Microwave disinfection as a treatment for blackwater from dewatered sludge [version 1; peer review: 3 approved with reservations]

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Abstract
Background: Fast and efficient on-site treatment of blackwater, rejected from the dewatering process, can decrease the costs associated with disposal of fecal sludge removed from pit latrines by reducing the volume of sludge transported to the disposal site.

Methods: In this study, we examine the potential use of low cost consumer microwave units for disinfecting pathogen-rich blackwater. Domestic bench top microwave units were modified to allow flow through and re-circulation of blackwater. Energy, throughput, and disinfection characteristics related to microwaves are studied and compared to conventional thermal heating. A custom flow through stack of 5 microwaves was designed and used to examine the feasibility of single pass, high throughput application.

Results and Conclusions: The results show microwave energy does not play a role in the disinfection of blackwater. The benefits of a microwave disinfection system are shown to be high energy efficiency, compact size, and cost efficiency.

Keywords
Microwave disinfection, blackwater

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1. Introduction
Under the Millennium Development Goals 2.1 billion people have gained access to improved sanitation facilities since 1990. The pit latrine, the most basic and inexpensive form of improved sanitation facility, was used by an estimated 1.8 billion people in 2013. Maintaining the beneficial health and environmental outcomes provided by pit latrines necessitates routine evacuation and hygienic disposal of highly pathogenic fecal sludge. The preferred method for pit latrine sludge removal is the vacuum tanker because it minimizes human contact with pit contents, is efficient at removing sludge, and allows sludge to be directly transported to a disposal or transfer site. The typical vacuum tanker can empty up to 4 pit latrines before transport to a disposal site or transfer station is required. In areas where transfer stations are non-existent a tanker can spend up to 60% of its operational time travelling between the facilities and the disposal site with fuel and tipping making up about three quarters of the total cost of pit emptying. Dewatering sludge can reduce volume and decrease the costs associated with transport and disposal. For example, if a pit has a moisture content of 97% only a 2% reduction in moisture would be needed to achieve a 50% reduction in sludge volume. However, the dewatering process also generates a large volume of nutrient and pathogen rich blackwater, which if left untreated poses significant health and environmental threats. The research presented in this study is focused on the fast and efficient disinfection of this blackwater.

Microwave volumetric heating (MVH) enables fast and uniform heating of liquids, suspensions, and semi-solids and is a popular method for sterilization and pasteurization in the food industry. Microwave radiation has also been used for the remediation of sludge, specifically, for the solubilization of COD, enhancement of anaerobic digestion, biogas production and nutrient recovery, improving the dewaterability of sludge and the dewatering of blackwater sludge. The main advantages of microwave heating compared to thermal heating, in the food industry, are the speed of the process and minimum come-up time, with high through-put systems showing successful disinfection at rates of up to 340 L/hr and temperatures of 145°C easily achievable. Heat inactivation of Helminths and E.coli has been shown to occur after 10 minutes at 65°C and 1 minute at 70°C respectively, therefore microwave heating of the blackwater from dewatered sludge has the potential to allow for fast and efficient onsite disinfection and discharge.

Though it is generally thought the destruction of microorganisms by microwave radiation is due solely to thermal effects, the possibility of non-thermal effects continues to be studied and debated. A recent study attempted to determine the role of microwave radiation in disinfection but did not account for variable time and temperature conditions between conventionally heated and microwave heated samples. The current study is intended to explore the feasibility of using microwave radiation for the disinfection of blackwater, with a focus on implementation in developing regions. Notably, we utilize off-the-shelf consumer microwave units due to their cost efficiency and ready supply chain, two factors that are critical for successful implementation in developing regions. Additionally, we directly compare disinfection by MVH and thermal heating by carefully controlling the heating rate to ensure comparable time-temperature dynamics in order to determine whether non-thermal effects of MVH play a role in blackwater disinfection. Furthermore, we determine if MVH provides enhanced disinfection of blackwater with varying solids contents, and test the practicality of a high throughput single pass system.

2. Methods
2.1 Bench top microwave apparatus
For bench top scale experiments, a domestic microwave oven (Panasonic NN-SA661S), 1200W, 2450 MHz, 1.2 ft³ was modified for continuous circulation of blackwater by drilling two holes into the back of the unit and inserting flared teflon feed through fittings that were then sealed with glue and microwave shielding adhesive tape to make inlet and outlet ports. Twenty-five feet of coiled Teflon tubing (ID 9.5 mm) was connected to the internal inlet and outlet ports with a teflon bracing spine to distribute the tubing evenly in the microwave. Two foot sections of prothane ii tubing (ID 7.95 mm) were used to connect the external inlet and outlet ports to an insulated reservoir (Figure 1A). A total tubing volume of 0.6 L was used. Type K thermocouples were inserted into the inlet and outlet ports, as well as in the reservoir. Temperatures were recorded with a FLUKE 54II B thermometer. A 24V Jabsco pump capable of delivering 2.9 GPM was inserted between the reservoir and inlet port and a metering valve, to control flow, was placed between the outlet port and the reservoir (Figure 1A). The reservoir was placed on a stir plate (Corning) and blackwater in the reservoir was agitated throughout microwave experiments to ensure a homogenous temperature. A total volume of 1.5 L of blackwater was re-circulated through the system at a flow rate of 1 LPM until the desired reservoir temperature was achieved. To ensure the integrity of the microwave compartment was not compromised a microwave leakage detector (EMF-810, Lutron Electronics) was used to confirm no electromagnetic radiation was detected outside of the unit during operation. The desired temperature and heating rate was achieved by controlling the microwave power setting.

