Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Institutionen för energi och teknik

Comparative study of bark, bio-char, activated charcoal filters for upgrading grey-water

From a hygiene aspect

Modibo Sidibe

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SUMMARY

The growth of the global population, the increasing need of water for agriculture and the increasing urbanization put great pressure on the existing resources of freshwater and the finding of news sources of freshwater become necessary. An alternative source of water can be to reuse wastewater.

Greywater is all wastewater from a household, with the exception of toilet water, which is called blackwater. Water from dishwashing, from kitchen sinks and from laundry machines constitute greywater and it account for 80% of the household wastewater.

Greywater can be reused in areas that do not require portable water such as irrigation and toilet flushing.

The reuse of greywater reduces the pressure on freshwater resources and thereby preserves the environment and decrease the cost of water. Greywater in this scenario is a resource of water rather than wastewater.

Unfortunately, greywater by its origins contains chemicals, bacteria and viruses. The reuse of raw greywater without a pretreatment can have negative impacts on the soil, can pollute the groundwater, the surfacewater or/and contribute to the transmission of diseases.

The high cost and the insufficiency of centralized wastewater treatment plants mainly in low income countries justify the choice of the onsite filtration system with local and inexpensive filter materials.

In this study, bark, bio-char and activated charcoal were used as filter media in column filters.

Some physical and chemical parameters of greywater that can have a negative environment impact were measured before and after filtration. The concentration of two bacteria and two viruses were estimated before and after filtration. The filters were run for ten weeks.

Differences between different bacteria, between different viruses and also between bacteria and viruses contribute to them having different properties that effect the way in which they interact with filter material. The filtration efficiency thus depends on both the microorganism and the filter material. Activated charcoal filter was found to be better in reducing some of the chemical parameters. The bark filters have an acidifying effect on the filtrated greywater and was less effective in reducing the viruses analyzed. The bio-char was found to be better at reducing the bacteria studied.

This study had contributed to the finding of methods to improve the quality of greywater for reuse. The study confirmed the possibility to improve the quality of greywater by filtration and showed that degree of the reduction depends of the filter material used and the characteristics of the microorganisms.

Abstract

The reuse of grey-water (GW) for non-potable water uses as in agriculture constitutes a great potential of water resources.

A laboratory study was carried out to investigate the upgrading potential of onsite bio-filtration in the purpose to minimize the health risk and harmful environmental impacts associated to the reuse of grey-water.

Nine laboratory columns (height 65 cm, diameter 4.3 cm) were filled by pine bark, bio-char and activated charcoal (three columns by material). The columns were fed by artificial GW mixed with real wastewater and microbial inoculate. The hydraulic loading rate was 0.032 m^3 m^{-2} day^{-1} and the organic loading rate was 76 mg BOD_5 m^{-2} day^{-1} (240g COD m^{-2} day^{-1}).

The concentration of *Salmonella* spp., *E. faecalis*, PhiX-174 and MS2; the percentage of COD, the changes in EC, pH and nitrogen have been assessed before and after filtration.

The bio-char filters showed the best performance in reduction of *Salmonella* spp., the difference between filters in reduction of *E. faecalis* was not significant, bark filters were less effective in removal of phage MS2.

Activated charcoal filters were better in reduction COD and tot-N, it occurred an increase in nitrogen in bio-char and bark filters, bark filters exhibited a drop of pH.

On the basis of the results of this study, the removal of microorganisms by filtration seems to be governed by the characteristics of the filters material, the features of the microorganisms and the environmental conditions as pH.

This thesis analyzed and discussed the performance of the filters materials in removal of bacteria and viruses from GW and supplied some recommendations for further studies on onsite bio-filtration.

Acronyms and abbreviations

BOD   biologic oxygen demand

COD   chemical oxygen demand

CFU   colony forming unit

EC   electric conductivity

E.coli   *Escherichia coli*

E. faecalis   *enterococcus faecalis*

GW   grey-water

HA   humic acid

PCU   plaque forming unit

TC   total coliforms

1. Introduction

Water is capital for the social and economic development of human beings and the preservation of a healthy environment (WHO, 2006) . About 1.2 billion people are facing physical water shortage, one quarter of the world's population is facing economical water shortage (WHO, 2010), in total 62% of the world population will face physical or economical water scarcity by 2030 (Rijsberman, 2006). The world's population is growing in particular in urban areas in developing countries (WHO, 2010) , which leads to the increase of the freshwater demand essential for the socio-economic development and make acute the finding of new water sources. The production of wastewater increases in parallel with the increase of the population and this fact make the wastewater a reliable source of water that can withstand the increase of freshwater demand when it is suitably reused (Bakir, 2001).

