Hygienic quality of source separated urine based on the analysis of faecal indicator bacteria

Qualité hygiénique de l'urine séparée à la source à travers l'analyse d'un indicateur bactérien fécal.

Hoglund C, Stenström T.A, Sundin A.
Departement of Water and Environmental Microbiology
Swedish Institute for Infectious Disease Control,
S-105 21 Stockholm, Sweden
E-mail : Caroline.Hoglund@smi.ki.se

Jönsson H, Vinneras B.
Department of Agricultural Engineering
Swedish University of Agricultural Sciences
PO Box 7033,
S-750 07 Uppsala, Sweden.

Abstract

Human urine is the largest contributor of nutrients to household wastewater. The re-use of urine as a fertiliser in agriculture would reduce the loads on wastewater treatment plants and the impact on recipients. An important criteria for recirculated products from a sewage system is that the risk for transmission of disease should be low. In a urine source separating system faeces may contaminate the collected urine through the toilet. Concentrations of faecal indicator organisms were measured in 14 urine collection tanks at two occasions. E. coli was seldom found; in 84% of the samples the concentration was below the detection limit of 10 cfu/ml. Clostridia was found in varying amounts, ranging from 1 cfu/ml to 2000 cfu/ml. Faecal streptococci occurred in large and varying amounts with 76% of the samples having concentrations above 1000 cfu/ml, thus indicating a growth within the systems. Total coliforms were found in varying amounts with an average of 470 cfu/ml. For all indicator bacteria, concentrations were generally higher in the sediment layer present on the bottom of the collection tanks. Samples were also collected from a urine storage tank during a period of four months. On the last sampling occasion concentrations of all indicator bacteria except clostridia were below detection limits. The results correspond well with laboratory experiments where the die-off of faecal indicator bacteria in urine were studied. At the conditions prevailing in the urine tanks E. coli had a D-value below one day. Faecal streptococci was reduced from 10^6 cfu/ml to <10 cfu/ml in approximately five months whereas clostridia shows no reduction during 36 days. Thus, after the six month storage generally applied for source separated urine, the hygienic quality of the urine can be considered as satisfactory.

Keywords : Hygiene, faecal indicators, source separation, urine separation.

Résumé

L'urine humaine contribue largement à l'apport en éléments nutritifs des eaux usées domestiques. La ré-utilisation de l'urine en tant que fertilisant en agriculture réduirait d'autant la charge sur les stations de traitement et l'impact du rejet en milieu naturel. Un critère important pour la recirculation des produits issus d'un système d'eaux usées est le risque de transmission de maladies qui doit être minimum. Dans un système de séparation de l'urine à la source, les faeces peuvent contaminer l'urine collectée via les toilettes. Les concentrations en indicateurs fécaux ont été mesurées dans 14 cuves de stockage à 2 périodes. *E.coli* était rarement présent ; dans 84% des échantillons la concentration se situait en dessous de la limite de détection soit 10 cfu/ml. *Clostridia* était présente à des concentrations variables, allant de 1 à 2000 cfu/ml. Les *streptococci* fécaux sont présents en grandes quantités dans 76% des échantillons avec des concentrations supérieures à 1000 cfu/ml, ce qui témoigne d'un développement dans le système. Les coliformes totaux étaient présents dans de larges proportions avec en moyenne 470 cfu/ml.

Ces résultats confirment les données de tests en laboratoires où l'on a étudié la survie (valeur D) des bactéries fécales présentes dans l'urine. Dans les conditions rencontrées dans les cuves de stockage de l'urine, *E.coli* présente une valuer D inférieure à 1 jour. Les *streptococci* fécaux étaient réduits de 10^6 cfu/ml à moins de 10 cfu/ml en 5 mois de stockage, alors que *clostridia* ne montre pas de réduction avant 36 jours.

Ainsi, après les 6 mois de stockage habituellement appliqués pour l'urine séparée à la source, la qualité hygiénique de cette urine peut être considérée comme satisfaisante.

Mots-clés : hygiène, indicateurs fécaux, séparation urine source.