2.2 Conventional thermal heating
For conventional thermal heating experiments a 2L glass beaker was placed on a combination stir/hotplate (Corning) and 1.5L of blackwater was heated under continuous agitation. A Type K thermocouple was placed in the center of the blackwater (Figure 1B). Temperature was recorded with a FLUKE 54II B thermometer. The desired temperature and heating rate were achieved by controlling the analogue heat setting (1–10) on the hotplate.

2.3 Microwave stack
To test for the applicability of microwave disinfection at a scale practical for implementation, a stack of 5 domestic microwave ovens (Panasonic NN-SA661S), 1200W, 2450 MHz, 1.2 ft³ was constructed by placing one microwave per shelf on a 5 shelf rolling cart. Two holes were drilled into the back of each microwave and flared Teflon feed through fittings were inserted and sealed with glue and microwave shielding adhesive
to create the inlet and outlet ports for each microwave. Five feet of coiled Teflon tubing (ID: 6.35 mm) was placed into the microwave compartment of each microwave. With the exception of the first and last microwaves in the stack, the outlet of the proceeding microwave was connected to the inlet of the next microwave in the series with the same Teflon tubing. The inlet of the first microwave was connected to an untreated blackwater reservoir and the outlet of the last microwave was connected to the treated blackwater reservoir using prothane ii tubing (ID 7.95 mm) (Figure 1C). The total tubing volume was 0.54 L. For some experiments an insulated copper coil was inserted between the outlet of the last microwave and the treated blackwater reservoir to increase retention time at temperature. A 24V Jabsco pump capable of delivering 2.9 GPM and a metering valve were inserted between the untreated reservoir and inlet port of the first microwave in the stack (Figure 1C). The desired temperature was achieved in a single pass through the stack by controlling the flow rate and microwave power setting. To ensure the integrity of the microwave compartments were not compromised a microwave leakage detector (EMF-810, Lutron Electronics) was used to confirm no electromagnetic radiation was detected outside of the units during operation.

2.4 Research design
This study was performed using blackwater obtained from a prototype toilet system, described elsewhere. The blackwater was composed of 25% urine in recycled flush water contaminated with feces and had a total solids (TS) content of approximately 0.7%. Simulated pit latrine sludge was made by mixing feces and urine to a TS content of 1–3%, in a 19 L polypropylene container. The simulated pit latrine sludge was then allowed to sit undisturbed for at least a month. The raw feces and urine were obtained from healthy anonymous volunteers 20–50 years in age. All feces and urine collection methods were reviewed and approved by RTI’s institutional review board. Blackwater or simulated sludge was used as collected or diluted with DI H₂O as necessary to achieve the desired TS content. The initial temperature of blackwater, simulated sludge, and feces, prior to all experiments, was room temperature.

Three separate microwave or heating systems were used in this study (Figure 1) and have been described previously in this publication (sections 2.1 – 2.3). The conventional hotplate heating system (Figure 1B) was used to determine the time and temperature kill dynamics of blackwater. The single microwave system (Figure 1A) was used to determine if microwave radiation played a role in disinfection by comparing to conventional hotplate heating. This was accomplished by maintaining comparable heating rates in the two systems (~1°C/min). The single microwave system was also used to determine the effect of TS on microwave sterilization of blackwater.

Intact feces was exposed to microwave radiation by evenly distributing an intact fecal sample in the bottom of a sealed microwave safe plastic container. Temperature was verified by removing the sample from the microwave and inserting a type K thermocouple.
thermocouple into the center of the fecal sample. Temperatures were recorded with a FLUKE 54II B thermometer.

The microwave stack system (Figure 1C) was used to determine the feasibility of a single pass high throughput system (i.e. practical application). All experiments were performed at least twice to confirm results, except for the intact feces trial, which was performed once.

2.5 Microbial enumeration and reported data

Lysongeny broth (LB) was used as culture media and consisted of 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl in deionized water. The broth was sterilized at 121°C for 15 minutes and then cooled to a temperature allowing handling. Samples for a three tube most probable number (MPN) assay were collected using clean sterile pipettes and 15 mL centrifuge tubes. For MPN quantification, the FDA method for MPN from serial dilutions was used by serially diluting \((10^{-1} \text{ to } 10^4)\) each sample with LB and culturing 1 mL of the diluted sample in a single well of a sterile 48-well plate\(^2\). Each dilution, at each time, point was run in 3 individual wells. Samples were incubated at 37°C for 48 hours before being analyzed. Reported values are the average of at least 2 individual trials.

TS were determined according to the EPA method 1684 “Total, fixed, and volatile solids in water, solids, and biosolids”. Briefly, new clean aluminum weigh boats were heated at 103–105°C for an hour, cooled to room temperature and used immediately. The weight of the empty dish was recorded as \(W_{\text{dish}}\) Next a 20–50 mL sample of blackwater was added to the dish and the weight recorded as \(W_{\text{out}}\). The sample was then dried at 103–105°C overnight, cooled to room temperature and the weight recorded. The sample was then returned to the oven at 103–105°C and heated for an hour, cooled and re-weighed. This process was repeated until the weight change was less than 1 mg/L, this was recorded as \(W_{\text{dry}}\). TS were determined as follows.

\[
\text{Total solids (\%)} = \left( \frac{W_{\text{dry}} - W_{\text{dish}}}{W_{\text{total}} - W_{\text{dish}}} \right) \times 100
\]

Redox potential (ORP), pH, and conductivity were all measured using a Myron L 6PFC\(^6\) Ultrameter II (Myron L Company, Carlsbad CA) according to the manufacturer’s instructions. The conductivity cell and pH/ORP sensor wells were rinsed 3 times with the sampled blackwater at each time point, prior to data recording. The heating rate was calculated by dividing the change in temperature (\(\Delta T\) in °C) over the time period (\(\Delta t\) in minutes) in which the change occurred.