Grey-water (GW) is all household wastewater from dishwashing, kitchen sinks, laundry machines (dark grey); showering, bathroom sinks (light grey); while water from toilets (black water) is excluded (Birks & Hills, 2007). It constitutes the largest amount of wastewater produced at household level (50-80% of domestic wastewater) and is less polluted (in term of microbial concentration) compared to blackwater as it does not include faeces (Al-Jayyousi, 2003). The daily production of GW is estimated to 20-30 L d $^{-1}$ pe $^{-1}$ in arid or poor areas, 100 L d $^{-1}$ pe $^{-1}$ in water ample areas as Sweden and Germany (Ridderstolpe, 2004) and up to 600 L d $^{-1}$ pe $^{-1}$ in USA (UNWATER 2013).

The largest consumer of fresh water is the agricultural sector, which account for about 70% of all freshwater use (UNWATER 2013). Water consumed in agriculture is not recoverable and will increase by 19% by 2050 (UNWATER, 2013). To withstand the shortage of water and save freshwater, the reuse of GW in areas that does not require potable water constitute an obvious alternative with a huge potential. Moreover, irrigation with GW can save freshwater, recycle nutrients and increase food and biomass production (Toze, 2006). However, irrigation with untreated GW can have negative environmental impacts and contribute to the spreading of diseases. To prevent the dangers due to the use of untreated GW, the on-site bio-filtration presents some environmental, economic and feasibility advantages compared to the centralized wastewater treatment and others filtration systems.

Bark (B), bio-char (BC) and activated charcoal (AC) were used as filters media. This study was a continuation of the study conducted by Ramiyeh (2013) with the same filters with the specificity of the addition of addition of real wastewater to synthetic GW.

In studies conducted by Stepanović et al. (2004), *Salmonella* spp. showed a high capacity of biofilm formation on plastic surface with preference for the media with low nutrients content. The formation of biofilm on the wall of the filter column should also be considered and influence the choice of columns material, as the features of the material -surface roughness and wettability

used for the column can influence the formation of biofilm (Van Houdt & Michiels, 2010) and have to be taken in account in the set-up of the filtration system. The column material will thus also contribute to the adsorption of microorganism and not merely serve as support for the filter media.

Differently from the attachment of bacteria, the electrostatic interactions and van der wall's forces are driving forces of the adsorption of viruses (Bradley et al., 2011).

A major difference between MS2 and PhiX-174 is their isoelectric point (IEP). The EIP is the pH at which the net surface charge of the virus is neutral, it is specific to a single virus, it determine the surface charge of viruses thereby their sorption aptitude (Michen & Graule, 2010). The surface charges of viruses have been used to detect, characterize viruses and study the adsorption to different surfaces and flocculation processes (Michen & Graule, 2010).

1.1 Meeting WHO guidelines

A judicious quantitative microbial risk assessment (QMRA) during the treatment and reuse of GW can be helpful for the setting of environmental and health based target for the upgrading of GW.

A QMRA followed by an application of the WHO guidelines stating that the localized irrigation can contribute for 2-4 log reduction, the washing of raw products can reduce by 1-2 log, the peeling by 2 log, the cooking by 5-6 log can complete the efficacy of filtration in the upgrading of GW to reach the level of total sanitization (WHO, 2006).

Those barriers preceded by a judicious choice of crop as non-food crops or crop which the harvested parts are not in contacts with irrigation water can significantly lower the risk of contamination by pathogenic agent in GW. So, even the filters upgrading capacity does not reach the total sanitization level, the filtration combined with appropriate agricultural practices such as sub-irrigation, restricted irrigation to crops that are cooked before consumption enable reduction values overlapping the international guidelines.

2. Background

2.1 Water scarcity

With an anticipated increase in world population by 2-3 billion people over the next four decades (WHO, 2010), the water demand is increasing twofold (64 billion cubic meter per years) the rate of the population increase (FAO 2012). Urbanization is increasing by 1.5% per years globally (WHO, 2010) and it is estimated that by 2050 the percentage of the total population that will live in urban centers is 98% in North America, 83% in Europe, 60% in Asia and 58% in Africa (UN 2012); which result in the decline in the groundwater level in urban areas (WHO, 2010).

