

# PATHOGEN REMOVAL IN THE PRODUCTION OF A URINE-BASED FERTILIZER

## INTRODUCTION

Centralised sanitation systems break the **nutrient cycle** : nitrogen, phosphorus and potassium found in urine are lost rather than recovered for agricultural purposes<sup>(1)</sup>.

The association Aneco has developed low-tech **charcoal biofilters** that **stabilise source-separated urine** into a liquid fertilizer called Pitribon, via biological nitrification.

To be certified and allowed on the market, Pitribon must meet safety standards such as sufficiently low pathogen levels. Despite a lack of strict regulations, we consider the **safety limit of 10 CFU\*/mL** that should be met in the final product, based on legislations and guidelines issued by the European Union<sup>(2)</sup>.

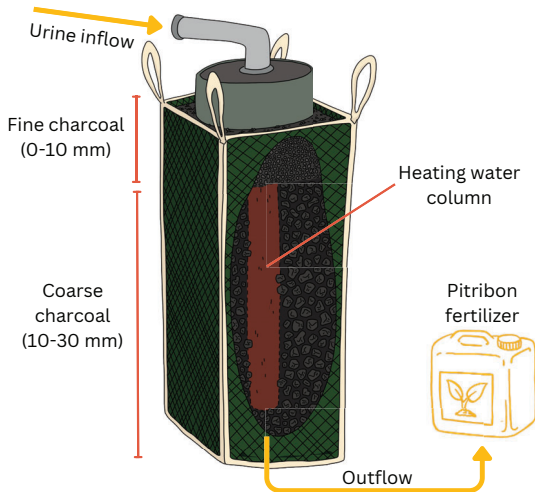


Figure 1 – Diagram of the production of Pitribon<sup>(3)</sup>

## 1. Flow rate

**Assess the effect of urine flow rate on pathogen indicators removal efficiency**  
Measuring concentrations of *Escherichia Coli* (*E.coli*) as gram-negative and *Enterococcus faecalis* (*Enc.*) as gram-positive bacteria indicators before and after filter in operation under different flow rates.

## OBJECTIVES

## 2. Storage

**Determine the decay rate of pathogen indicators in storage**  
Regular measurements of *E.coli* and *Enc.* concentrations in different storage conditions over time.

## METHODOLOGY

### Conditions

- **Two filters** (F1 and F2)
- **Three flow rates** assessed
  - Normal flow: 17.5 L/d (week 1)
  - Decreased flow: 12.8 L/d (week 2)
  - Increased flow: 27.2 L/d (week 3)

- **Storage 1: Pitribon** collected from mature filter
- **Storage 2: Fresh urine** collected from first collection tank
- Stored at room temperature (22.5°C)

### Sampling strategy

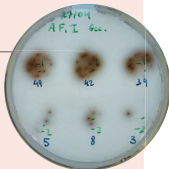
- Urine samples taken at **inflow** and **outflow** of F1 and F2 for 3 days in a row per flow regime
- Two **duplicates** per day (A and B)

- One sample per day on the following days:
  - Storage 1: day 0, 1, 2, 5, 6, 7, 8, 9, 12
  - Storage 2: day 0, 1, 2, 3, 6, 7, 8, 9, 10

### Plating strategy

- Plating ~2h after sampling
- Week 1 (normal flow):
  - *E.coli* dilution 0 & -1 (10 µl)
  - *Enc.* dilution -1 & -2 (10 µl)
- Week 2 and 3 (decreased and increased flows):
  - *E.coli* dilution 0 (50 µl)
  - *Enc.* inflow dilution -1 & -2 (10 µl)
  - *Enc.* outflow dilution 0 (50 µl)

- Plating directly after sampling
- Storage 1:
  - *E.coli* dilution -1 & -2 (10 µl)
- Storage 2:
  - *E.coli* dilution -1 & -2 (10 µl), from day 6 dilution 0 (50 µl)
  - *Enc.* dilution -1 & -2 (10 µl)



## RESULTS

### Influence of flow rate on removal

- Poor *E.coli* data → focus on *Enc.*
- Comparison of filters:
  - **F2 was more effective** at reducing *Enc.* concentrations due to **nitrification**<sup>(4)</sup> (the filter explains 31% of variance of log reduction values and p-value = 0.04)
  - higher removal would be expected with fully **mature filters**
- Comparison of flow rates:
  - **Increased flow** provides the **lowest *Enc.* reduction efficiency** (average log reduction = 1.09)
  - No significant difference between Decreased and Normal flow rates (average log reduction ≈ 1.4)

$$\text{Decay rate in storage: } \ln\left(\frac{C}{C_0}\right) = -k \times t$$

Concentration at specific time t (CFU/mL)      Decay rate constant (d<sup>-1</sup>)  
Concentration at time t=0 (CFU/mL)      Time (d)

