WATER RESEARCH 40 (2006) 3109–3114

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Bacteria removal in septic effluent: Influence of biofilm and protozoa

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ARTICLE INFO

Article history:
Received 6 September 2005
Received in revised form
5 June 2006
Accepted 11 June 2006
Available online 8 August 2006

Keywords:
Protozoa
Bacteria removal
Grazing
Predation
Biofilm
Septic effluent

ABSTRACT

Numerous biological, physical and chemical parameters are involved in the retention and removal of bacteria in wastewater treatment systems. Biological parameters, such as biofilms and protozoa grazing activity, are often mentioned but few studies provide a better understanding of their influence. In this study, the effect of bacterivorous protozoa on pathogenic indicator bacteria removal was investigated in septic effluent and in the presence of a biofilm coating glass slides. Endogenous bacteria from septic effluent were quantified. First, bacteria removal was compared between septic effluents treated or not with an inhibitor of protozoa (cycloheximide). The mortality rates were 10 times lower in treated effluent (96 CFU $mL^{-1} d^{-1}$) than in untreated effluent (1100 CFU $mL^{-1} d^{-1}$). Secondly, the efficiency of bacteria removal was studied (i) with a biofilm surface and active protozoa, (ii) with a biofilm surface and inactivated protozoa, (iii) with a clean surface. Protozoa in the presence of a biofilm were responsible for 60% of bacteria removal. Biofilm without protozoa and a clean surface each removed similar quantities of bacteria. Grazing by protozoa could be an important biological mechanism for bacterial elimination in wastewater treatment systems.

1. Introduction

In on-site wastewater treatment plants, domestic wastewater is pre-treated by septic tank and treated by soil infiltration or sand filtration systems, with unsaturated flow conditions. This process occurs in the presence of air and water and can remove carbon, nitrogen, and suspended solids efficiently (Pell and Nyberg, 1989a,b). The purification of septic wastewater involves physical, chemical and biological mechanisms. A significant reduction in the number of bacteria occurs in these treatment systems but some pathogens can migrate through the porous media over long distances. This diffusion leads to the contamination of groundwater resulting in the outbreak of water-borne diseases (Yates and Yates, 1988). Several studies have focused on the transport and fate of bacteria in porous media (Gerba and Bitton, 1984; Schwager and Boller, 1997). The two mechanisms involved in the retention of bacteria are filtration and adsorption. The factors influencing these phenomena in porous media are widely discussed in the literature (Stevik et al., 1999a,b; Sélas et al., 2002; Bomo et al., 2003). Among those that affect the filtration of bacteria, the most important are: grain size, bacterial cell size and shape, hydraulic load, water saturation and the degree of clogging layer development in the filter. Meanwhile, cell adsorption onto porous media is influenced by the content of organic matter, the degree of biofilm development, temperature, water flow, ionic strength, pH, hydrophobicity and bacterial concentration (Stevik et al., 2004). However, the

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doi:10.1016/j.watres.2006.06.008

mechanisms of bacterial elimination are less studied than those of retention. Temperature, pH, moisture content, organic matter levels and bacterial species are the most reported parameters (Stevik et al., 2004). Little information is available about microbial interactions, such as predation by protozoa (Bomo et al., 2003; Weber-Shirk and Dick, 1999), species competition, substrate competition, antibiosis by phages and synthesis of inhibiting substances (Stevik et al., 2004). Sélas et al. (2002) observed in a sand column that bacteria removal was better in the presence of a biofilm than without it. They attributed this effect to the action of predatory micro-organisms.

In this study, the objective was to show the qualitative effect of protozoa grazing on the removal of pathogenic bacteria in septic effluent and in the presence of a biofilm. For this purpose, the survival of endogenous faecal and total coliforms in a septic effluent was compared with active and inactivated endogenous protozoa. The removal of total and faecal coliforms and aerobic bacteria was investigated by applying septic effluent to glass slides (i) with a biofilm and active protozoa, (ii) with a biofilm and inactivated protozoa, and (iii) without a biofilm.