The combined effect of the population growth, the urbanization, industrial development and climate change increases the water demand and makes the water shortage more severe in most places around the world (Vörösmarty et al., 2000). The amount of wastewater and GW in particular increases in parallel with the increase of the population, its reuse can alleviate the shortage or stress on water resources around the world.

2.2 GW potential

GW can be reused in irrigation of garden, of sport fields, for toilet flushing at home or in a hotel (Christova-Boal et al., 1996) and for landscaping, car washing, in industry, cleaning of floor, groundwater recharge (March et al., 2004). It is reused in different regions around the world for different reasons: alleviate the shortage of water in arid and semi-arid areas (Jordanian, USA, Australian, Saudi Arabian, Mali, Oman, Israel, Australia, USA, India) as reported by Al-Jayyousi (2003) and Hadadin et al. (2010); to ease the pressure of excessive water demand in areas with high density of population such as Japan (Al-Jayyousi, 2003); or as respond to environmental or economic considerations as in Germany or in Sweden.

The reuse of GW can considerably reduce household water consumption: savings between 30-50% of their potable water consumption has been reported by Jeppesen (1996). It also decrease the sewage loading by 47% and thereby decrease the pressure on centralized wastewater treatment plants; alleviate the stress in water deficient areas; reduce water cost by reducing water and sewage bills, save energy for potable water production; recycle nutrients, and constitute a stable water supply even in times of drought (Christova-Boal et al., 1996). The reuse of GW brings about costs in term of implementation, maintenance and manpower but it is profitable in long term. Friedler and Hadari (2006) found that in conditions of water and sewage prices of 1.16 and 0.3 US$ m^{-3} respectively in Israel, the onsite treatment by rotating biological contactor (RBC) become economically feasible when the building is seven storeys (28 flats) in size. Monetization of GW reuse in toilet flushing, cleaning of floor and irrigation in a residential school in India, give an annualized benefit of 705 US$ in availability of GW, 1176 US$ in avoidance of water infrastructure, 1035 US$ in environmental benefits, 18659 US$ in health benefits (Godfrey et al., 2009).

2.3 GW reuse challenges

The reuse of GW introduce many challenges that have to be overcome. The variations in volume is one issue: e.g. the weekly GW generated at the Al Hail South mosque varied between 4.1 m^3 and 17.6 m^3 (Ahmed et al., 2005). There are great variations in the composition of GW that depend on: the quality of the water supply, the type of distribution, and the activities of the households. It also undergoes dynamic chemical changes (decrease in pH, dissolved oxygen) and microbial growth (total and fecal coliforms) (Dixon et al., 2000) during storage. GW contains suspended solids (food particles, hair, raw animal, fibers), xenobiotic organic compounds, heavy metals or hazardous substance (Al, Fe, Mn, Cd, Cu, Pb, Hg, Zn, Ni, Cr) (Palmquist and Hanæus (2005)), can have a high biologic oxygen demand (BOD) and chemical oxygen demand (COD), carry pathogenic bacteria, protozoa, viruses and helminths but can also contains nutrients as nitrogen and phosphate (Eriksson et al., 2002).

More general issues exist, such as public acceptance (Ahmed et al., 2005), political constraints (lack of guidelines) and economic constraints in term of initial investment in the separation, transport, storage and treatment (Al-Jayyousi, 2003).

Untreated GW - by its physical, chemical and biological composition - can cause alkaline conditions toxic to plants, aesthetic concerns as offensive odor, clogging of the distribution system, oxygen depletion in nature, accumulation of heavy metals and XOCs in soil and groundwater and soil hydrophobicity (Travis et al., 2010). It also includes a sanitary risk of spreading of diseases through direct contact or the consumption of contaminated products. The growth of microorganisms - rise of TC from 50 to $> 10^5$ 100 mL^{-1} after a few days - decrease of DO (anoxic conditions) after 3 days of storage and the decrease of pH (acidification) (Dixon et al., 2000) during the storage or in the distribution system constitute an additional source of health and environmental risks.

2.4 GW reuse systems

GW can be reclaimed by three main mechanisms: physical treatments (coarse sand filtration, soil filtration and membrane filtration); chemical processes (coagulation, photo-catalytic oxidation, ion exchange and granular activated charcoal); or biological treatments (rotating biological contactor (RBC), the sequencing batch reactor (SBR), anaerobic sludge blanket (UASB), the constructed wetland (CW) , the membrane bioreactors (MBR)) (Li et al., 2009).The physical systems are affective in improving the aesthetic quality of the effluent but can be subjected to fouling by pollutant and are energy demanding, the biological treatments reduce to some extents all components of the GW but they are costly (Jefferson et al., 2000).