1. Introduction

By the re-use of wastewater or other separated parts of human wastes in agriculture, there is a possible risk for transmission of microbial contaminants to grazing cattle, to vectors, to surface and ground water and through standing crops to humans. The present laws and regulations for the re-use of human wastes vary between countries. In Sweden they are related to the national implementation of the EC Urban Wastewater Treatment Directive (EC, 1991). Also WHO has published guidelines for the safe use of wastewater (WHO, 1989). Some countries specify analyses of indicator bacteria, viruses and parasites while others put more effort into methods for treatment and restrictions for the use of wastewater products. According to Morsing (1994) there are four approaches to minimise the risk for contamination through sludge: (1) reduction of pathogens, (2) reduction of the vector attraction (rats, flies etc), (3) soil treatment and (4) restrictions on cultivation methods and crops.

In several countries the use of untreated sludge in agriculture is not permissible. In

Sweden it is permitted if the sludge is injected or worked into the soil within 24 hours after application. However, the main practises have been an anaerobic treatment of the sludge from wastewater treatment plants or, for single households, storage of the sludge for six months before re-use in agriculture. The latter has initially been the general rule also for the re-use of source separated human urine in Sweden.

A urine source separating sewage system is based on a toilet which has the bowl divided into two parts; the front part collects the urine and the rear one collects the faecal material. The urine is lead to a collection tank usually buried in the ground. When the tank is full the urine is transported to a farm where it is stored until used as a fertiliser, mainly for cereal crops. The faecal material is either mixed with the greywater and transported to a sewage treatment plant or collected separately and dry for composting.

Human urine is the largest contributor of nutrients to household wastewater. Approximately 80% of the nitrogen in wastewater and 60% of the phosphorus origins from urine if no phosphorus detergents are used (Sundberg, 1995). The total quantities of nutrients in human urine are significant when compared to the quantities of nutrients in mineral fertilisers used by agriculture. In Sweden, the yearly production of human urine equals 15-20% of the mineral fertiliser consumption 1993 (Jönsson *et al*, 1996), referring to nitrogen, phosphorus and potassium. Fertilising experiments have indicated that the fertilising effect of stored human urine is comparable to that of mineral fertilisers for wheat and barely (Kirchmann and Pettersson, 1995; Kvarmo, 1998). Furthermore, the concentrations of heavy metals in the urine solution are very low, for example it contained 3.2 mg cadmium/kg phosphorus (Jönsson *et al*, 1997). This can be compared to mineral fertilisers containing 26 mg Cd/kg P (Jönsson *et al*, 1997), and to sludge from Swedish sewage treatment plants containing an average 55 mg Cd/kg P (Swedish EPA,1995).

Urine normally contains low amounts of transmissible microorganisms. It is therefore mainly the possible contamination of the urine by displaced faecal material that involves a risk. Due to the low amount of flush water used, a rather high concentration of microorganisms of faecal origin in the collection tank may occur even if minor amounts of faecal material enters the front part of the toilet bowl. We wanted to investigate the level of faecal contamination in urine collection tanks based on faecal indicator bacteria. Survival studies were performed in order to estimate how valid a risk assessment based on these indicator bacteria would be. From these results it also would be possible to determine optimal storage conditions for source separated human urine.

2. Methods

Sampling - *Urine collection tanks* Eleven different urine separating sewage systems located at eco-villages, one-family houses and others like schools were included in the study. Samples were collected at two occasions from 14 different

urine collection tanks within these systems, both directly beneath the liquid surface and an additional sample including sedimented material from the bottom of the tank. The samples were transported cold to the laboratory where they were analysed the same day or within 18 hours.

Urine storage tanks Urine solution from one of the eco-villages was sampled after transportation to a storage tank. Samples were taken from the storage tank at three different occasions; after 17, 57 and 126 days of storage from four different levels; at the surface, in the middle of the liquid phase, five cm above the bottom of the tank and right at the bottom. The samples were transported cold to the laboratory and analysed the same day or within 18 hours.

Analysis of physical and chemical parameters - pH and temperature of the urine solution were measured during the sampling procedure using a portable pH-meter (Orion 250 A). The conductivity was measured simultaneously using a portable conductivity meter (Hanna Instruments 8733).