2.6 Statistical analysis

For experiments involving comparison of two or more disinfection protocols over time at different temperatures, log-transformed MPN data were compared by two-way ANOVA with a Sidak’s multiple comparison test. For experiments comparing the effect of heating method (microwave versus hot plate) or solids content on disinfection at a given temperature, mean log reductions upon reaching that temperature were compared by Student’s t test. A p value of < 0.05 was considered statistically significant. Statistical calculations were performed with GraphPad Prism v 7.01.

2.7 Energy calculations

Energy usage measurements were calculated by averaging the wall current draw for a single microwave running at 100% power for five minutes using Mastech MS 2138R inductive ammeter. For hotplate energy the total time needed to heat the blackwater to 95°C, on the highest analog heat setting (10), was used. For microwave stack energy the total time each microwave was required to be on, at full power, was used. The energy required to heat 1L of water from room temperature to 95°C was used to determine the energy efficiency of each system.

3. Results

3.1 Time-temperature disinfection kinetics of blackwater

The time-temperature disinfection kinetics of blackwater conventionally heated on a hot plate under constant agitation are shown in Figure 2. The blackwater was obtained from the prototype toilet system as described in the Methods section 2.4. The average initial thermal heating rate and TS across all trials were 1.01 ± 0.15 °C/min, and 0.55 ± 0.16 % respectively. The average initial pH, conductivity, and ORP were 8.73 ± 0.12; 14651 ± 3631 µS/cm; and -224 ± 82 mV respectively. Heating resulted in complete disinfection (MPN below the disinfection threshold of 5/ml) upon reaching 95°C. Heating to either 70°C or 80°C resulted in a significant reduction in MPN, but did not reach the disinfection threshold. The reductions in MPN upon achieving 70°C and 80°C (5.2 and 5.4 log reduction, respectively) were not significantly different from each other suggesting that below 95°C a hotter temperature did not result in a faster kill (Figure 2). Maintaining the blackwater at 70°C or 80°C for a further 100 minutes resulted in an additional 0.6 log and 0.9 log reduction, respectively; however, this difference was also not statistically significant (Figure 2). Therefore, we propose, 95°C should be the target temperature for sterilization of blackwater.

2.8.1 Energy required and throughput of the microwave stack system

For experiments involving comparison of two or more disinfection protocols over time at different temperatures, log-transformed MPN data were compared by two-way ANOVA with a Sidak’s multiple comparison test. For experiments comparing the effect of heating method (microwave versus hot plate) or solids content on disinfection at a given temperature, mean log reductions upon reaching that temperature were compared by Student’s t test. A p value of < 0.05 was considered statistically significant. Statistical calculations were performed with GraphPad Prism v 7.01.

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Figure 2. Time and temperature disinfection kinetics of blackwater conventionally heated on a hotplate under constant agitation. *Indicates time when temperature was achieved. Dotted line at MPN = 5/ml indicates disinfection threshold. Data are mean MPN versus time of heating ± standard deviation, n = 3 for 70°C and 80°C. Data presented for 95°C are representative data from n = 2.
3.2 Effect of electromagnetic radiation on disinfection
Using the bench top single microwave system (Figure 1A) we sought to determine if microwaves could affect disinfection through a non-thermal mechanism by comparing disinfection of microwave irradiated fecal contaminated blackwater at a sub-lethal temperature to that of conventionally heated blackwater (Figure 3A). A sub-lethal temperature of 40°C was chosen because it is was not expected to affect cell viability and any disinfection could be attributed to a non-thermal mechanism provided by the microwaves. Initial blackwater conditions can be found in Table S1. A temperature of 70°C (Figure 3B) was chosen to investigate if microwave radiation had a synergistic effect on disinfection at elevated temperature (i.e., whether microwaves plus heat provide enhanced disinfection over heat alone). Initial blackwater conditions can be found in Table S2. No reduction in MPN was seen under either condition (MVH or conventional heating) at 40°C. Heating to 70°C resulted in significant reductions in MPN in both conditions that were not significantly different from each other. The heating rates of both systems were maintained at approximately 1°C/min to ensure equal time-temperature exposure and that the only difference between samples was the addition of microwaves in the MVH system. Thus, our data show that microwaves provided no enhancement of disinfection under non-lethal or lethal temperature conditions when compared to conventional heating.

3.3 Effect of total solids on volumetric heating
Figure 4 shows the effect of TS content on disinfection after MVH to 70°C of intact feces, high (2.3%), and low (0.19%) TS samples in the bench top microwave apparatus. Initial blackwater conditions can be found in Table S3. The bacterial counts in the low TS sample decreased an average of 3.8 log orders while the high TS sample decreased 3.7 log orders whereas intact feces had a 3.0 log decrease, with final MPN’s of $1.8 \times 10^3$. 

![Figure 3](image)

**Figure 3.** Effect of microwave radiation on blackwater disinfection at 40°C (A) and 70°C (B). Data are mean MPN versus time of heating ± standard deviation, n = 3 for 70°C microwave condition, n = 2 for all other conditions.

