Impact of certain water quality parameters on *Enterococcus faecalis* JH2-2 transport in sandy sediments

A. Chandrasekar[*], T. Berendonk[#], I. Kampouris[#], D. Cacace[#], D. Kneis[#], M. Binder[*,+], R. Liedl[*]

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INTRODUCTION

Re-use of treated waste water (TWW) is a practice adopted by many countries to combat water scarcity, and enable a more sustainable economy. However, we must study and understand the risks associated with this practices and the possible spread of contaminants of emerging concern into the environment: food crops and groundwater. (Berendonk, et al., 2015).

The possible spread of antibiotic resistance is studied in this poster using *Enterococcus faecalis* JH2-2. *Enterococcus faecalis* are opportunistic pathogens, and their high resilience to antibiotics make them ideal indicators of fecal contamination in water, and for spread of antibiotic resistance in the environment.

Considering the context of waste water reuse, the objective of this study is: to investigate impact of Dissolved Oxygen (DO) and nutrients(N) on the fate and survival of *Enterococcus faecalis* JH2-2 in both the water phase and the sediment phase.

METHODS

Experimental set-up, conditions, sediment and water characteristics

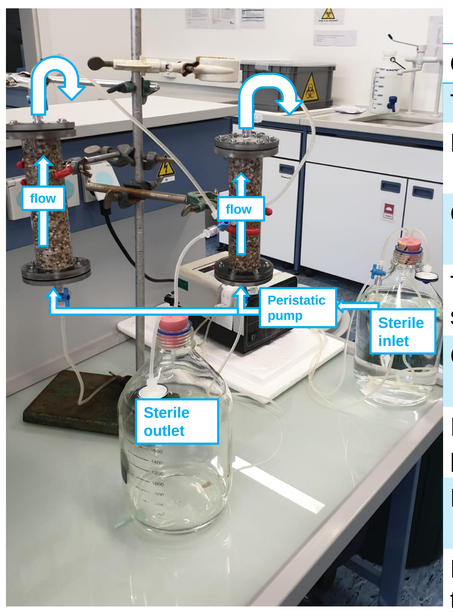

Figure 1: Experimental Set-up

Table 1:Experimental conditions

Condition	Value
Temperature	20±2°C
Model bacteria	*Enterococcus faecalis* JH2-2
Column type	Acrylic glass (L=15cm, d=3cm)
Type of sterilization	Autoclavation at 121°C for 20 minutes
Growth media	Brain Heart infusion media (30°C for 18h)
Injection period (t)	240 minutes
Flow rate (F_{in})	0.85±0.2mL/min
Residence time (RT)	30-40 minutes
Reproducibility	3 times

Table 2: Sediment Characteristics

Characteristic	Values
pH	5.4
Bulk density	1.7($\frac{g}{cm^3}$)
Grain Density	2.63($\frac{g}{cm^3}$)
Sediment packing method	Fully saturated
Total Porosity	0.34
Effective porosity (θ_e)	0.33
Loss on ignition	<0.1%
Grain Size fraction	1mm-4mm

Figure 2: Wet packing of column with sediment

Table 3: Water characteristics for each of the test conditions

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Without DO	De-oxygenated sterile water with nutrients (Condition 3) **[C3]**	Has no dissolved oxygen or nutrients (Condition 4) **[C4]**

Non dimensionalisation equations

1. $\frac{C}{C_o}(-) = \frac{conc.of\ water\ phase\ bacteria\ (^{CFU}/_{mL})}{conc.of\ inlet\ bacteria\ at\ t=0\ (^{CFU}/_{mL})}$

2. $\frac{CFU\ retained}{CFU\ injected}(-) = \frac{conc\ from\ plate\ count * (\frac{9mL}{1g}) * weight\ of\ sedimentsection}{\sum_{i=0}^{i=t_{end}} C_{in}(t_i) * t_i * F_{in} * \theta_e}$

3. $pore\ volume\ (pv)(-) = \frac{time(mins)}{residence\ time\ in\ column\ (mins)}$

