

GROWTH OF BACTERIA IN TREATED GREYWATER IN THE CONTEXT OF BLUE DIVERSION AUTARKY TOILET

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Abstract

In the Autarky project (www.autarky.ch), a toilet with on-site treatment is being developed to provide a safe and affordable sanitation technology for people who lack access to sewer-based sanitation. Water from flushing, hand washing, and personal hygiene is treated, and recycled using a biologically-activated membrane bioreactor (BAMBi) followed by a secondary treatment technology. To determine the most appropriate treatment, granular activated carbon (GAC), ultraviolet C (UVC), chlorine (sodium hypochlorite), and electrolysis are benchmarked based on performance inactivating and reducing growth of four different bacteria (*Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, and *Salmonella typhimurium*). *E. coli*, *P. aeruginosa*, and *S. typhimurium* demonstrated potential for limited growth in water following treatment with the BAMBi. Adding a carbon (*E. coli* and *P. aeruginosa*) or iron (*S. typhimurium*) source increased growth potential, suggesting carbon or iron limitation. The addition of GAC after BAMBi contributed up to 94% removal of dissolved organic carbon (DOC), 96% reduction of assimilable organic carbon (AOC), and reduced growth potential of *E. coli* by 2 log₁₀. The indigenous community did not have significant effect on the growth of *E. coli* or *P. aeruginosa* in water after BAMBi, while decreased *E. coli* growth and increased *P. aeruginosa* growth in water after BAMBi+GAC. UVC irradiation (after 15 min), chlorination, and electrolysis (after 5 min) achieved more than 5 log₁₀ inactivation of *E. coli* in water after BAMBi+GAC. Regrowth of *E. coli* was observed after 72 h after chlorination or UVC irradiation, but not after electrolysis. Treatment including the BAMBi, GAC, and electrolysis appear to be promising technologies for inactivating and reducing growth of bacteria for greywater reuse.

Keywords

Bacterial growth, greywater treatment, water reuse, safe sanitation

METHODS

Samples were collected from the clean water tanks of two Blue Diversion Autarky Toilets (BDT), one operated with only the BAMBi (after BAMBi), and one with the addition of a GAC column after the BAMBi (after BAMBi+GAC). Samples were prepared by pre-filtering through a 1 µm-pressurized filter, pasteurizing (80°C for 60 min), and re-filtering through a 0.2 µm membrane filter (Vital et al., 2008). Growth potential of bacteria was determined by measuring the increase of bacterial concentration after being spiked into prepared water samples at 30°C in 60 – 72 h. Indigenous bacterial community, or nutrient solutions (sodium acetate, ammonium sulfate, sodium phosphate dibasic, and ferric chloride as the form of C, N, P, and Fe, respectively) were added to test for their effects on bacterial growth. Inactivation experiments were conducted with *E. coli* exposed to chlorine (sodium hypochlorite, 0.5 mg Cl₂/L), UVC (JBL, 9 µW/cm²), and electrolysis (Condias, 8V, 2.4A) in water after BAMBi+GAC. Regrowth of *E. coli* was measured by incubating the inactivated water at 30°C in dark for 72 h. Flow cytometry measurement (BD, Accuri C6) for total cell counts and intact cell counts, and culture-based method using selective media were used to measure bacterial concentrations.

RESULTS AND DISCUSSION

Growth potential of bacteria. *P. aeruginosa* and *E. coli* grew in both water after BAMBi and after BAMBi+GAC, while *Enterococcus* spp. and *S. typhimurium* did not grow. The net growth of *E. coli* in water after BAMBi+GAC was significantly lower (2 log₁₀) than in water after BAMBi, aligning with the decrease of DOC (17 times lower) and AOC concentration (25 times lower). However, there was no significant difference in net growth of *P. aeruginosa* in both waters, signaling that AOC concentration is not the only factor of bacterial growth, AOC quality may also play a key role.

