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Removal of micro-organisms in a small-scale hydroponics wastewater treatment system

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ABSTRACT

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Aims: To measure the microbial removal capacity of a small-scale hydroponics wastewater treatment plant.

Methods and Results: Paired samples were taken from untreated, partly-treated and treated wastewater and analysed for faecal microbial indicators, i.e. coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens* spores and somatic coliphages, by culture based methods. *Escherichia coli* was never detected in effluent water after >5·8-log removal. Enterococci, coliforms, spores and coliphages were removed by 4·5, 4·1, 2·3 and 2·5 log respectively. Most of the removal (60–87%) took place in the latter part of the system because of settling, normal inactivation (retention time 12·7 d) and sand filtration. Time-dependent log-linear removal was shown for spores ($k = -0{\cdot}17$ log d^{-1}, $r^2 = 0{\cdot}99$).

Conclusions: Hydroponics wastewater treatment removed micro-organisms satisfactorily.

Significance and Impact of the Study: Investigations on the microbial removal capacity of hydroponics have only been performed for bacterial indicators. In this study it has been shown that virus and (oo)cyst process indicators were removed and that hydroponics can be an alternative to conventional wastewater treatment.

Keywords: coliforms, coliphages, enterococci, hydroponics, removal, spores, wastewater treatment.

INTRODUCTION

More than 90% of Swedish inhabitants have their wastewater treated in municipal wastewater treatment plants. Most of them have at least secondary treatment including physico-chemical treatment (chemical precipitation and sedimentation) and biological treatment (activated sludge). To improve nutrient recycling and decrease eutrophication of receiving waters, other systems are developed and implemented in Sweden. Most of these are based on source separation of different waste streams, i.e. black (faeces and flush water), yellow (urine) and greywater (bath, dish and laundry water) (Vinnerås and Jönsson 2002). Another alternative has been developed in the USA where hydroponics are integrated with conventional systems as a means to increase nutrient recirculation (Todd and Josephson 1996). In order to evaluate such a wastewater treatment technology under Swedish conditions, a small-scale system was constructed where conventional biological methods were combined with hydroponics and microalgae (Norström *et al.* 2003). The aim was to treat domestic wastewater while making use of the nutrients in the water for cultivation of valuable plants. At the time of this study the system treated a part of the wastewater flow, in average 559 l d^{-1}, from a small local catchment area outside Stockholm, Sweden. Removal of nonmicrobial parameters have been measured giving 90% removal of the chemical oxygen demand (COD), 72% removal of total nitrogen and 47% removal of phosphorus (Norström *et al.* 2003). In terms of micro-organism removal, a study on another Swedish hydroponics system have reported 2–3-log reduction of coliforms and 3–4 log of *Escherichia coli* and enterococci (Guterstam *et al.*

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1998). The sufficient removal of indicator bacteria does however not necessarily mean that parasitic (oo)cysts and human viruses are as efficiently removed. Studies have shown (oo)cyst and virus removal to differ from that of faecal coliforms and enterococci (Aulicino *et al.* 1996; Rose *et al.* 1996; Bonadonna *et al.* 2002; Jacangelo *et al.* 2003). *Clostridium perfringens* spores have been used successfully as an indicator of (oo)cyst removal in water treatment processes (Payment and Franco 1993; Hijnen *et al.* 2000). Bacteriophages have been proposed as an indicator to determine human enteric virus removal in wastewater treatment processes (Havelaar *et al.* 1991). To be able to estimate the removal of (oo)cysts and viruses, we have therefore added spores of sulphite-reducing anaerobic bacteria and somatic coliphages to the analysis of the most commonly-used faecal indicators, i.e. total coliforms, *E. coli*, enterococci, as process indicators for pathogenic protozoa and human virus removal.