Microbiological indicator analysis - The samples were homogenized and diluted in tenfold steps. For each dilution three parallel agar plates were streaked with 0.1 mL of the suspension. Total coliforms were enumerated on mEndo Agar Les (Difco). The plates were incubated at 35±0.5°C for 44±4 hours and the number of shiny colonies was recorded after 24±4 and 44±4 hours of incubation. Colonies were confirmed by a negative oxidase test (Organon Technika). For quantification of *E. coli* mFC Agar (Difco) was used. The plates were incubated at 44±0.5°C for 24±4 hours and blue colonies counted thereafter. Confirmation was performed by fermentation of lactose and formation of indol from tryptophan (LTLSB, Oxoid). For faecal streptococci mEnterococcus Agar (Difco) was used. Typical colonies were counted after incubation at 35±0.5°C for 44±4 hours. Selected colonies were confirmed on Esculin plates that were incubated in 44±0.5°C over night and by a negative catalase test. The vegetative cells of clostridia were inactivated by heating the samples to 75±5°C for 15 minutes. The inactivated samples were diluted in tenfold steps and clostridia spores were analysed by the pour plate method with 1 mL of the diluted sample in Perfringens Agar Base (Oxoid). The plates were incubated anaerobically at 37±0.5°C (BBL GasPak) for 48±3 hours and the number of black colonies were recorded after 24±3 and 48±3 hours.

Survival studies of indicator bacteria - Survival studies of faecal indicator bacteria in source separated human urine were performed at two temperatures (4°C and 20°C), at three different dilutions (undiluted, 1:1 and 1:9), and at four different pH-values (4.5, 6.0, 8.9 and 10.5). Urine solution was collected from two urine collection tanks at two different housing areas and mixed. The undiluted urine solution contained approximately 1-2 parts of urine per part flush water. Sterile water (Pharmacia & Upjohn) was used as diluent to prepare the 1:1 and 1:9 dilutions. pH of the collected urine solution was 8.9. To obtain pH 4.5 and 6.0 the urine solution was mixed with concentrated HAc. pH was adjusted to 10.5 by adding 1M NaOH. *Escherichia coli* and faecal streptococci were isolated from source separated human urine and added to the urine solutions to a final concentration of 10^6/ml. *Clostridium perfringens* was isolated from faeces in a dry

sewage system and added to a concentration of 10^2/ml. Die-off of normally occurring faecal streptococci was also studied in urine solution with an initially high concentration of these bacteria at 4°C and 20°C. In this urine solution the proportion of urine and flush water was approximately 3:1. Enumeration of the indicator bacteria was performed as described above.

3. Results

Figure 1
Concentrations of clostridia in samples from beneath the surface (surface) and from the bottom including sedimented material (bottom) from sampling round two. The values are expressed on a log_{10} scale as cfu/ml urine solution.

Collection tanks - Among the indicator bacteria *E. coli* was found in the lowest concentration. In 84% of the samples the concentration was below the detection limit of 10 cfu/ml. Faecal streptococci were by far present in the highest concentrations; 76% of the samples having concentrations above 1000 cfu/ml. Coliforms were found in varying amounts, calculated on the positive samples (55%) the mean value was 470 cfu/ml. Sulphite reducing clostridia were found in 76% of the samples with concentrations ranging from 1 to 2000 cfu/ml. All bacteria except coliforms were present in higher numbers in the bottom sediment samples than in the samples collected from beneath the surface.

Collection tanks that contained urine with high concentrations of faecal streptococci in the first round of sampling often showed similar results in the second round of sampling (figure 2). The corresponding was valid for low concentrations.

Table 1. Mean[1] and median concentrations (median concentrations in parenthesis) of faecal indicator bacteria in source separated human urine. n equals the number of samples included in the calculation of the mean value (samples with concentrations detection limit) and the number in parenthesis equals the total number of sampling results (all included in the calculation of the median value). Samples from beneath the surface (surface) and samples from the bottom of the collection tanks including sedimented material (bottom). Mean concentrations are listed with two significant numbers.