A high acceptance for GW from bathroom and laundry reuse for garden watering, toilet flushing, has been experienced in Melbourne in Australia where people prefer a reuse system with a short payback time of about 2-4 years (Christova-Boal et al., 1996). In a survey conducted by Mourad et al. (2011), 83% of Sweida (Syria) inhabitants were favorable to the reuse of treated GW for

toilet flushing or irrigation while 10% were already using untreated laundry water for irrigation or cleaning of the house.

2.5 Bio-filters

The term bio-filtration, in this thesis, refers to the biodegradation of pollutants by microorganisms fixed to a porous media. Bio-filtration has been used removing many substances. Bio-filters can be used for treatment of heavy metals from industrial wastewater (Srivastava & Majumder, 2008), for abatement of odor (McNevin & Barford, 2000; Nicolai & Janni, 2001) , for upgrading of GW (Mourad et al., 2011) and denitrification of aquaculture (Dalahmeh et al., 2012; Saliling et al., 2007) . The bio-filters media are various: wood chips, wheat straw, kaldnes plastic (Saliling et al., 2007),;a mixture of compost and wood chips (Nicolai & Janni, 2001); bark, sand, foam and activated charcoal , have all been used as support media in bio-filtrations systems (Dalahmeh et al., 2012). The bio-filters media can be found on the location of the treatment unit or be bought from industry (Mourad et al., 2011). According to Saliling et al. (2007), the abundance, the cost and the expected life are important criteria in the choice of filter media. The efficacy of a bio-filter is largely depends on the properties and characteristic of the filter media (Srivastava & Majumder, 2008).

On-site treatment systems have the potential to remove both the chemical and microbial pollutants in remote areas or in areas with low or lacking infrastructure. The centralized wastewater treatment stations are insufficient, costly in term of implementation and mainly in transport (pipes), often overloaded and require specifics skills for operation and maintenance (Massoud et al., 2009). Furthermore, they have negative environmental impacts because they require majors excavations for piping and manholes for access (Massoud et al., 2009) and are not suitable for areas with small communities and disperse individuals household because of the high cost to build them and the technical expertise to operate them (Bakir, 2001). The on-site treatment is an affordable option that can deal with specific issue at local level (Massoud et al., 2009), as excess chemicals and predominance of pathogenic agent is treated on site and the effluent is used on site, the pollutants will not be spread to the rest of the community.

2.6 Pathogens and indicator organisms

Microorganisms in GW are mainly originated from fecal contamination, food handling, and opportunistic pathogens from body or respiratory organs. The organisms of interest of the thesis are:

2.6.1 *Salmonella* spp.

Salmonella spp. are gram negative aerobic, bacteria, rod-shaped infecting humans and animals (Ottoson & Stenström, 2003) with a size varying between 0.7-1.5 nm in diameter and 2.0-5.0 nm in length.

Salmonella spp. are originated from contaminated animal foods (meat, poultry, eggs, milk), they are major cause of foodborne disease with fever, abdominal pain, diarrhea, nausea, vomiting as symptoms and can be severe with children and elderly. In 2008 an outbreak of *Salmonella*

typhimurium in Sweden, Denmark, and Norway occurred, originated from Danish pork meat and pork products (Bruun et al., 2009).

2.6.2 *E. faecalis*

Since it is impossible to test GW for all bacteria, *E. faecalis* - natural occurring bacteria in the human intestines - can be used as indicator organism and their presence in GW indicates a fecal contamination and thereby potential health risks. They are spherical Gram positive bacteria and can be grouped in short chains, in pairs or as single cell (Murray, 1990). Their size varies between 0.6-2 µm by 0.6-2.5 µm and have been observed in raw GW by Birks and Hills (2007) in a concentration of $2.5*10^3$ CFU100 mL^{-1} . Their presence indicates fecal contamination of GW.

2.6.3 Bacteriophages

MS2 and PhiX-174 are bacteriophages (viruses that infect bacterial cell) with different surface charges. They are similar in structure, morphology and size to many human enteric viruses (Bradley et al., 2011). They have been regularly used in studies to analyze sorption aptitude in aquatic environment (Michen & Graule, 2010). Their small size, their survival in harsh conditions and their resistance to different treatments make them a good indicator to predict the behavior of enteric viruses (Ottoson & Stenström, 2003). Furthermore, the detection of bacteriophages is simpler and cheaper than others methods of detection of enteric viruses.

