

## (Hyper-)thermophilic anaerobic digestion of concentrated BW for pathogen removal and safe nutrient recovery

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**Abstract:** Source separated toilet water contains a high concentration of nutrients, which can be recovered to produce fertilizer products. To safely recover and reuse these nutrients, pathogens present in concentrated black water (BW; toilet fraction of domestic wastewater) should be removed. In this study, results showed that thermophilic (55 °C) and hyper-thermophilic (70 °C) conditions increase the removal of (antibiotic-resistant) pathogen indicator organisms, compared to mesophilic conditions. Furthermore, the potential of BW as source for safe nutrient recovery has been demonstrated.

**Keywords:** (hyper-)thermophilic anaerobic digestion; pathogens; nutrient recovery

### Introduction

Nutrient recovery from waste streams is crucial to ensure sufficient fertilizer production for agricultural use, since conventional resources of nitrogen (N), phosphorous (P) and potassium (K) are either depleting or require energy intensive processes (Zeeman and Kujawa Roeleveld, 2011). The majority of nutrients excreted by humans can be found in the toilet fraction of domestic wastewater. By separation at the source, black water (BW; toilet fraction of domestic wastewater) is diverted from other domestic waste streams. Utilisation of vacuum toilets (flush volume <1L) further increases the organics and nutrients concentrations in BW compared to conventional toilets.

Within the H2020 RUN4LIFE project ([www.run4life-project.eu](http://www.run4life-project.eu)) this concentrated BW (typical composition shown in Table 1) is treated in high-rate upflow anaerobic sludge blanket (UASB) reactors under thermophilic and hyper-thermophilic anaerobic conditions, to ensure pathogen removal and safe nutrient recovery. During anaerobic digestion (AD) biogas is produced for energy recovery and nutrients as N, P and K are released.

**Table 1** Typical composition of vacuum collected black water

Parameter	Concentration	In this study, BW and effluent samples from four UASB systems (HRT 6-11
COD <sub>total</sub>	20 ± 3.6	days)
COD <sub>soluble</sub>	4.1 ± 0.7	
N <sub>t</sub> (gN/L)	1.8 ± 0.4	
P <sub>t</sub> (gPO <sub>4</sub> <sup>3-</sup> -P/L)	0.44 ± 0.2	

days) operating at mesophilic (35 °C), thermophilic (55 and 60 °C) and hyper-thermophilic (70 °C) conditions were analysed for pathogen concentrations to determine the pathogen removal efficiency of each condition.

## Material and Methods

Concentrated BW was collected by ultra-low flush volume vacuum toilets, and treated at lab-scale under thermophilic (55/60 °C) and hyper-thermophilic (70 °C) conditions in UASB reactors at the department of Environmental Technology of Wageningen University and Research and the offices of DeSaH B.V. Mesophilic conditions were studied in pilot-scale in a 40,000 L UASB reactor treating vacuum collected BW from a neighbourhood of 232 households in Sneek, The Netherlands. *Escherichia coli* and extended-spectrum  $\beta$ -lactamases (ESBL) producing *E. coli* were selected as pathogen and antibiotic-resistant pathogen indicator organism respectively. *E. coli* species were selected because of their prevalence in the human gut, thermotolerant properties and easy detection method. The colony forming unit (CFU) concentrations were determined by standard plating assays (ISO 8199).

## Results and Discussion

In anaerobic treatment at mesophilic conditions, *E. coli* CFU concentrations (TBX agar) in the effluent of the reactor were reduced by 2.7 log (Table 2) compared to levels in the influent. During both thermophilic and hyper-thermophilic treatment the removal was higher (3.9-5.6 log) and mean CFU effluent concentrations were <2 CFUs/mL. For the ESBL *E. coli*, the log removal for mesophilic treatment was again 2.7. During thermophilic treatment, ESBL CFU concentrations were reduced to below the detection limit. For hyper-thermophilic treatment the ESBL log removal was 3.9 resulting in effluent concentrations of <1 CFU/mL. Statistical analyses showed that thermophilic and hyper-thermophilic conditions significantly reduced the CFU concentrations of the (antibiotic-resistant) *E. coli* ( $p < 0.05$ ), and that there is a significant difference between mesophilic and (hyper-)thermophilic anaerobic treatment (Moerland et al., 2020).

**Table 2 Average CFU concentrations per sampling site and plate type (Adapted from Moerland et al. 2020)**

Reactor	Temperature (°C)	Influent		Effluent			
		Mean concentration (CFUs/mL)	Standard deviation	Mean concentration (CFUs/mL)	Standard deviation	Log removal	
TBX	Noorderhoek (N)	35	1.80E+06	6.52E+05	3.93E+03	9.29E+02	2.66
	DeSaH (D)	60	3.68E+05	1.52E+05	<detection limit		5.57
	WUR (W)	55	1.47E+04	5.65E+03	4.04E-01	4.04E-01	4.56
	WUR (W)	70	1.47E+04	5.65E+03	1.86E+00	1.38E+00	3.90
ESBL	Noorderhoek (N)	35	1.57E+04	5.07E+03	2.89E+01	7.44E+00	2.73
	DeSaH (D)	60	7.86E+03	6.80E+03	<detection limit		3.90
	WUR (W)	55	3.29E+02	1.15E+02	<detection limit		2.52
	WUR (W)	70	3.29E+02	1.16E+02	4.04E-02	4.04E-02	3.91

Previous work on BW treatment mainly focus on mesophilic operating conditions (de Graaff et al., Cunha et al., 2019). At these mesophilic conditions, P removal has shown to reach up to 90% during concentrated BW treatment (Cunha et al. 2019). The current study is the first to show that thermophilic conditions are sufficient for pathogen free recovery of these recovered nutrients.

## Conclusions

Thermophilic digestion of concentrated BW increases the removal of selected (antibiotic-resistant) pathogen indicator organisms compared to mesophilic treatment. BW contains high nutrient concentrations and the recovery potential has already been demonstrated. Treatment at (hyper-)thermophilic conditions thus has high potential for safe recovery of these nutrients.

## References

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