MATERIALS AND METHODS

System description

The treatment system was constructed on Överjärva Gård, which is a small area outside Stockholm, Sweden, that is not connected to the municipal water and sewer systems. Wastewater from the catchment area was collected in a sedimentation tank, and thereafter stored in an equalizing tank. From the equalizing tank one-third of the total daily flow was pumped into the hydroponic treatment system. The flow was applied as batchflow by a Grundfors KP 150 pump (Siemens; Nurenberg, Germany) that worked for 95·5 s every second hour, resulting in an average daily flow of 559 l to the system. Excessive wastewater was treated in a sequence batch reactor. The treatment system was composed of several parts is presented in Fig. 1. The system was operated with pre-denitrification by recycling of nitrate-rich water from the second hydroponics to the anoxic tank. The total hydraulic retention time in the system was 12·7 d. Temperature in the system varied between 19·5–22·7°C, pH between 7·4 and 8·7 with the highest values measured in the algal ponds. A detailed description of the system, its function and components can be found in Norström *et al.* (2003).

Sampling

Four hundred millilitre grab samples were taken from untreated (in), partly-treated (1 and 2) and treated wastewater (out) (Fig. 1). Removal was measured from paired samples. There was no adjustment for the retention time in the system.

Microbial analyses

Analyses of total coliforms, *E. coli* and enterococci were performed with Colilert^{TM} 18 and Enterolert^{TM} (IDEXX; Westbrook, ME, USA) according to the manufacturer's instructions. Sulphite-reducing anaerobes were analysed with standard method (ISO 6461/2). The samples were heated (70°C, 20 min) and thereafter analysed with the pour plate method (Perfringens agar base, 44 h, 37°C). Somatic coliphages were analysed by plaque assay with the double agar layer method according to ISO 10705-2 using *E. coli* C (ATCC 13706) as host strain.

Data analysis

The microbial distribution in the environment is often well described by a log-normal function (Hirano *et al.* 1982;

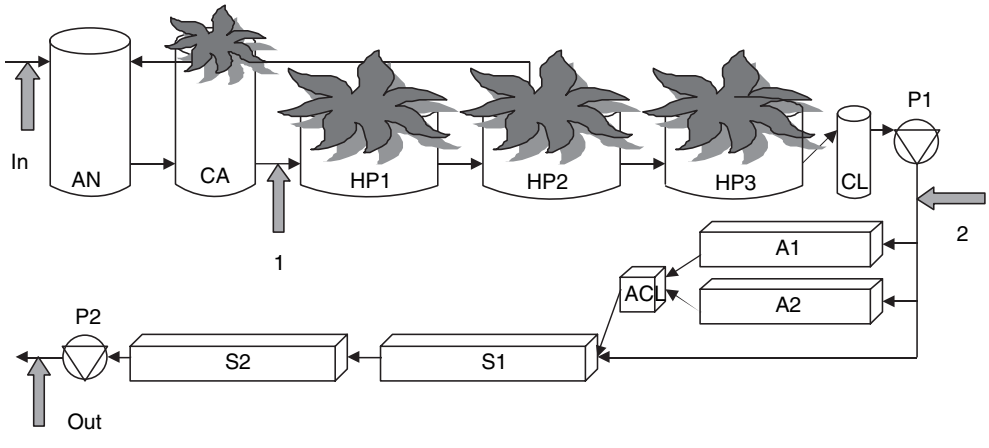

Fig. 1 The studied system was built up of several parts. Anoxic tank (AN), closed aerobic tank (CA), hydroponics (HP1–HP3), clarifiers (CL, ALC), peristaltic pump (P1), algal steps (A1–A2), sand filters (S1–S2) and effluent pump (P2). The four sampling points (in, 1, 2 and out) are indicated with arrows

Loper *et al.* 1984). Hence numbers are presented as $\log_{10}$-values (mean ± SD) per volume. As a consequence, removal is measured as $\log_{10}$-reduction. Removal was quantified from paired samples. For negative samples the limit of detection was used. Statistical analyses were performed in SigmaStat 3·0 (SPSS Inc., Chicago, IL, USA). Plots were made in SigmaPlot 2001 (SPSS Inc.).