![Figure 4](image)

**Figure 4.** Effect of volumetric heating on varying solids contents. Low total solids (TS) (0.19 %), High TS (2.3 %), Feces (19.9 % TS).
were heated \(9.4\) minutes and the time above 95°C to 2.8 minutes however, increased the length of time the blackwater was above 80°C to drop from the inlet to outlet of the coil was 1°C. The coil temperature for an additional amount of time. The temperature microwave five to allow the blackwater to remain at an elevated temperature for an additional amount of time. The temperature was chosen based on the data from Figure 2 showing complete blackwater disinfection at 95°C. Initially, an 18 L volume of tap water was passed through the system to determine the optimal flow rate (0.624 LPM) for achieving 95°C from the 5 microwave stack under full power. However, the heating profile of blackwater differed from pure water mostly due to the instability particles in the blackwater caused in the flow meter, resulting in unstable flow rates and temperature fluctuations and highlighting the need for caution when designing water treatment systems based off of simulated or surrogate effluents, a challenge this group has encountered and addressed previously\(^{(15)}\). Once the system was adapted for blackwater, a flow rate of 0.82 LPM was used with the average time in the microwave stack being 39.7 seconds. Under these conditions the blackwater remained above 80°C for 12 seconds and 95°C for 6 seconds, however this was not sufficient to achieve full disinfection (Trial 1, Table 1). For Trials 2–5 (Table 1) a 50 ft length (2.4L) of coiled insulated copper piping was added to the output of microwave five to allow the blackwater to remain at an elevated temperature for an additional amount of time. The temperature drop from the inlet to outlet of the coil was 1°C. The coil increased the length of time the blackwater was above 80°C to 2.9 minutes and the time above 95°C to 2.8 minutes however, complete disinfection was still not achieved. This process of increasing the time above 95°C was continued up to 9.4 minutes (Table 1). When sterilization was still not achieved, the length of copper tubing was increased to 100 ft, allowing the blackwater to remain at temperature for an additional 18.8 minutes (Trials 7 and 8) where full sterilization was achieved (Table 1).

When we compare these results to those obtained in Figure 2 we can infer the difference in the time-temperature kinetics is due to the difference in come-up time. The samples in Figure 2 were at a temperature above 80°C for approximately 15 minutes before 95°C was achieved, while the samples in Table 1 were heated rapidly using the full power of the microwave. These results tell us the advantage of rapid heating microwaves provide is limited because an extensive time at elevated temperature is still needed for sterilization; however, this limitation can be overcome by the addition of insulated tubing allowing the effluent to be exposed to the proper time-temperature conditions without additional energy input.

### 3.5 Energy and throughput evaluation

The time required by the hotplate to heat 1L of water on the highest setting was 48 minutes, while the 5 stack of microwaves needed only 2.14 minutes. Overall the wall plug power measured for the microwave system was higher than for the hotplate heating system, 1920 W vs 313 W respectively. However, due to the longer time required by the hotplate to heat 1L of blackwater, the total energy required was 32% greater than that used by the 5 stack microwave system (Table 2). The throughput of the microwave system was 15 LPH. These results show a microwave heating system for the disinfection of blackwater has the potential to be an energy efficient field option, however throughput conditions need to be optimized.

### Table 1. Time-temperature, energy applied, and disinfection in microwave flow through system.

| Trial # | Time over 90°C (min) | Total kJ/L | MPN ≤ 5 |
|---------|----------------------|------------|---------|
| 1       | 0.2                  | 702        | No      |
| 2       | 2.8                  | 676        | No      |
| 3       | 3.4                  | 645        | No      |
| 4       | 3.1                  | 730        | No      |
| 5       | 9.4                  | 776        | No      |
| 6       | 9.4                  | 685        | No      |
| 7       | 18.8                 | 685        | Yes     |
| 8       | 18.8                 | 685        | Yes     |

### Table 2. Comparison of energy expenditure to heat 1L of blackwater to 95°C and energy efficiency of each heating system used.

|              | Hotplate | Microwave |
|--------------|----------|-----------|
| Energy (kJ/L)| 901      | 685       |
| Efficiency (%)| 35       | 46        |
4. Discussion
In this study we examined the potential use of low cost off-the-shelf microwave units for the treatment of the pathogen-rich blackwater by-product obtained from the sludge dewatering process. The objectives of this work were to determine the time and temperature kill dynamics of blackwater, to determine if microwave radiation plays a factor in pathogen inactivation, and to identify energy consumption and throughput trade-offs as well as the feasibility of a practical single-pass system. We utilized a domestic bench top microwave modified to allow flow through and re-circulation of blackwater to understand the energy, throughput, and disinfection characteristics related to microwaves. A custom flow through stack of 5 microwaves was designed and used to examine practical application.

The blackwater used in this study had a TS between 0.18 and 2.28%. Previous studies have shown TS removal for various dewatering methods to be between 80–90%. Assuming an initial pit latrine TS content of 3% the TS used in this study spans and exceeds the range predicted for the liquid fraction of dewatered sludge. The initial concentrations of bacteria used for testing are in line with those previously reported for sewage. This suggests the blackwater in this study mimics expected field conditions as well as tests the boundaries for use.

Here we report a temperature of 95°C is needed for complete disinfection of blackwater (Figure 2). These findings are in contrast to results by Feachem et al. 1983, showing pathogens in night soil and sludge are killed within 60 minutes at 70°C. Additionally, the US EPA requires disinfection of wet sludge by pasteurization at 70°C for 30 minutes. However, in Europe this pasteurization practice has resulted in the regrowth of certain pathogens and is no longer used as a terminal treatment process. This taken with our results suggest more stringent conditions may be needed to disinfect blackwater and are in line with a study by Hong et al. 2004 that found 99°C was necessary for disinfection of total and fecal coliforms in conventionally heated sludge.

