Ecological Engineering 81 (2015) 363–372

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Fate of faecal indicator organisms and bacterial diversity dynamics in a series of continuously fed aerated tank reactors treating dairy manure

Anni Alitalo [a,*], Johanna Nikama [b], Erkki Aura [c]

[a] Natural Resources Institute Finland (Luke), Koetilantie 5, 00790 Helsinki, Finland
[b] Natural Resources Institute Finland (Luke), Tietotie, 31600 Jokioinen, Finland
[c] Biotaito, Killintie 14, 31300 Tammela, Finland

ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form 2 February 2015
Accepted 5 April 2015
Available online 17 April 2015

Keywords:
Aerobic treatment
Dairy
Cow
Manure
Hygiene
16S rRNA

ABSTRACT

This study aimed to investigate the fate of faecal indicator organisms and to characterize microbial diversity dynamics in dairy manure and in aerated liquid manure in different treatment reactors in a series of continuously fed aerated tank reactors using low intensity aeration and ammonia-reduced feedback. The bacterial diversity in the treatment reactors was also compared with that in the soil from which the process inoculant originated. The fate of enteric indicator organisms was studied by the enumeration of viable counts. Total bacterial community DNA was extracted, amplified using 16S rRNA primers, cloned, sequenced and identified by comparison to known sequences. At best over 90% reduction was observed in the numbers of enteric indicator organisms. However, the result obtained varied depending on treatment run and indicator organism. Firmicutes were the dominating phyla of the untreated slurry; whereas; Proteobacteria and Deinococcus-Thermus dominated in the treatment tanks. Soil had the broadest phylogenetic diversity: the major phylums were Acidobacteria, Proteobacteri and Gemmatimonadetes. At the genus level, raw manure and treatment tanks shared a number of sequences. Soil, the origin of the inoculant, and treatment tanks did not share any sequence at the genus level, although some was observed at the family level.

1. Introduction

Slurries and manures are valuable nutrients but may also be potential sources of pollution and contamination of the human food chain. Livestock manure can harbor a wide range of bacterial, viral, and parasitic pathogens (McAllister and Topp, 2012). Therefore, it can be a significant microbiological risk. There are reports of epidemics caused by enteric microorganisms common to cattle and humans, such as the bacteria *Salmonella* (Velge et al., 2005), *Mycobacterium bovis* (Etter et al., 2006), and Shiga toxin-producing *Escherichia coli* (Henderson, 2008), as well as protozoa such as *Cryptosporidium* (Bodley-Tickell et al., 2002). Moreover, new patterns of resistance to antimicrobial agents and new forms of virulence may emerge in old pathogens and influence the risk of epidemics in the future (e.g., Velge et al., 2005).

Microbiological, chemical and physical methods have been applied to reduce and control pathogenic contamination. The varying efficiencies, as well as the advantages and disadvantages of these methods, have been reviewed e.g., by Heinonen-Tanski et al. (2006). Several conditions may influence the survival of pathogens in manure or slurry. These include temperature, pH, solids content, microbial content/microbial competition (=numbers and types of pathogens present and presence of competing organisms (Jones, 1976; Jones et al., 1977)), oxidation–reduction potential (ORP) and time.

A pronounced effect of aeration on the rate of inactivation of enteric organisms has been reported by several authors (e.g., Heinonen-Tanski et al., 2006; McGarvey et al., 2007). It is possible to achieve from 90 to 99.9% reduction in the count of intestinal bacteria or viruses at low temperatures (0–30 °C). Continuous or discontinuous aeration (in batch processes) can be performed either at a mesophilic temperature or alternatively at a higher temperature in a thermo-insulated reactor, because the process itself generates heat.

Pathogen reduction during aeration can be due to a variety of factors. In addition to O_2 sensitivity and temperature, microbial inactivation can be caused by the combined effects of a high concentration of free ammonia and high pH (Jenkins et al., 1998). However, the tolerated ammonium ion concentration varies between studies and may be dependent on such factors as solution buffer capacity and the hydraulic retention time (HRT) of

* Corresponding author at: Natural Resources Institute Finland (Luke), Koetilantie 5, PL 28, 4th floor, room 410, FI-00790 Helsinki, Finland. Tel.: +358 29 5326059.
E-mail address: anni.alitalo@luke.fi (A. Alitalo).

http://dx.doi.org/10.1016/j.ecoleng.2015.04.050
0925-8574/

the system. Long HRT may increase the potential for acclimatization and minimize the severity of response to toxicity (Marchaim, 1992). Bacteria that are inhibited by a given concentration of a toxic substance can often become acclimated to grow in that concentration upon prolonged exposure (Lee et al., 2011; Rajagopal et al., 2013). Acclimation can involve alterations of metabolism and molecular structures, and increase in the number of microbes that can lessen the effect of the toxin (e.g., Liebert et al., 1991; Margesin and Schinner, 1996).

Traditional cultivation-based methods (using specific media and conditions) probably reveal the level of hygienic quality attained in a system, although today there are molecular techniques, such as viability real time PCR, that can help to improve our knowledge on the real microbial risk. New nucleic acid-based methods have provided new insight into the diversity and composition of communities and can even detect differences in the functioning and interactions of the communities. Recently, Hanajima et al. (2011) and McGarvey et al. (2007) observed changes in microbial community composition during a batch aeration treatment. Furthermore, when using different aeration intensities, shifts in clones over time were delayed under reduced aeration rates (Hanajima et al., 2011). They suggested that the microbial sub-population that develops under aeration may be responsible for a variety of environmental parameter changes.