RESULTS

Escherichia coli was never detected in the treated wastewater, enterococci 3/9 times after >3·8 log (99·98%) reduction. Coliforms were subsequently detected in the treated wastewater after average 4·1 log reduction (Table 1). Spores and phages were removed significantly less than the bacterial parameters ($P < 0·001$), 2·3 and 2·5 log respectively. The mean/SD removal ratio varied between 5·4 (spores) and 10 (enterococci) (Table 1). In the first two closed tanks, between 13% (spores) and 40% (total coliforms) of the total removal took place (Fig. 2). Spores followed a time-dependent loglinear removal with a decay rate of 0·17 log d^{-1} (Fig. 2). Vegetative bacteria were removed significantly more per time unit ($P < 0·001$) in the closed tanks and algal tanks or sand filters than in the nitrifying zone between sampling point '1' and '2'. Most of the somatic coliphage removal, 51%, took place in the algal tanks or sand filters (Fig. 2).

Table 1 Mean $\log_{10}$ removal of microbial indicators in hydroponics wastewater treatment

Micro-organism	Mean	SD	Minimum	Maximum	Mean/SD	*n*
Total coliforms	4·1	0·41	3·1	4·5	10	9
Escherichia coli	>5·8	0·22	>5·6	>6·3	–	9
Enterococci	4·5	0·44	3·8	4·6	10	9
Somatic coliphages	2·5	0·39	2·1	3·1	6·3	9
Spores of sulphite-reducing anaerobes	2·3	0·42	1·6	2·9	5·4	9

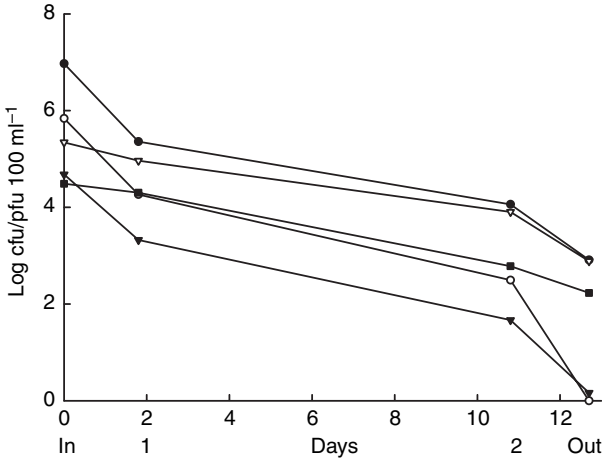

Fig. 2 Log number of faecal indicators in hydroponics wastewater treatment as a function of treatment and hydraulic retention time. Total coliforms (●), *Escherichia coli* (○), enterococci (▼), somatic coliphages (▽) and spores of sulphite-reducing anaerobes (■) were measured after anoxic tanks (1), hydroponics treatment (2), algal tanks and sand filters (out)

DISCUSSION

Coliforms and enterococci were detected with Colilert 18 and Enterolert. Studies have shown that these methods are equivalent to standard methods for analyses of drinking and bathing water (Edberg *et al.* 1994; Eckner 1998). We have used Colilert and Enterolert successfully on wastewater. The advantage is that 100 ml can be analysed with the same method as 10^{-3} ml and thus >5 log removal of coliform bacteria and enterococci is possible to measure. Still, *E. coli* was never detected in the treated wastewater after >5·8 log removal. This figure can be compared with the removal of 2–3 log in common Swedish wastewater treatment plants for the same parameter (Stenström 1986). Also coliphages and spores of sulphite-reducing anaerobes were removed significantly more than in secondary wastewater treatment. However, they were not as efficiently removed as the bacterial indicators. The high mean/SD ratio indicates reliable removal during the time of the study. The hydraulic retention time in the studied system was 12·7 d compared with 12 h in ordinary secondary treatment plants. Thus, most of the removal was attributed to settling and thermo-mediated die-off of phages and spores, also shown in wetland treatment (Quinonez-Diaz *et al.* 2001; Stenström and Carlander 2001; Falabi *et al.* 2002), which may be the most appropriate treatment to compare hydroponics with in terms of removal mechanisms. All the studied organisms, with the exception of spores of sulphite-reducing anaerobes, were however, removed more efficiently in the closed tanks, because of anaerobic conditions and/or higher biomass in these tanks. The removal in the latter part, algal tanks and sand filters, was also higher than in the nitrifying zone, probably because of high pH in the algal tanks and adsorption to the sand filters.

