


Title	Fate of faecal pathogens and indicator bacteria in urea treatment
Keywords	Biowaste, Nutrient recycling, Pathogens, <i>Salmonella</i> , Sanitation
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Short CV for Introduction Purposes (100 words max)	Björn Vinnerås is a PhD in environmental engineering and work as a researcher at Swedish University of Agricultural Sciences and at the National veterinary institute. His main research topic is secondary treatment of different wastewater fractions for safe nutrient recycling.
Photograph attached (jpg)	

Introduction

In today's society, nutrients are transported from arable land by harvested foods and feedstuffs, distributed into the society and consumed by people. After digestion, most of the nutrients are directly or indirectly transferred to the water recipients via the excret.

Nutrient resources are scarce and those most used (macronutrients) are in danger of becoming scarce within the coming century (Jönsson et al., 2004). Today, the energy usage both for nutrient removal from wastewater and for production of new fertilisers is high. If some of the nutrients, i.e. nitrogen and phosphorus, are not removed from the wastewater they can cause eutrophication in water recipients. To increase the sustainability of society, these nutrients have to be recycled. However, when closing the loop the risk for transmitting pathogens will also increase.

Objective

Evaluate urea treatment of faecal matter for removal of pathogenic bacteria.

Methods

The material is collected both as mixed fractions (blackwater) and as sorted fractions. Generally the stabilisation of the collected material is performed via biological treatment, either anaerobic or aerobic digestion or via long term storage.

Treatment by urea addition

The chemical treatment of faecal matter (Vinnerås et al., 2003), of manure (Vinnerås, 2005) and of blackwater (Vinnerås & Svensson, 2005) is based on addition of the fertiliser urea to the material to be treated. The biowaste naturally contains the enzyme urease, which degrades the urea to ammonia and carbon dioxide, while the pH rises to almost 9. For the faecal matter, holding 10% total solids (TS), approximately 6% wet weight of urea was added at a temperature of 20°C. Similar tests with treatment of cattle manure (TS=12%) and of pig manure (TS=5%) adding 2% wet weight have been performed at 4°C and at 14°C (Vinnerås, 2005). For the treatment of blackwater (TS=0.2%) urea was added in concentrations between 0.0125% and 0.1%. Of the added urea 28/60 is ammonia nitrogen, i.e. 1% added urea corresponds to 0.47% nitrogen.

Methods for analysis of organisms present

Salmonella spp. were always monitored in the studies and in most cases survival of *Enterococcus* spp. and *E.coli* spp. was also studied. Samples extracted were diluted 10-fold in physiological saline solution.

The *Salmonella* spp. analysis for the faecal material was performed as a qualitative measurement, where the presence of organisms was analysed using an ISO two-step enrichment method (Vinnerås et al., 2003). In the studies of survival during storage, the analyses were quantitatively enumerated with the MPN method using an ISO two-step enrichment method. In the blackwater and the manure studies, the material was directly surface spread onto xylose lysine desoxycholate (XLD) agar containing novobiocin, followed by incubation at 37°C for 24h.

Enterococcus spp were analysed by surface spreading onto Slantz-Bartley agar and incubating at 44°C for 24h. *E.coli* was cultured in double agar layer using violet red bile agar (VRB) at 44°C for 24h. *E.coli* O157:H7 was analysed by surface spreading onto chrome agar containing 0.25 µl/ml, chloramphenicol and incubating at 37°C for 24h.

Results

Storage at controlled temperatures resulted in significantly longer survival time during storage at 4°C compared to storage at 21°C, with decimal reduction (Dr) values for *Salmonella* spp of 50 days and 30 days, respectively (Table 2). The difference at the two temperatures for *Enterococcus* spp. was smaller, with a Dr of 50 at 7°C and 38 at 21°C.

Table 2. Dr (decimal reduction) in days for different treatment alternatives and organisms in sewage sludge, faecal matter and manure

Method of treatment	<i>Salmonella</i> spp	<i>Enterococcus</i> spp	<i>E.coli</i>
Storage, sewage sludge 7 °C	50	50	-
Storage, sewage sludge 21°C	30	38	-
Storage, blackwater 15°C	24	22	20*
Storage, faecal matter 20°C	>9	51	8
Urea 6% 20°C, faeces	<0.7	<3	<0.7
Urea 0.1% 15°C, blackwater	3	10	3*
Urea 2% pig manure 4°C	7	-	-
Urea 2% pig manure 14°C	3	-	-

**E.coli* O157:H7

Treatment of the faecal matter with 3% ammonia nitrogen at 20°C resulted in a rapid reduction of the investigated microorganisms (Table 2) (Vinnerås et al., 2003). When blackwater was treated using considerably lower concentrations, by wet weight, of urea compared to the faecal treatment, the reduction effect was slower compared to that for the faecal matter. For *Enterococcus* spp Dr was halved, for *E.coli* Dr was decreased by a factor of 40 and for *Salmonella* spp Dr was decreased by a factor of 6 (Table 2).

