anaerobically stabilized sludge. Main process characteristics of the wastewater treatment plants evaluated in the scope of the study and the number of samplings are shown in Table 1.

2.2. Sample processing and enumeration of Fecal Coliform and E. coli by Standard Culture Methods

Five different samples taken from WWTP A and WWTP C, three different samples taken from WWTP D and WWTP E and two different samples taken from WWTP B were analyzed for