Figure 3 shows microwave energy does not play a role in the disinfection of blackwater at sub-lethal or lethal temperatures. Many non-thermal effects of microwaves have been reported in literature including changes in cell morphology, poration of the cell membrane, and effects on metabolic activity. Interestingly, many of these effects were reversible after a short duration and showed no long-term differences to conventionally heated cells. Other reported effects include changes in cell growth rate and absorption of microwaves by DNA. However, these effects appear to be dependent on frequency of exposure with higher frequencies causing changes in growth rate and DNA structure and lower frequencies resulting in metabolic and cell wall alterations. Our results show that 2.4 GHz microwave radiation does not cause or enhance bacterial disinfection compared with thermal heating to comparable temperatures at comparable rates, and match with previously published studies. We therefore postulate that microwaves have non-thermal effects on bacteria that affect cell metabolism and integrity, but that heat remains the dominant mechanism for disinfection by microwave radiation.

We tested the effect of volumetric heating on disinfection of blackwater with various TS contents and intact feces (Figure 4). The effect of TS content on microwave disinfection was minimal. This result is not entirely unexpected, despite the fact that sludge and blackwater are complex media containing microorganisms, as well as organic and mineral components, water is the main component affected by microwaves. Because global feces and urine contents are very diverse our results show microwave heating can provide an efficient way to deliver thermal disinfection from the variety of pit contents that might be encountered, microwaves therefore have a broad application potential in sanitation applications.

5. Conclusions
This work shows that despite a lack of evidence for non-thermal mechanisms of disinfection by microwaves in this application, microwaves are nonetheless a good candidate for delivering thermal energy to sludge for disinfection. The single pass, five microwave stack showed the main advantage to microwave heating is the minimum come-up time provided by the microwave system, however we show an extended exposure to elevated temperature is still needed. The benefits of a microwave disinfection system are high energy efficiency, compact size, and cost efficiency of a standard off the shelf magnetron. Future work should include optimizing throughput and testing other microorganisms such as helminths and viruses as well as organic and nutrient content of microwaved blackwater to determine its suitability for discharge.

Data availability
Dataset is available from OSF: https://osf.io/2fr4x

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Competing interests
No competing interests were disclosed.

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Supplementary material
Supplementary file 1: Initial Conditions for Figure 3A, Figure 3B and Figure 4.

Click here to access the data.