Quantification methods

A. Sediment extraction

The column is divided into 5 equal sections (~3cm per section) at the end of the experiment → 1g sediment per section is suspended in 9mL of sterile 0.85%NaCl solution → Suspension is vortexed at 13,400 rpm for 30 seconds → Shaken in an orbital shaker for 30 minutes → 100µL plated

Figure 3: Flowchart representing the extraction of bacteria from sediment at the end of the experiment (Negreanu et al., 2012)

B. Plating in selective media

- 100µL of sample is plated using spread plate method in m - Enterococcus agar with Triphenyl tetrazolium chloride (TTC)
- Incubated at 41°C for 48 hours
- Samples are stored at 4°C for up to a week, for further dilutions
- Concentration of the bacteria is calculated according to the formula below

$$C\left(\frac{CFU}{mL}\right) = \frac{no.of\ colonies * dilutionfactor}{100\mu L}$$

Figure 4: Quantification of water and sediment phase bacteria

RESULTS

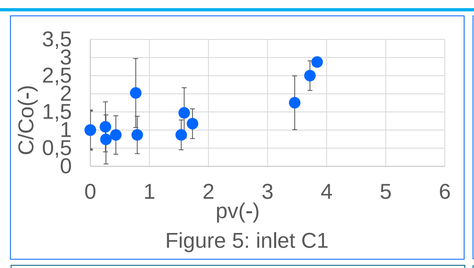

Figure 5: inlet C1

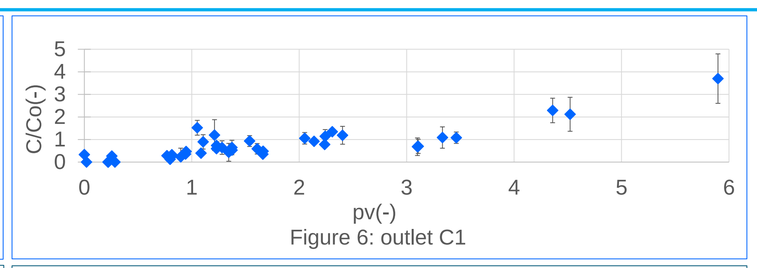

Figure 6: outlet C1

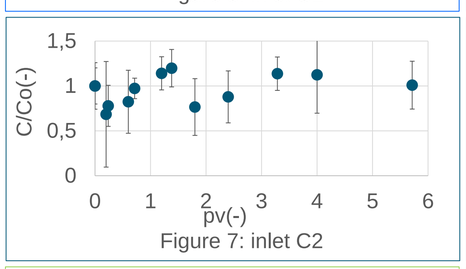

Figure 7: inlet C2

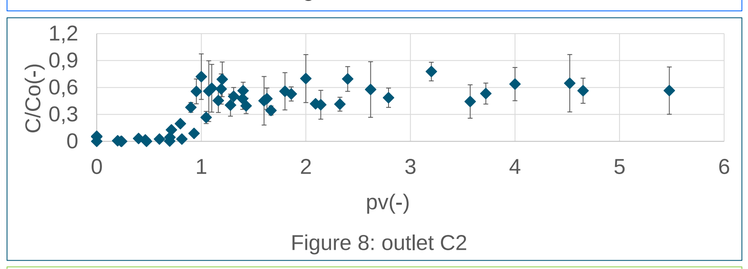

Figure 8: outlet C2

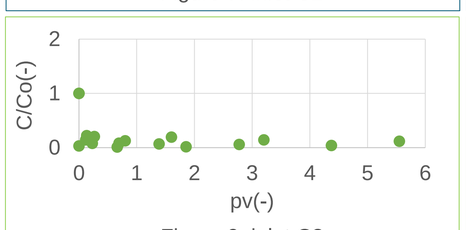

Figure 9: inlet C3