Discussion

In systems where biowaste is recycled, pathogens can be found. Studies have shown that the larger the system, the larger the risk for pathogens. Investigations in Sweden, a country that holds a naturally low load of *Salmonella* infections, show that even in these conditions a constant load of *Salmonella*, *Campylobacter* and helminths are found in wastewater products (Sahlström et al., 2004). Of the treatments used today, only long-term storage for more than one year and pasteurisation at 70°C for over one hour gives sufficient pathogen reduction (Table 1). In warmer regions, especially in developing countries, the load of pathogens in the biowaste is even higher (Gantzer et al., 2001; Estrada et al., 2004; Heinonen-Tanski & Wijk-Sijbesma, 2004) as it is in freshwater reservoirs (Obi et al., 2003). Obi et al. (2003) found among other organisms *Campylobacter*, *Shigella* and *Vibrio cholerae* in rivers in South Africa.

For successful recycling of plant nutrients, waste material has to be properly treated regarding the pathogen content. Otherwise, it will pose a risk for transmission of diseases. According to Faechem et al. (1983) the three major transmission routes for *Ascaris* are: 1) contact with raw faecal material, 2) contact with unclean equipment and 3) food fertilised with pathogen-containing biowaste.

Generally all these three transmission routes have to be taken into account for reducing the risk when using biowaste as a nutrient source in agriculture. In our studies we found that factor (2), use of unclean equipment, is one of the main sources for recontamination of sanitised material and thereby increased risk of transmission of diseases. The number of *Salmonella*-containing samples of biowaste was doubled at satellite storage compared to the fresh, untreated biowaste (Bagge et al., 2005).

The simplest method of treatment is storage. However, storage has not been shown to be a reliable method as re-growth can occur, especially when the external conditions are altered, e.g. a shift in temperature (Shidu et al., 1999). Recommendations for reuse of faecal matter devised by Schönning & Stenström (2004) are that storage at 2-20°C for between 1.5 to 2 years or at a temperature above 20°C for more than one year should be enough. Our investigations support this for bacteria, as *Salmonella* spp. had a Dr value of 24 days (Table 2) in blackwater under Swedish summer conditions (average temperature approximately 15°C). For *Enterococcus* spp and *E.coli* O157, the Dr values were 22 and 20 days, respectively (Table 2). Long-term (> 1 year) outdoor storage of sewage sludge in piles proved to be efficient in reduction of *Salmonella* spp and *Enterococcus* spp, as no viable organisms could be found (Table 1). However, *Ascaris suum* investigated at the same storage (Berggren et al., 2004) showed only a small reduction during the storage time. This indicates that some pathogens may survive long-term storage. (# give the reduction in manure too)

The reduction in *Salmonella* spp. during storage of sewage sludge under summer conditions (14°C) and fall/spring conditions (7°C) corresponded to a Dr value of 30 and 50, respectively (Table 2) This reduction corresponds well with other studies in warmer regions where the Dr values are lower at higher temperatures (Jeppsen et al., 1997).

Biological treatment can give some protection towards re-growth/contamination of pathogenic organisms by competition from the indigenous organisms in the biowaste (Sidhu et al., 2001). This is probably due to higher load of competitive microorganisms in the material, as sterilised, composted material has been shown to have significantly higher contamination levels compared to unsterilised material (Sidhu et al., 2001). However, in our studies we found that even though the material was properly sanitised via pasteurisation, there was an underlying risk for contamination when handling the sanitised biowaste. The transport truck and satellite storage were identified as key factors for the contamination. To avoid contamination in transport, a selected truck should be used for transporting treated material only. Furthermore, the contamination in satellite storage often seems to originate from prior contamination in the tank, which then continuously holds a pathogen seeding culture for the treated biowaste.

The use of urea for sanitation of biowaste has its origins in ammonia addition (Himathongkham & Reimann, 1999; Vinnerås et al., 2003). The advantage with urea is that it is easy to handle (granules) and globally available, one of the most common fertilisers in the world. In addition, the treatment does not require skilled personnel, in comparison to composting and anaerobic digestion. Upon addition of urea, the enzyme urease degrades the urea to ammonia and carbon dioxide. The enzyme is generally present in biowaste. Therefore, within a few hours after application to the biowaste, depending on temperature, the urea is degraded to ammonia, followed by an increase in pH. The free ammonia combined with increased pH sanitises the material even at the pH of 9-9.5 reached by the urea addition. The urea treatment shows a rapid reduction in organisms, including non-spore forming bacteria, viruses and parasites (Vinnerås et al., 2003).

Using urea for biowaste sanitation results in an increased fertiliser value of the material, as no ammonia is consumed during the treatment provided it is performed in a closed container. As long as the ammonia remains in the material, there is no risk for re-growth or contamination of the treated material. Both the economic and environmental costs of the treatment are low, as the ammonia holds a fertiliser value when the material is recycled.

The urea treatment does not require any extra stabilisation as the treatment itself stabilises the material. Investigations on human urine storage show that the ammonia losses can easily be kept below 1%, even if the material is stored for 6 months or more (Bagge et al., 2005). If the storage is not properly done, there is a major risk for ammonia emissions followed by a

decreased protection against pathogen contamination of the biowaste.

Conclusions

Storage is the simplest treatment method, but prior to the storage the material has to be stabilised. If the biowaste is not stabilised, there is a risk of odour and high attraction to potential disease vectors such as flies and rodents.

Urea treatment is the most efficient and safe of the three treatment alternatives compared, one of the main advantages pointed out in Table 3 being the protection towards re-growth of pathogenic bacteria and the short time of treatment needed, i.e. D_r value of 1.2 days when adding 1% urea-nitrogen and less than 0.7 days when adding 3% urea-nitrogen.

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