References

1. UNICEF and W.H. Organization: Progress on Sanitation and Drinking Water - 2015 Update and MDG Assessment. WHO Press: United States of America. 2015. Reference Source
2. Graham JP, Polizzioto ML: Pit latrines and their impacts on groundwater quality: a systematic review. Environ Health Perspect. 2013; 121(5): 521–530. PubMed Abstract | Publisher Full Text | Free Full Text
3. Montgomery MA, Elmleeh M: Water and sanitation in developing countries: Including health in the equation. Environ Sci Technol. 2007; 41(1): 17–24. PubMed Abstract | Publisher Full Text
4. Eales K: Sanitation partnership series: Bringing pit emptying out of the darkness: A comparison of approaches in Durban, South Africa, and Kibera, Kenya. BFD Sanitation Series, 2005. Reference Source
5. Boot NL: The use of transfer stations for faecal sludge management in Accra, Ghana. Waterlines. 2008; 27(1): 71–81. Publisher Full Text
6. Klingsf, Montagne A, Koné D, et al.: Fecal Sludge Management in Developing Countries. A planning manual. First edition. 2002. Reference Source
7. Hawkins P: Emptying on-site excreta disposal systems in developing countries: An evaluation of the problems. IRCWD News, 1982; 17: 1–9.
8. Nakato T: Calorific characterization of faecal sludge and its use as a fuel. Lambert Academic Publishing. 2015. Reference Source
9. Smollen M: Moisture Retention Characteristics and Volume Reduction of Municipal Sludges. Water SA. 1988; 14(1): 25–28. Reference Source
10. Coronel P, Truong VD, Simunovic J, et al.: Aseptic processing of sweetpotato purees using a continuous flow microwave system. J Food Sci. 2005; 70(9): E531–E536. Publisher Full Text
11. Tang ZW, Mikhaylenko G, Liu F, et al.: Microwave sterilization of sliced beef in gravy in 7-oz trays. J Food Eng. 2008; 89(4): 375–383. Publisher Full Text
12. Gentry TB, Roberts JS: Design and evaluation of a continuous flow microwave pasteurization system for apple cider. LWT-Food Sci Technol. 2005; 38(3): 227–238. Publisher Full Text
13. Nikdel S, MacKellar DG: A microwave system for continuous pasteurization of orange juice. Proc CF State Hort Soc. 1992; 105: 108–110. Reference Source
14. Budra T, van de Voort FR, Raghavan VG, et al.: Heating Characteristics of Milk Constituents in a Microwave Pasteurization System. J Food Sci. 1991; 56(4): 931–934. Publisher Full Text
15. Mawio PM, Reweyenami A, Garcia HA, et al.: Evaluation of a microwave based reactor for the treatment of blackwater sludge. Sci Total Environ. 2016; 548–549: 72–81. PubMed Abstract | Publisher Full Text | Free Full Text
16. Tyagi VK, Lo SL: Microwave irradiation: A sustainable way for sludge treatment and resource recovery. Renew Sust Energ Rev. 2013; 18: 288–305. Publisher Full Text
17. Steed LE, Truong VD, Simunovic J, et al.: Continuous flow microwave-assisted processing and aseptic packaging of purple-fleshed sweetpotato purees. J Food Sci. 2008; 73(9): E455–E462. PubMed Abstract | Publisher Full Text
18. Feechem RG, Bradley DJ, Garelick H, et al.: Sanitation and disease: Health aspects of excreta and wastewater management. In World Bank Studies in Water Supply and Sanitation. New York. 1983. Reference Source
19. Bank S, Bandyopadhyay S, Ganguly S: Bioeffects of microwave—a brief review. Bioreavour Technol. 2003; 87(2): 155–159. PubMed Abstract | Publisher Full Text
20. Goldthi SA, Wang Di: Effect of Microwaves on Escherichia coli and Bacillus subtilis. Appl Microbiol. 1967; 15(6): 1371–5. PubMed Abstract | Free Full Text
21. Shams Y, Taube A, Milik-Dineva N, et al.: Specific Electromagnetic Effects of Microwave Radiation on Escherichia coli. Appl Environ Microbiol. 2011; 77(9): 3017–3022. PubMed Abstract | Publisher Full Text | Free Full Text
22. Woo IS, Rhe P, Park HD: Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Appl Environ Microbiol. 2000; 66(5): 2243–2247. PubMed Abstract | Publisher Full Text | Free Full Text
23. Moore HA, Raymond R, Fox M, et al.: Low-intensity microwave radiation and the virulence of Agrobacterium tumefaciens strain B6. Appl Environ Microbiol. 1979; 37(1): 127–130. PubMed Abstract | Free Full Text
24. Sato S, Shibata C, Yazu M: Nonthermal killing effect of microwave irradiation. Biotechnol Techn. 1996; 10(3): 145–150. Publisher Full Text
25. Hong SM, Park JK, Lee YC: Mechanisms of microwave irradiation involved in the destruction of fecal coliforms from biosolids. Water Res. 2004; 38(6): 1615–1625. PubMed Abstract | Publisher Full Text
26. Sobsey MD, Stuber CE, Casanova LM, et al.: Point of use household drinking water filtration: A practical, effective solution for providing sustained access to safe drinking water in the developing world. Environ Sci Technol. 2008; 42(12): 4261–4267. PubMed Abstract | Publisher Full Text
27. Sellgren K, Gregory C, Hunt M, et al.: Development of an electrochemical process for blackwater disinfection in a free-standing, additive-free toilet. RTI Press, In Press. 2017. Publisher Full Text
28. Blodgett RJ: BAM Appendix 2: Most Probable Number from Serial Dilutions. 2010. Reference Source
29. Merin S: Rejection water management at biogas plants and the utilization of reject water as a culture medium for microalgae. Environ Technol. Lappeenranta University of Technology. 2016. Reference Source
30. EPA: Dewatering Municipal Wastewater Sludges. 1987. Reference Source
31. Edberg SC, Rice EW, Karlin RJ, et al.: Escherichia coli: the best biological drinking water indicator for public health protection. Symp Ser Soc Appl Microbiol. 2000; 88(29): 106s–116s. PubMed Abstract | Publisher Full Text
32. EPA: Process design manual for sludge treatment and disposal. 1979. Reference Source
33. EPA: Control of pathogens and vector attraction in sewage sludge. 2003. Reference Source
34. Dreyfuss MS, Chipley JR: Comparison of effects of sublethal microwave radiation and conventional heating on the metabolic activity of Staphylococcus aureus. Appl Environ Microbiol. 1980; 39(1): 13–16. PubMed Abstract | Free Full Text
35. Grunder W, Kelmann F, Putterlic V, et al.: Resonant-like dependence of yeast growth rate on microwave frequencies. Br J Cancer Suppl. 1982; 8: 206–208. PubMed Abstract | Free Full Text
36. Takashima S, Gabriel C, Sheppard RJ, et al.: Dielectric behavior of DNA solution at radio and microwave frequencies (at 20 degrees C). Biophys J. 1984; 46(1): 29–34. PubMed Abstract | Publisher Full Text | Free Full Text
37. Rose C, Parker A, Jefferson B, et al.: The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology. Chf Rev Environ Sci Technol. 2015; 45(17): 1827–1879. PubMed Abstract | Publisher Full Text | Free Full Text
38. Sellgren KL: Microwave Disinfection Paper Submitted to The Gates Foundation Open Access. 2016. Data Source
Open Peer Review

Current Peer Review Status:  ?  ?  ?

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Damir Brdjanovic
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2 Delft University of Technology, Delft, The Netherlands

I read with pleasure this interesting article covering an emerging topic of treatment of a waste stream generated from an onsite sanitation system. As late reviewer, I had the advantage of reading the feedback from other two reviewers who covered most of my comments. Therefore I opted to focus my response on few other points not or partially addressed by the fellow reviewers.

In general, the main outcomes of the work are expected. The most interesting development is the laboratory scale experimental stacked MW arrangement that apparently worked out well under given conditions. Authors postulated that elevated temperature and not exposure to MW irradiation was the main reason for the observed reduction in the number of cultivated microorganisms without providing scientific evidence for the latter based on observation from comparative experiment at sub-lethal temperature. It would be very interesting in discussion to read whether there is another better method which would decouple the effect of temperature and MW irradiation.