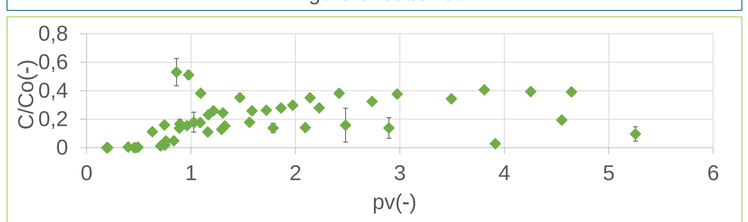

Figure 10: outlet C3

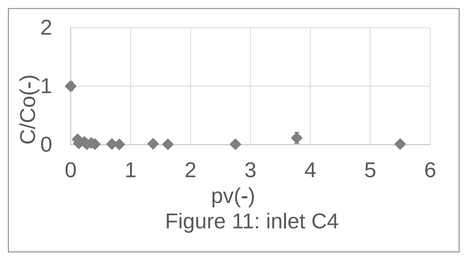

Figure 11: inlet C4

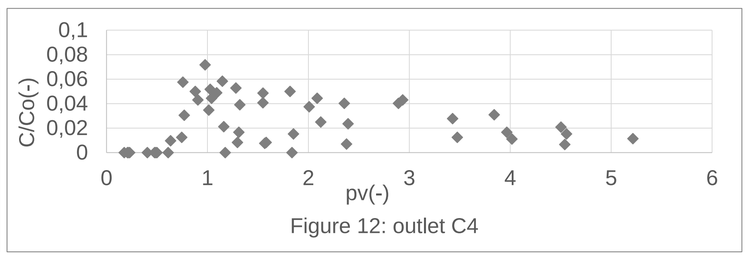

Figure 12: outlet C4

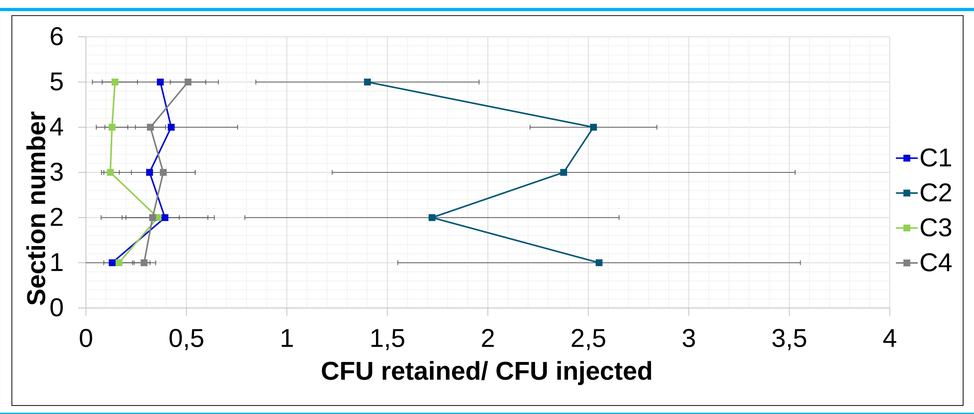

Figure 13: Mass retained in the column subject to the different conditions *(1: outlet→5: inlet)*

DISCUSSION, CONCLUSIONS AND OUTLOOK

Discussion and Conclusions

- Bacteria are cultivated aerobically, and their lower survival rates in the absence of dissolved oxygen could be due to the sudden change of conditions
- Presence of dissolved oxygen leads to an increased metabolic activity of bacteria (even for facultative anaerobes)
- Dissolved oxygen is more important for bacterial survival when compared to nutrients under the given growth and experimental conditions

Outlook

- A combined modelling and experimental approach could give more information about the processes driving the survival of bacteria under the studied conditions
- Impact of flowrate on the fate of bacteria under the aforementioned conditions, would be important to understand relative importance of the reactive processes to the transport processes

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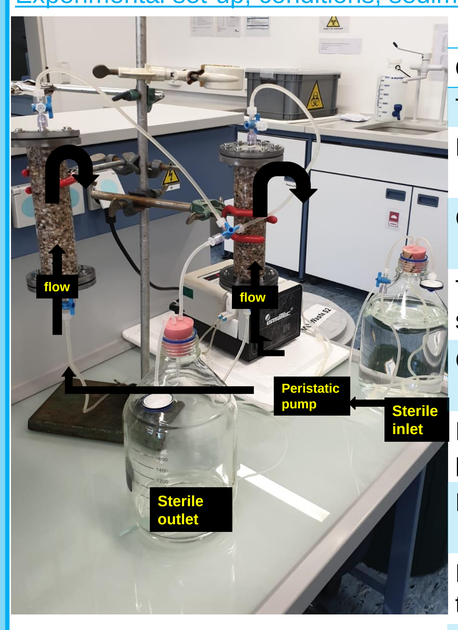