The addition of C in water after BAMBi significantly increased net growth of *E. coli* and *P. aeruginosa*, suggesting that C limits further growth of both bacteria (Figure 1). For *S. typhimurium*, Fe

was the limiting factor; the addition of FeCl_3 increased *S. typhimurium* concentrations by more than 3 \log_{10} . None of the additives showed any positive effect on the growth of *Enterococcus* spp. The indigenous community in water after BAMBi did not seem to have any significant effect on the growth of all four tested bacteria. However, the presence of the indigenous community in water after BAMBi+GAC slightly decreased the growth of *E. coli* but enhanced the growth of *P. aeruginosa*.

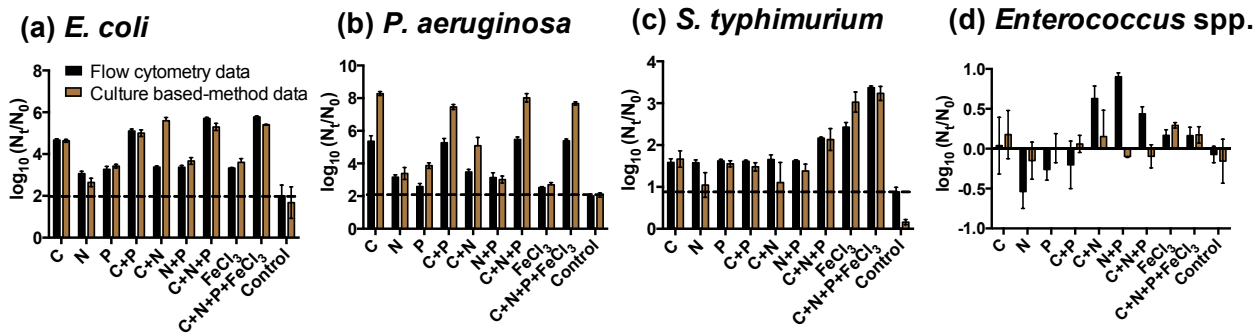


Figure 1. Net growth [$\log(N_t/N_0)$] of four tested bacteria in the addition of C, N, P, and Fe in water after BAMBi after 72 h. Controls were samples that did not have any addition. Dashed lines were drawn for comparison with the highest value of the controls in each experiment.

Inactivation and regrowth. More than 5 \log_{10} inactivation of was achieved after 5 min for chlorination and electrolysis, and after 15 min for UVC. While there was no reactivation or regrowth of *E. coli* after 72 h after electrolysis treatment, 100% of *E. coli* reactivated was measured after UVC and chlorination treatments (culture-based method). High concentration of free chlorine during electrolysis ($>2 \text{ mg Cl}_2/\text{L}$) explained for the lack of reactivation or regrowth after the treatment. However, when chlorine was quenched by sodium thiosulfate, *E. coli* was able to reactivate to reach the initial concentration after chlorination treatment. This result agrees with previous research showing a wide range of bacteria, including total coliforms, *Enterococcus* spp., and *Salmonella* spp. reactivated after exposing to low concentration of chlorine (Li et al., 2013). It was also documented that *E. coli* cells were able to repair the DNA damages and reactivate after UVC irradiation (Jagger, 1958).

CONCLUSION

Despite the growth of *E. coli* and *P.aeruginosa* in both greywater samples, we identified C as the factor limiting additional growth of these two bacteria, whereas Fe limited growth of *S. typhimurium*. The presence of the indigenous community showed effect on the growth of *E. coli* and *P. aeruginosa* in water after BAMBi+GAC, but not in water after BAMBi. Chlorination, electrolysis, and UVC were effective to inactivate more than 5 \log_{10} of *E. coli* in water after BAMBi+GAC. Bacterial cells damaged during UVC irradiation and chlorination, however, were able to repair and reactivate after 72 h in dark condition and in the absence of chlorine. The combination of the BAMBi, GAC and electrolysis appear to be the most suitable approach to inactivate and prevent the growth of bacteria in greywater reuse.

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