Samples	Coliforms[2] (cfu/ml)	E.coli[2] (cfu/ml)	Faecal[3] Streptococci cfu/ml	Clostridia[4] (cfu/ml)
Sampling round 1, surface	700 (100) n=8 (14)	58 (<10) n= 1 (14)	13000 (3135) n=11 (14)	140 (4) n=10 (14)
Sampling round 1, bottom	250 (<10) n=3 (7)	1200 (<10) n=1 (9)	200000 (7600) n=7 (9)	190 (86) n=8 (9)
Sampling round 2, surface	440 (22) n=6 (12)	5 (<10) (12)	16000 (2900) n=10 (12)	19 (2) n=7 (11)
Sampling round 2, bottom	230 (18) n=7 (11)	38 (<10) n=3 (9)	92000 (8000) n=12 (12)	340 (35) n=7 (8)
Total	470 (21) n=24 (44)	270 (<10) n=5 (44)	70000 (3000) n=40 (47)	340 (11) n=32 (42)

[1]Samples with concentrations below the detection limit are not included in the calculation of the mean values.
[2]The detection limit was 10 cfu/ml.
[3]The detection limit was 100 cfu/ml.
[4]The detection limit was 1 cfu/ml.
[5]All samples had concentrations below the detection limit.

Physical and chemical analysis - pH-values were generally around nine with a low of 8.30 and a high of 9.30. The temperature varied between 2.6°C and 18.5°C with a mean of 14.6°C and 8.0°C during the first and second round of sampling, respectively. The conductivity which can be seen as an indicator of the dilution varied between 1.76 mS/cm and 53.3 mS/cm with a total mean of 24.5 mS/cm. No correlation could be found between concentrations of indicator bacteria and pH, temperature or conductivity.

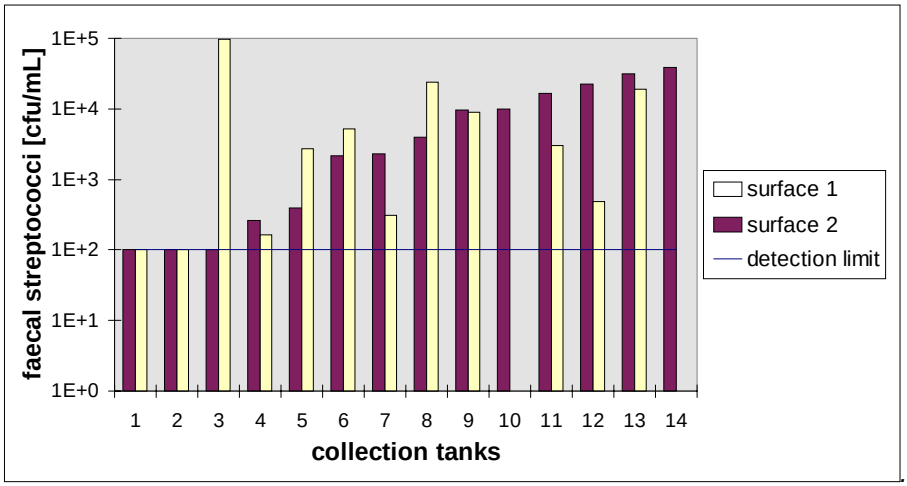

Figure 2
Concentrations of faecal streptococci in samples from beneath the surface in sampling round one and two.
The values are expressed on a log_{10} scale as cfu/ml urine solution.

Storage tanks - After four months (126 days) of storage, concentrations of all indicator bacteria except clostridia were below their detection limit of 10 cfu/ml. *E. coli* was not found in the collection tank (day 0) and not in the storage tank either. The amounts of bacteria were generally higher at the bottom in the storage tank as was the case in the collection tanks. For faecal streptococci and coliforms concentrations were higher at the surface than in the middle and close to the bottom.

	Storage days	Surface (cfu/ml)	Middle (cfu/ml)	5 cm above bottom (cfu/ml)	Bottom (cfu/ml)
Coliforms	0	250	82	<10	300
	17	<10	<10	<10	<10
	57	<10	<10	<10	<10
	126	<10	<10	<10	<10
Faecal streptococci	0	2400	1600	610	9500
	17	690	600	550	1600
	57	<10	<10	<10	110
	126	<10	<10	<10	<10
Clostridia	0	64	68	97	610
	17	140	130	110	240
	57	73	88	110	550
	126	15	59	88	290
E. coli	< 10 cfu/ml at every analysis.				