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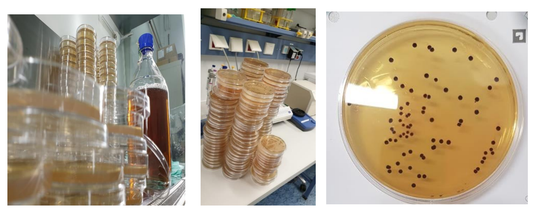


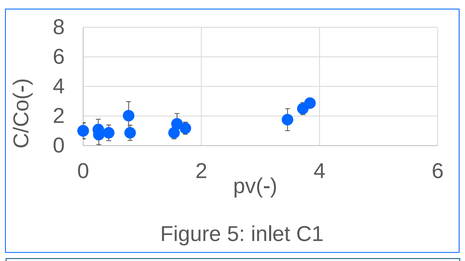
Figure 4: Plating method

RESULTS

Non-dimentionalisation of the axis:

$$\frac{C}{C_o}(-) = \frac{\textbf{Concentration of water phase bacteria}\left(\frac{CFU}{mL}\right)}{\textbf{Concentration in the inlet at t=0}\left(\frac{CFU}{mL}\right)}$$

$$\textbf{Pore volume}\ [pv(-)] = \frac{\textbf{time (mins)}}{\textbf{RT (mins)}}$$



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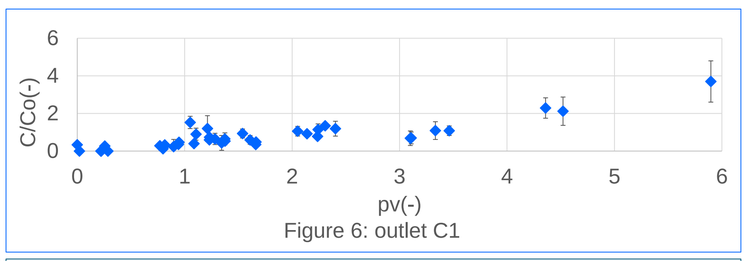