Looking at virus occurrence and removal to assess risks with wastewater reuse and discharge has been suggested (Pina *et al.* 1998; Hot *et al.* 2003). However, in absence of the possibility somatic coliphages are a good choice of indicator, if looking for specific virus is not possible. It has been suggested that F-specific phages and phages infecting *Bacteroides* spp. are possible indicators (Havelaar *et al.* 1991). F-specific phages are however, reported to be more sensitive than coliphages in wastewater at 22°C (Gantzer

et al. 2001). Phages infecting *Bacteroides* occur in lower numbers than coliphages (Lucena *et al.* 2003), which made them unsuitable for this study. Coliphage removal was 2·5 log, compared with 1·3 log in secondary treatment (Stenström 1986). In the anoxic and hydroponics treatment, 49% of coliphage removal took place at a mean decay rate of 0·11 log d^{-1}, similar to what Gantzer *et al.* (2001) reported in standing wastewater at 22°C. Additional removal can be attributed to either settling of phages bound to particles (Stenström and Carlander 2001) or adsorption to vegetation (Nokes *et al.* 2003; Vidales *et al.* 2003). Gersberg and Silvaggio (1992) and Quinonez-Diaz *et al.* (2001) did however, find UV and temperature to be more important than vegetation as removal mechanisms. Removal between sampling point '2' and 'out', where more than half of the coliphage removal took place, is attributed to high pH in the algal ponds or, most likely, adsorption of phages to the sand filter.

Spores were suggested as a process indicator for (oo)cyst removal in the studied system. The removal in this hydroponics system was 2·3 log, compared with 1·3 log in secondary treatment (Stenström 1986). In terms of long-term storage, spores are a good indicator of the removal of parasitic (oo)cysts. However, they are not likely to settle as easy as the (oo)cysts and will probably underestimate parasite removal, thereby overestimating the risk, in the studied system. In duckweed ponds, (oo)cyst removals exceeded bacterial indicator removal (Falabi *et al.* 2002). Because of the long-retention time, spores are preferred to the bacterial parameters as a conservative process indicator for (oo)cyst removal in the studied system.

In conclusion, we can say that micro-organisms are removed satisfactorily in the studied integrated hydroponics wastewater treatment system. It may be an alternative to traditional treatment at least to treat the wastewater from smaller populations, or in less densely populated areas, because of the long-retention time. Risks that have to be taken into account are if plants grown in the wastewater are eaten or sold. Plant uptake and adsorption can only explain a fraction of the total removal in the system. Enterohaemorragic *E. coli*, did however, adsorb better to roots of salad crops than did nonpathogenic *E. coli* (Wachtel *et al.* 2002). Because of adsorption of micro-organisms to the roots (Nokes *et al.* 2003), possible uptake of micro-organisms and uptake of heavy metals, it is recommended to grow ornamental or nonedible crops for wastewater treatment (Rababah and Ashbolt 2000). Risks must also be considered in the open tanks in the early part of the system if it is possible for human contact with the partly-treated wastewater. Most of the removal took place in the latter stages of the treatment. Almost no removal was shown for spores or coliphages, which were suggested as process indicators for pathogenic virus and protozoa removal, in the first two closed tanks of the system. Safety measures should be considered, preferably in accordance with hazard analysis and critical control points (HACCP), for example, as described in Westrell *et al.* (2004).

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