Table 2.
Concentrations of faecal indicator bacteria in source separated human urine during storage. Concentrations are listed with two significant numbers. The detection limit was 1 cfu/ml for clostridia and 10 cfu/ml for the other organisms.

Survival experiments - *E. coli* had the fastest die-off in the urine solution among

the bacteria studied. At all pH-values except at pH 6.0, *E. coli* had D-values below one day, i e a reduction from 10^6 cfu/mL to below the detection limit occured within five days of incubation for all other samples. In the 1:9 dilution *E. coli* had approximately five times longer survival than in undiluted urine solution, both at incubation temperatures 4°C and 20°C. Faecal streptococci had a slower reduction than *E. coli* in the urine solution. In the pH experiment all faecal streptococci had died within 20 days except in the two samples with pH 6.0 and 8.9 that were kept at 4°C. In these samples approximately one $\log_{10}$ reduction had occured after 27 days of incubation. Temperature had a significant effect on survival. In the samples with initially high concentrations of faecal streptococci a reduction from 10^6 cfu/ml to <10 cfu/ml was obtained after 25 days at 20°C and after 150 days at 4°C. *Clostridium perfringens* showed no reduction in any of the urine solutions during 36 days of incubation. Thus, no effect of pH, temperature or dilution on the survival of clostridia spores was noted.

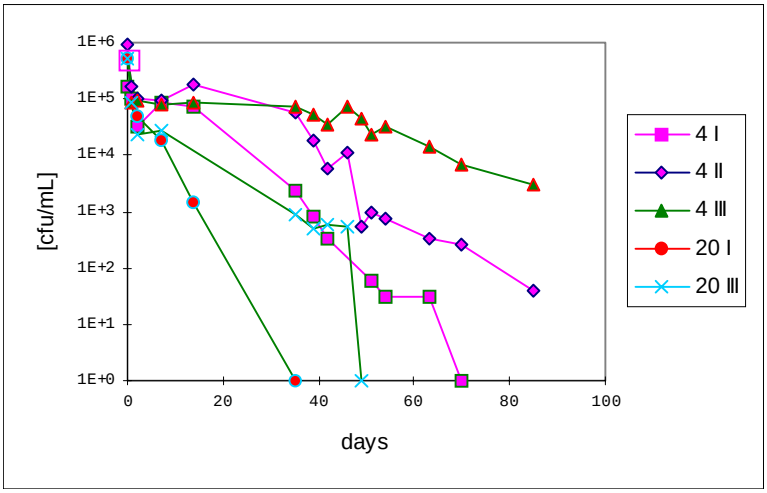

Figure 3.
Die-off of faecal streptococci in source separated human urine at different dilutions (I=undiluted, II=1:1, III=1:9) at 4°C (4) and 20°C (20). The values are expressed on a $\log_{10}$ scale as cfu/mL urine solution. (Also published in Jönsson et al, 1996.)

4. Discussion

Source separated human urine is a liquid solution rich in plant available nutrients. It contains very low concentrations of heavy metals, especially compared to sludge. Also, it is the fraction of human excreta containing the lowest amounts of microorganisms. Thus, by separating urine from other household wastes there is much to gain with regards to recycling nutrients to agriculture, eutrophication, water saving and hygiene. Urine separation has been suggested as one part of a sustainable future in Sweden and about 3000 source separating toilets have been

installed so far. However, it is inevitable that some contamination of the urine through mixing in of faecal material occurs and due to the low amounts of flush water used for the urine, concentrations of faecal bacteria might be as high as in untreated wastewater. Therefore a storage period of six months has been recommended before the urine is used as a fertiliser. If this period is reasonable depends on the initial level of faecal contamination as well as on the possibilities for various microorganisms to survive in the urine solution.