- **Faster decay of *E.coli* in fresh urine** (more hostile conditions<sup>(5)</sup>) ( $k = 1.88 \text{ d}^{-1}$ ) compared to Pitribon ( $k = 0.212 \text{ d}^{-1}$ )
- **Higher resistance of *Enc.*** ( $k = 0.115 \text{ d}^{-1}$ ) compared to *E.coli* in fresh urine<sup>(4)</sup>
- Required fresh urine **storage of ~ 37 days** before treatment (at normal flow rate) to reach safe pathogen levels in the final product (< 10 CFU\*/ml)

\*CFU = colony forming units

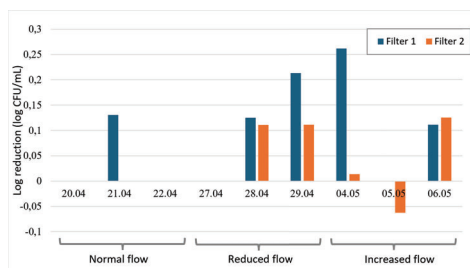


Figure 1 – Log reduction of *E. coli* over time

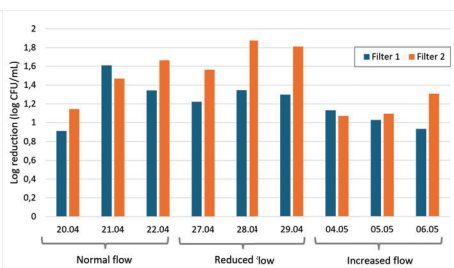


Figure 2 – Log reduction of *Enc.* over time

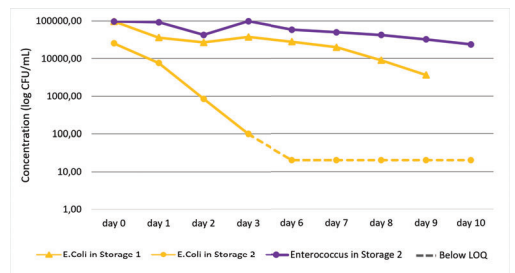


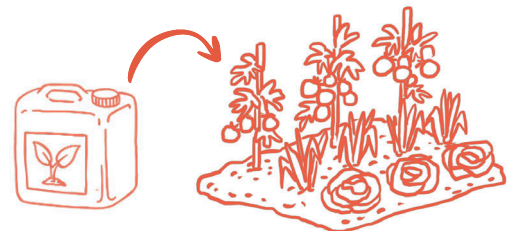
Figure 3 – Log concentration of *E. coli* and *Enc.* in both storage conditions over time

## CONCLUSION

- **Nitrification** in the filter actively contributes to pathogen reduction
- Within this study, the **EU safety limit of 10 CFU/mL** was not met by *Enterococcus*, unknown for *E.coli*
- *Enc.* is a more persistent and conservative indicator for pathogen presence in urine
- **Lower flow rate** = better pathogen removal

### Design indications

- *Enc.* should be used as the **reference indicator** for pathogen assessments in urine treatment systems
- Maturity of the filters is crucial : the product of immature filters should not be used
- Optimal flow rate : ~17.5 L/d → the flow should not exceed 27 L/day



## LIMITATIONS

- Filters were not sufficiently mature at time of sampling → results are not representative of steady-state performance
- *E. coli* concentrations frequently below detection limit (LOQ) & changes in plating method → unreliable statistical analysis for this indicator

## REFERENCES

(1) C. Hoglund. "Evaluation of microbial health risks associated with the reuse of source-separated human urine." Doctoral thesis. Stockholm: Royal Institute of Technology (KTH), Swedish Institute for Infectious Disease Control (SMI), 2001.  
 (2) European Commission. "Proposal for a Regulation on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2008 and (EC) No 1107/2009." COM(2016) 157 final, annex I. Product Function Categories (PFC) of CE marked fertilising products.  
 (3) Aneco. "Note applicative pour la construction d'une unité de filtre à Pitribon 2023."  
 (4) A. Schertanlieb. "Inactivation of pathogens in urine nitrification reactors." 2014.  
 (5) M. Maurer, W. Pronk, and T. A. Larsen. "Treatment processes for source-separated urine". In: Water Research 40,17 (Oct. 2006), pp. 3151-3166. issn: 0043-1354.