2. Materials and methods

2.1. Protozoa inactivation

Protozoa are eukaryotic predators that can be inhibited chemically by cycloheximide (Davies et al., 1995; Le Bihan and Lessard, 2000; Bomo et al., 2004). In a preliminary experiment, the effect of two cycloheximide concentrations, 200 and 1000 mg L^{-1}, on endogenous protozoa activity from septic effluent was assessed under an optical microscope (Leica DMLB, GMBH, Germany). The septic effluent came from a septic tank of a scale 1 experimental treatment plant fed by domestic wastewater. For information, some average values characteristics of septic effluent are: 84 ± 25 mg L^{-1} suspended solids, 72 ± 25 mg L^{-1} TOC, 437 ± 122 mg O_2 L^{-1} COD, 215 ± 75 mg O_2 L^{-1} BOD, 52 ± 15 mg N L^{-1} NH_4^+, 1 ± 0.5 mg L^{-1} dissolve oxygen and pH 7.5 ± 0.5. In septic effluent, bacterial concentrations are 10^6–10^7 UFC 100 mL^{-1} for total coliforms, 10^5–10^6 UFC 100 mL^{-1} for faecal coliforms and 10^7–10^8 UFC 100 mL^{-1} for total aerobic bacteria.

2.2. Pathogenic bacteria removal in septic effluent

A die-off study was performed to investigate the influence of bacterivorous protozoa on pathogenic indicator bacteria present (i) in a septic effluent with endogenous protozoa and (ii) in a septic effluent supplemented with 200 mg L^{-1} cycloheximide to inhibit protozoa at the beginning of the experiment. The survival of total and faecal coliforms was followed during 26 days. Flasks containing about 150 mL of effluent were continuously stirred at room temperature (20 ± 2 °C) in a dark room.

2.3. Pathogenic bacteria removal in the presence of biofilm

2.3.1. Experimental procedure

The experiment consisted of providing a septic effluent on a glass surface (i) without a biofilm (configuration 1), (ii) with a 36-day-old biofilm and inactivated protozoa (configuration 2), and (iii) with a 36-day-old biofilm and active protozoa (configuration 3) (Fig. 1).

Biofilm on glass slides was grown at room temperature (20 ± 2 °C) in a dark room using a septic effluent changed daily. Each glass surface area of 98.8 cm^2 was composed of five coupled glass slides (7.6×2.6 cm). They were placed in cellular culture flasks, open at the top. In order to ensure unsaturated flow conditions, the flasks were inclined so that effluent could run off the surface. For each configuration, septic effluent was distributed with a peristaltic pump at 0.23 L h^{-1}. Cycles of 30 min of supply and 30 min of time off were applied during 24 h. The estimated retention time in our experimental design was measured at about 5 min.

Three days before measurements were made, septic effluent supplying configuration 2 and colonized glass slides

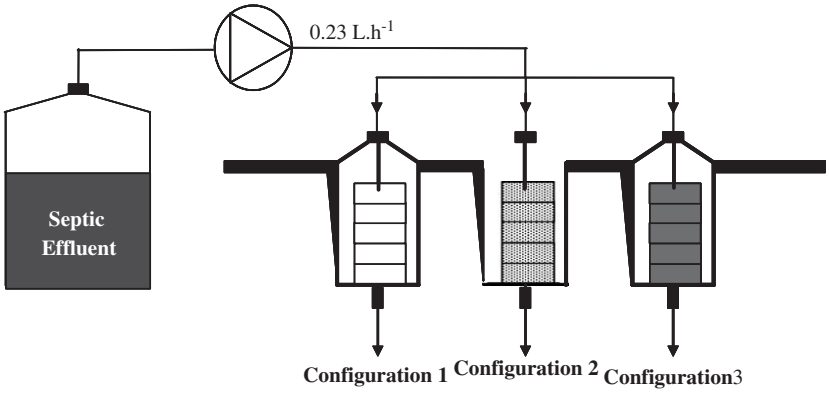

Fig. 1 – Configuration of the experimental design and conditions.