As has been reported before (Höglund *et al*, 1998) the different faecal indicator bacteria were present in various amounts. *E. coli* was seldom found, thus indicating no faecal contamination whereas faecal streptococci most often was found in high numbers indicating a significant faecal contamination. However, the concentration of faecal streptococci was in some collection tanks as high as 10^6 cfu/mL, which would correspond to 100% faeces (Geldreich, 1978). This indicates that there is a possible growth within the pipes leading from the toilets to the collection tank.

The higher concentrations found in samples from the bottom of the collection and storage tanks are explained by bacteria adsorbing to particles that sediment. Also the higher concentrations right at the surface in the storage tank can be explained by adsorption. Here bacteria bind to hydrophobic particles (Kjelleberg and Stenström, 1980). The variation in concentration of bacteria with depth illustrates the importance to collect representative samples. A calculated risk in this case would be underestimated if the bottom sediment was not included, and the other way around.

Many times concentration of *E. coli* is used as a single parameter for judging if hygienisation has occurred. In systems like this it is important to analyse several different indicator organisms rather than a single indicator bacteria. If only enumerating *E. coli* the hygienic quality of the urine solution would be considered as satisfactory without storage, whereas if enumerating clostridia the opposite would be the case.

The fast die-off of *E. coli* in human urine explains the above and further implies that this organism is useless when estimating the faecal contamination of the urine. Clostridia, which shows no reduction in urine, could possibly be used to calculate the degree of contamination. However, as previously discussed (Höglund *et al*, 1998) clostridia is not alone reliable as a faecal indicator. The frequency in human faeces is only 13-35% (Geldreich, 1978) and the spore can be found in various environments as well. The high concentrations of faecal streptococci and their slow reduction imply that they could be valuable as an indicator for sufficient storage.

Some of the urine separating sewage systems investigated were included in a previous study (Olsson, 1995). The systems having high concentrations of faecal streptococci in the presented study usually had high concentrations in the previous as well. As shown in figure 2 the concentrations of faecal streptococci also correlated between sampling occasions within systems. Families could have their own strains of faecal streptococci more adapted to this environment or depending on the feature of the system, including piping, some systems are more likely to

have higher concentrations of faecal bacteria present. Another reason could be that a sludge is formed at the initial use of the system. The special features of this sludge then determine future potentials for growth of different bacteria.

According to the commonly used faecal indicator bacteria the hygienic quality of the urine solution after storage would be equal to or better than recreational water guideline values. However, the true meaning of these indictor bacteria have been widely discussed and questioned. Are their survival coherent with the survival of pathogenic bacteria or not? In the case of shorter survival of indicator bacteria than of pathogens an estimation of the risk in a special case would be underestimated. There are known cases (Goldstein *et al*, 1996) where waterborne illnesses have been transmitted even though no indicator bacteria were present. This is important to recognise especially when evaluating the risk of newly recognised pathogens like the parasitic protozoa Giardia and Cryptosporidium known to be very resistant in the environment.

The results obtained from the survival experiments imply that a high temperature, a low dilution and a pH far from neutral shorten the survival of indicator bacteria. Survival of some pathogenic bacteria as well as other pathogens in urine has been studied (Jönsson *et al*, 1996, Höglund *et al*, 1998). Results indicate that the urine solution has a detrimental effect on microorganisms. So far no bacteria, excluding clostridia spores, seem to survive longer than faecal streptococci. Thus a storage period of six months would be sufficient. Data on the survival of *Cryptosporidium* in urine are currently being evaluated. It would also be valuable to study the behaviour of human viruses in urine. A salmonella phage showed no reduction during a 50-day period in urine, however, no coliphages have been found in urine collection tanks (Jönsson *et al*, 1996).

The inconsistency in the results from analysis of indicator organisms implicates that an alternative to the commonly used faecal indicator bacteria could be necessary for evaluating the function of complementary sewage systems and the quality of the product obtained. Faecal sterols have been suggested (Höglund *et al* ,1998; Sundin *et al*, in preparation) as a chemical indicator, suitable for estimating the faecal contamination of source separated urine. However, if sufficient storage is applied a control system might not be needed due to the germicidal effects of source separated urine as indicated by the above results.

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