The prolonged gravity settling and decanting (1 month) as applied here, is bit misleading as term 'dewatering' is rather used in the context of forced and substantial reduction of the water content in the sludge. I would appreciate that authors adjust the title accordingly. Also term 'blackwater' does not reflect the commonly accepted definitions in FSM practice, specifically in the context of developing countries¹. It is rather a supernatant from a storage tank content than blackwater from dewatered sludge.

Authors manipulated the TS content in the sludge in the range 1-3% which is by far below the (faecal) sludge TS in many places, especially in Africa. This fact should be mentioned in the text.

Beside various suggestions from fellow reviewers on the design of the system, I would ad a practical comment on the high risk of clogging/sedimentation/growth in tubes (despite being made from Teflon) and its possible influence on design and scaling up.
Energy consumption is ultimately the parameter which will determine the success of this technology. I assume that calculations used are based on the properties of water. Are authors of the opinion that presence of TS will influence calculations and whether some other important properties will need to be determined and adjusted (e.g. dielectric properties)?

Authors mentioned that by the prolonged exposure they achieved further dewatering of the media but did not reflect how the system handled such a high TS content. I assume that the present concept is only suitable for rather liquid media which limits it application to disinfection.

It seems that the only temperature measurement point (T1?) was at the outlet of the system; it would be interesting to have several measuring points along the stacked system (e.g. at the outlet of each microwave unit. In discussion section, it would be useful to have a short reflection on their experimental design and some other options for extending the retention time to boost the efficiency (e.g. step-wise increase in the diameter of tubing as opposite to increase in length).

It would be also interesting information to get about the efficiency of MW in such a setup and whether they examined the optimal location of the spiral within the unit.

Indeed the study focuses on bacteria only, while other (pathogenic) microorganisms are not studied and are mentioned as such only at the very end of the paper instead being part of the discussion.

In general it is nice practical paper where the main advantage of MW is reported to be a comparatively lower energy consumption, however only a conventional thermal heating system was used for comparison. Readers would benefit from more extended comparison. As the energy consumption is the prime competitive parameter, author should elaborate more extensively and bring more convincing arguments that would support the application of this particular technological arrangement.

The two main messages: that the main mechanism for bacterial kill is a thermal action and not MW irradiation, and that the advantage of rapid heating by MW is lost by the demand for prolonged retention/exposure to reach desired efficiency, are not really supporting the presented technology. The third message argues that the MW is comparatively more energy efficient, even if the prolonged exposure is required. My opinion is that the MW treatment has the real competitive advantage only when sludge drying is included, which automatically include pathogen kill and solves problem of supernatant and sludge in one go (but also creates problem of condensate etc). It would be good that the authors more clearly state the possible scope and limitations of the proposed technology in discussion and conclusion sections.

In conclusion, I wish to see the paper indexed after authors consider the comments from reviewers.

References
1. Strande L, Ronteltap M, Brdjanovic D: Faecal Sludge Management: Systems Approach for Implementation and Operation. IWA Publishing, 2014.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 09 May 2018

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Ewan McAdam
Cranfield Water Sciences Institute, Cranfield University, Cranfield, UK

General comments:

This manuscript reports on an important subject area, which has been widely discussed and trialled but not for this specific problem set, for which there is significant need for such a solution. The manuscript structure is clearly laid out, providing clarity to the problem whilst also articulating the results in a logical and lucid text. The data presentation is clear, including use of data replication. The areas for improvement I would suggest for the manuscript would be to: (i) more clearly describe the mechanisms underpinning why differences occur in this matrix versus previous research; (ii) provide a little more text clarifying the structural properties of the set-up that would enable a stronger view on the heat and mass transfer principles underpinning the design that might help influence translation to the broader research community; and (iii) provide a short paragraph in the discussion where the authors could describe how they might translate
their data into a field ready system (e.g. dimensional consideration and boundary condition selection), were one to be developed in a next stage of development. Overall the research is convincing, and represents a new contribution to the field. Minor comments are listed below.

Specific comments:

Introduction
[1] Whilst I appreciate the term ‘blackwater’ is often used generically for this type of work, I think it is misleading for a general audience. Specifically, ‘blackwater’ has historically been used to describe waste arising from conventional systems in which a typical flush volume of around 5L would be used. In this case, and in the case for activities across the Gates sanitation portfolio, the flush volume to waste ratio would be much lower, and in the case of unlined pits maybe lower still due to leaching. This has obvious significance in the typical solute concentrations that may be experienced in this ‘blackwater’ compared to a typical domestic ‘blackwater’ reported in the European or North American context. It would help to provide commentary early on that underpins this distinction to emphasise the difference.
[2] ‘Microwave radiation has also been used for the remediation of sludge, specifically, for the solubilization of COD, enhancement of anaerobic digestion, biogas production and nutrient recovery, improving the dewaterability of sludge and the dewatering of blackwater sludge’ – whilst this statement outlines the output, it does not stipulate the mechanism for how such results arise. Therefore, a brief additional statement on why these outcomes are possible would be beneficial, e.g. how microwave radiation is thought to induce solubilisation of COD etc.
[3] ‘to 340 L/hr and temperatures of 145°C easily achievable’ Can a comment on normalised energy be added to this statement to connect flow to temperature?
[4] ‘fast and efficient onsite disinfection and discharge’ – the text has clarified the ‘efficient’ term – perhaps a short sentence added on this (connected to previous comment) would be beneficial; specific reference to comparison with conventional thermal routes may present an avenue to explore this area.