MS2 phages, they are bounded to temporary bacterial receptors (present during the growth of bacteria) and called F-specific RNA (F-RNA) viruses (Elving, 2012) with a diameter of 24 to 26 nm (Ellis & Winters, 2007).

PhiX-174, they bounded to permanent bacterial receptors at the cells surface and are called somatic coliphages (Elving, 2012) with a diameter of 25 to 27 nm, single-stranded DNA viruses.

This study is a continuation of the experiment conducted by Lalander et al. (2013) for the aerobic treatment of GW by different bio-filters media. Sand filters are not included in this study and the organic loading will remain constant during the experiment.

2.7 Reuse requirements

WHO recommends an upgrading of GW prior to a reuse in irrigation, to a level that ensures the health protection of $\leq 10^{-6}$ Disability-Adjusted Life Year (DALY) pe^{-1} yr^{-1} and minimize the negative environmental impacts on soil (salinization, accumulation of heavy metal or persistent organic compounds) and on water bodies (eutrophication). The DALY being one lost year of healthy life, the sum of these DALYs across the population is the burden of disease in the population and express the gap between the current health status and the situation where the entire population is free from diseases and disability (WHO, 2006).

Thus, a treatment system with an efficiency in reduction of pathogens of 4-5 log units for unrestricted irrigation and able to maintain the pH above 6.5 and an EC below 3 mS/cm is considered as suitable for the reclamation of GW (WHO, 2006) .

2.8 Aim and objectives

The aim of the thesis was to evaluate the performance of different filters materials in upgrading of GW for a reuse in agriculture and the effect of natural wastewater in the formation of biofilm.

The objectives of this thesis were:

- To compare the effectiveness of the pine bark, bio-char and activated charcoal filters in the remove of bacteria and viruses from GW;

- To assess the in performance of the filters compare to previous experiment;

- To analyze the impact of added wastewater in the performance of the filters;

- To monitor the consequences of the different filter materials;

- To assess the potential of sanitization of the different filters for a future reuse of GW in agriculture.

The physic-chemical parameters pH, total nitrogen (N-tot), ammonia (NH_4^+), nitrates contents (NO_3^-), chemical oxygen demand (COD), and the electric conductivity (EC) was measured before and after filtration.

The concentration of the bacteria (*Salmonella* spp, *E. faecalis*) and the bacteriophages (PhiX-174, MS2) were assessed before and after filtration.

3. Materials and methods

3.1 Experimental set-up

Vertical columns were fed with artificial GW twice daily for 10 weeks to compare the filtering properties of pine bark, activated charcoal and bio-char. The filters had already been under operation for 10 weeks, fed with artificial GW with no addition of wastewater (Ramiyeh, 2013). Each filtering material was set-up in triplicates.
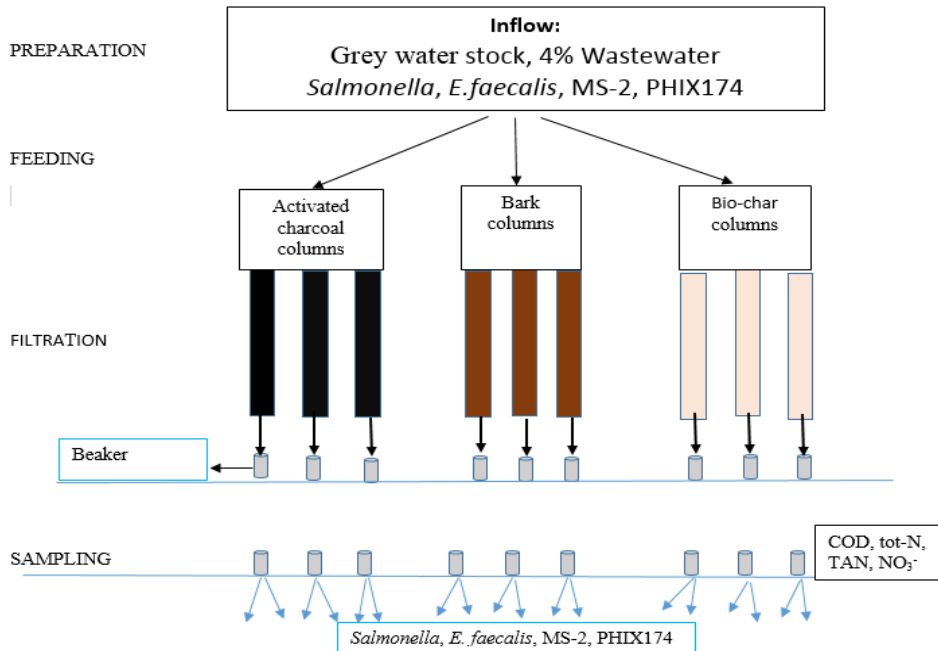