Whereas, in the previous studies hygienic state and/or community compositional changes have been monitored during batch aeration treatments, in this study aeration was used in a series of continuously fed aerated tank reactors in a continuous regime. Simultaneously, treated effluent, the ammonia content of which had been reduced by ammonia stripping, was fed back to the first tank with a volume slightly higher than the feed-in volume. The ammonia-reduced feedback was used because it had been found to have process-improving properties, such as improved odor reduction and reduced HRT time, and allowed higher process loading (unpublished data).

The hygienic state of the process was monitored by measuring viable counts of bacteria, including total counts and counts of enteric indicator microorganisms. In addition to traditional cultivation-based methods, the changes in microbial community composition were studied by 16S rRNA gene sequence analysis in different treatment tanks of the system and were compared to the community composition of untreated slurry and soil (the origin of the used inoculant).

This study was part of a wider study conducted in order to investigate operational conditions of the serial treatment system and the influence of operational conditions on the treatment process conducted with ammonia-reduced feedback (Alitalo et al., 2013a).

2. Materials and methods

The treatment system used is described in detail elsewhere (Alitalo et al., 2013a; Supporting information and Fig. 1). Briefly, the continuous system consisted of six 600 l (actual liquid volume 400 l) aeration tanks connected in series. After the biological treatment, stripped solution was transferred to the first treatment tank of the serial system as feedback solution with the purpose of lowering the ammonia concentration. The serial treatment system had been first filled with sub-cultured inoculant prepared from a soil–water suspension (Alitalo et al., 2013b). Soil was from sites where the field had a long fertilizing history with cattle manure (clay soil, Vertic Cambisol (FAO/Unesco, 1994)).

2.1. Experimental design

Studying trials were carried out during a six month period, further divided into four periods (Table 1). During these periods, the serial treatment system was loaded increasing loading from 50 to 350 ld-1 with varying TS load. The pH, ORP and TS content was measured daily in treatment tanks during the six month study period (Alitalo et al., 2013a). Slurry samples were also collected for total ammoniacal nitrogen analyses (Periods III and IV). Analyses were performed as described by Alitalo et al. (2012). Hygienic indicator samples were taken on four different sampling occasions (Table 1). DNA was extracted from the day 130 sample.

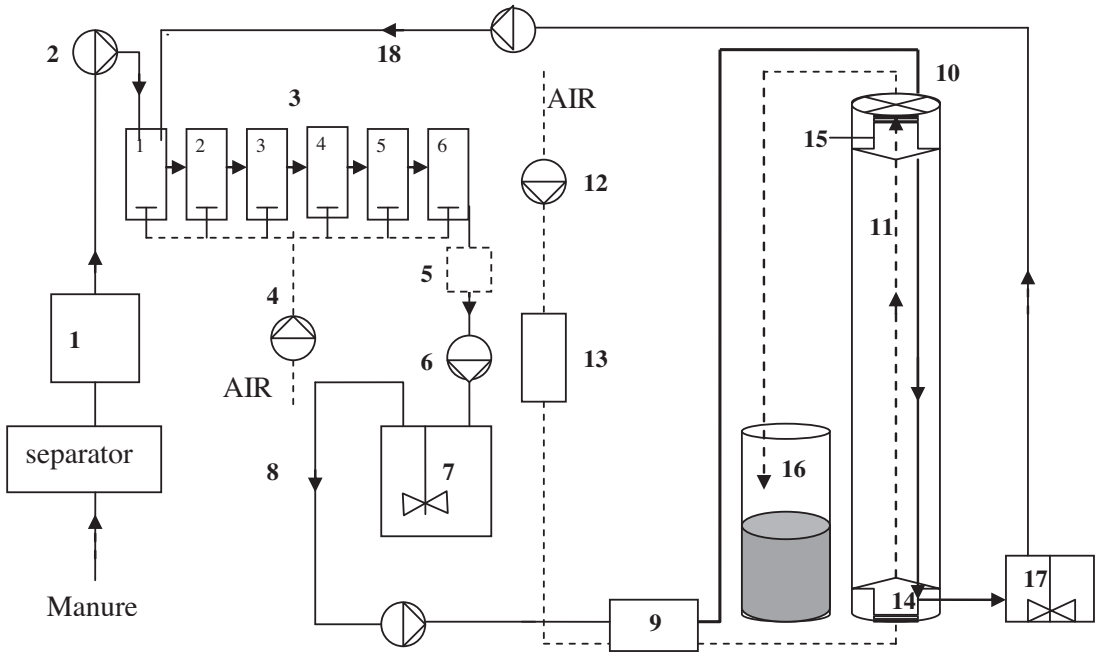

Fig. 1. Schematic prepresentation of the treatment system. (1) Separated slurry storage tank, (2) feed-in pump, (3) serial tank system, (4) blower, (5) overflow container, (6) pump, (7) intermediate container (MgO addition), (8) effluent to the stripping tower, (9) thermostatic path, (10) liquid distributer, (11) stripping tower, (12) blower, (13) flow meter, (14) air flow, (15) liquid flow, (16) ammonia wet washer, (17) stripped effluent storage tank, (18) feedback.