Methods
[1] I appreciate this is a retrofit to discern feasibility but if it is at all possible, for a more general audience it may be useful to describe features that will influence heat transfer in the setup. e.g. wall thickness of the tubing, thermal conductivity of the material, distance of the tubing from the source, orientation and location of the tubing and how it was evenly distributed – I assume this means using a structure to ensure the outer surface of the wound tubing was not in contact (which would expectedly influence heat transport). Total surface area of the tubing that has been exposed to microwave radiation should be calculated and included, together with calculation of the specific surface area (surface area to volume ratio). It would also be useful to convert the flow rate into fluid velocity and determine the Reynold’s number, as the fluid regime would also influence heat transfer. Perhaps a more detailed diagram outlining some of this detail would be instructive to the general reader.
[2] In multiple systems, were thermocouples sited between stages to account for transient temperatures? Useful to add to methods section.
[3] It would be beneficial to report the limit of detection for MPN and why this is discriminated (e.g. minimum viable count per plate). Useful to add to methods section.

Results
[1] Figure 4. Was the same source water used for each line? If so, What was the time between data
points; maybe a short comment in method section on whether any transition in broad sanitation parameters were observed.

[4] Figure 4. The difference in heating rate for faecal sample is classified which is good. In addition to the difference in methodology, it might be useful to explore a in a brief commentary within the discussion, on the influence of viscosity (as well as other rheological/structural parameters) that might influence heat transfer, in order to help discern the wider exploitability and impact of the results on the design of a field-ready system.

[5] Section 3.4. It would again be useful to convert the flowrate to apparent fluid velocity and Reynold’s number.

[6] ‘However, the heating profile of blackwater differed from pure water mostly due to the instability particles in the blackwater caused in the flow meter, resulting in unstable flow rates and temperature fluctuations and highlighting the need for caution when designing water treatment systems based off of simulated or surrogate effluents, a challenge this group has encountered and addressed previously’. – Can I suggest checking the language and recommend splitting into two sentences; currently a little tough to digest.

[7] ‘...however, this limitation can be overcome by the addition of insulated tubing allowing the effluent to be exposed to the proper time-temperature conditions without additional energy input’ – this point is creeping into discussion. I’d also suggest that there are more factors to highlight that influence heat transfer in the system that would be useful to describe in the discussion.

[8] Table 2. It would be useful to add the calculations and assumptions made underpinning the efficiency calculations.

**Discussion**

[1] In the final results section, the authors describe a classical heat transfer problem. The heating rate determined is a function of the heat and mass transfer characteristics employed (surface area to volume ratio, wall thickness, tube ID and fluid velocity). It may be worth adding a commentary on this together with a normalised energy assessment versus conventional techniques, to help provide some general guidance on how such features could shape and influence the design of a field ready system.

[2] Is it at all possible to infer the uniformity of microwave energy across the cavity – or is there any literature that we might be able to lend from to determine whether this may also be of significance to the final result?

[3] ‘...more stringent conditions may be needed to disinfect blackwater and are in line with a study by Hong et al. 2004 that found 99°C was necessary for disinfection’ – this section of the discussion is useful. It would also be useful for the authors to provide a brief sentence on what they think is the underlying differentiator between their research and other authors work: e.g. Physical (heat transfer), structural (rheological), or biological (microbiome).

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly
If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Reviewer Report 20 April 2018**

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**Susan K. De Long**

Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, CO, USA

**General Comments:**

This work investigated microwave disinfection of simulated blackwater and blackwater from a prototype toilet system. The paper explored the mechanisms involved (heat vs. microwave radiation) and reported a feasible design for field implementation. Energy use was also considered. This work addressed a critical problem, and the work offers useful contribution to the body of knowledge on microwave disinfection and presents a highly practical design. The manuscript is well-written, and easy to follow and seems to cite appropriate literature, although the references list is not long and some useful papers may have been overlooked (see suggested reference provided).

I do have one overarching concern about the experimental design and conclusions. Although the paper is focused on disinfection, the authors only used a single plate-based method to assess bacterial inactivation: planting on LB. It is well known that only a small fraction of environmentally relevant microorganisms can grow on conventional plate media. Thus, this plating-based approach does not provide a complete picture of the viable microorganisms that may still be present in the treated blackwater post disinfection. It would have been desirable to monitor some specific pathogens, via microbial spiking assays, and to monitor fecal coliform bacteria. Additionally, disinfection methods must address helminths, protozoa, and viruses, which are not studied herein. While the work adds value despite these shortcomings, the authors should do a
better job of acknowledging limitations throughout the manuscript. For example, all instances of the wording “complete disinfection” should be changed to reflect the limitation of the methods. I have some additional specific comments as well.

Specific comments:
1. Please provide more information on the prototype toilet system.
2. The total solids analysis methods section could reference Standard Methods for the Examination of Water and Wastewater.
3. The discussion section is a bit redundant with earlier text.
4. The point regarding disinfection of Helminths and viruses noted in the Conclusions section should be acknowledged in the Discussion section.
5. As noted above, the wording “complete disinfection” needs to be changed throughout as it is misleading. As a matter of ethical consideration, I suggest the authors be careful not to overstate the safety offered by their method until further testing is completed.
6. Additionally, the term “disinfection threshold” should be changed to “detection threshold” at all instances, as the original term is misleading.
7. The authors should also acknowledge the possibility of Viable but Not Culturable organisms.

References
1. Zimmermann K: Microwave as an emerging technology for the treatment of biohazardous waste: A mini-review. *Waste Manag Res*. 2017; 35 (5): 471-479 PubMed Abstract | Publisher Full Text

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Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental Microbiology, Environmental Engineering, Molecular Biology Tool Applications
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.