Figure 6: outlet C1

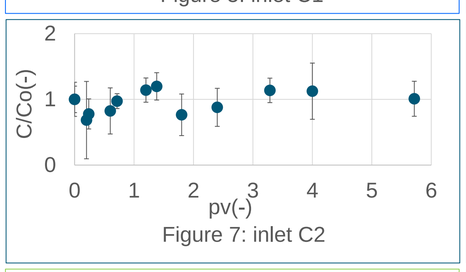

Figure 7: inlet C2

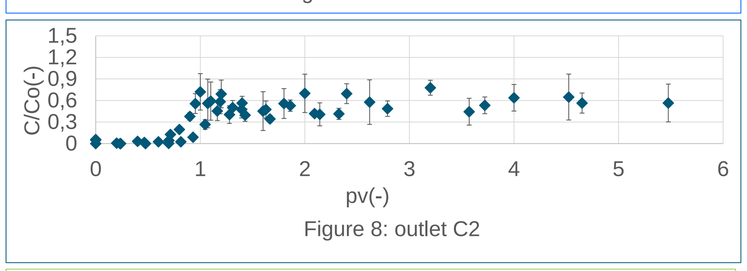

Figure 8: outlet C2

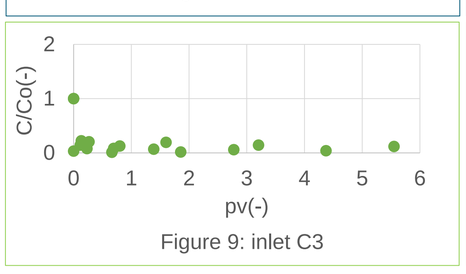

Figure 9: inlet C3

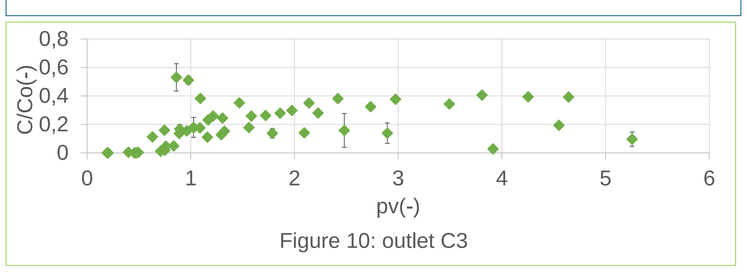

Figure 10: outlet C3

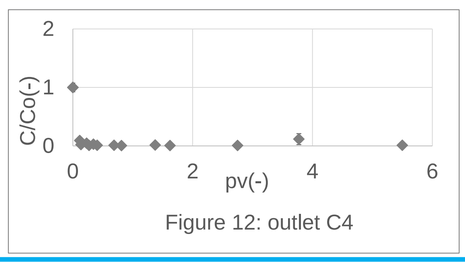

Figure 12: outlet C4

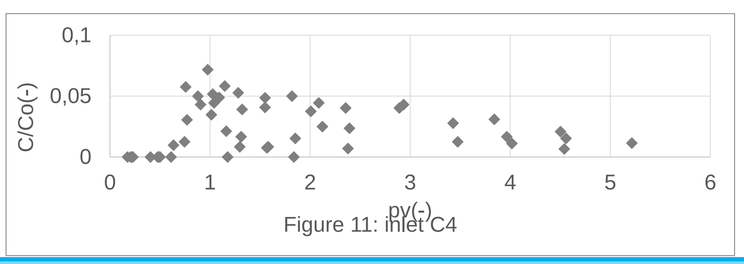

Figure 11: inlet C4

RESULTS

Concentration of bacteria in sediment phase:

$$\frac{\textbf{CFU retained}}{\textbf{CFU injected}} = \frac{\frac{\textbf{CFU}}{\textbf{mL}}\ \textbf{from plates} * \left(\frac{\textbf{9mL}}{\textbf{1g}}\right) * \textbf{Weight of sediment section}}{\textbf{C}_{\textbf{inlet}} * \textbf{t} * \textbf{F}_{\textbf{in}} * \boldsymbol{\theta}_{\textbf{e}}}$$

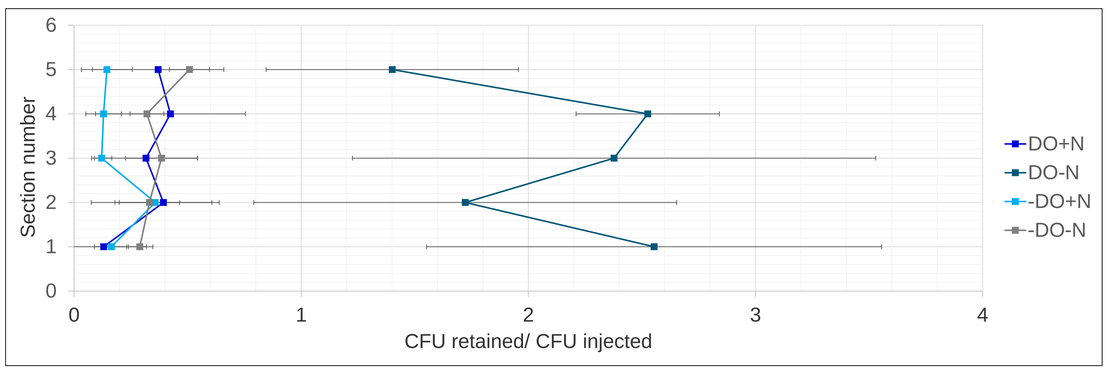

Figure 12: Mass retained in the column subject to the different conditions (1: outlet → 5: inlet)

DISCUSSION AND OUTLOOK

Discussion

- Dissolved oxygen leads to an increase in the survival of bacteria (even for facultative anaerobes)
- The bacteria survive the highest in the presence of both dissolved oxygen and nutrients
- Dissolved oxygen is more important for bacterial survival when compared to nutrients
- The amount of bacteria retained in the sediment is higher,

Outlook

- A combined modelling and experimental approach could give more information about the processes driving the survival of bacteria under the studied conditions
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