Figure 1. Experimental scheme presenting the experimental set-up showing the nine columns used - three were filled by activated charcoal, three by pine bark and three by bio-char-the - different steps of the experiment (preparation of GW, feeding of the filters, the filtration process and the sampling), and the microbial and chemical parameters analyzed.

3.2 Filters materials

The bark originated from an undefined mixture of pine bark was air-dried and sieved through 7, 5, 3.15 and 1 mm screens. The bark retained on the 3.15 mm and 1 mm screens was mixed in a 3:2 ratio by weight.

The activated charcoal obtained from Merck with two different sizes (diameter 1.5 mm and, length 1.5 or 3.5 mm) was mixed in a 2:3 ratio by weight.

The bio-char originated from Salix leaves in Germany were sieved through 7, 5, 2.8, 1.4, and 1 mm screens.

Table 1. Physical properties of the bark, activated charcoal and bio-char.

Filter material	Particle size (mm)	Effective size (mm)	Bulk density $(g\ m^{-3})$	Particle density $(g\ m^{-3})$	Total porosity (%)	Water content (%)
Bark	1-3.15	1.4	0.36	1.3	73	7.6
	3.15-5					
Activated charcoal	1.5	1.2	0.56	1.89	70.6	1.9
	3-5					
Bio-char	1-1.4	1.4	0.27	0.74	63.3	0.06
	2.8-5					

3.3 Columns

The preparation of the columns, the columns material, size and set-up are described in detail by Ramiyeh (2013).

3.4 Preparation of artificial GW

Three liters of artificial GW was prepared once a week in a sterile beaker with tap water according to the follow recipe and ingredients: 1.92 g YES dishwashing gel (YES Original, Protector and gamble, Stockholm, Sweden), 1.92 g hair shampoo (VO5, Upplands-Väsby, Sweden), 1.92 g washing powder (Ariel, Germany), 1.2 g cooking corn oil (EL Nada, Al-Asher for products, 10th Ramadan City, Egypt), 13.44 g standard nutrient broth (OXIOD, Sollentuna, Sweden). This mixture was stored at 4 °C during the rest of the week in order to minimize microbial development and the deterioration of the GW (Ramiyeh 2013).

The dose of artificial GW (without pathogen inoculation) for the next day was measured and stored in a beaker at room temperature (RT) to allow the GW to reach RT to mimic conditions in warm countries.

3.5 Inoculum

Some colonies of *Salmonella enterica* subspecie-1 serovar Typhimurium phage type-178 isolated from sludge (Sahlström et al., 2004) and *Enterococcus faecalis* (ATCC 29212) were grown separately in nutrient broth (Oxiod AB, Sweden) at 37 °C overnight. Each morning 1% of the bacterial solution was added to the daily dose of GW.

Each morning the daily dose of GW is prepared by mixing predefined amount of synthetic GW with 4% of wastewater from Uppsala's WWTP "Kungsängen" in the purpose to inoculate and enhance the establishment of indigenous microbial flora, 1% *E. faecalis* solution, 1% *Salmonella*

spp solution, 1% bacteriophage PhiX-174 (ATCC 13706-B1), 1% bacteriophage MS2 (ATCC 15597-B1).

3.6 Experimental execution

The first week the filters were fed with 45 ml per day with artificial GW without any inoculation of microbes in order to wash any potential previous bacterial flora before the start of the experiment.

The columns were fed daily with 45 ml daily of artificial GW with wastewater and inoculum of *Salmonella enterica*, *Enterococcus faecalis,* PhiX-174 and MS2: 30 ml in the morning and 15 ml in afternoon.

The filtered effluent was collected in the sterile beakers placed below each column. The beakers were emptied each morning before new feeding of the columns and were reused during a week. On sampling days, new sterile beakers were used.

3.7 Analysis of physic-chemical parameters

The N-tot, TAN, NO_3^-, COD, pH and the EC of the GW and the filtered effluent from each column were measured once each two weeks in order to appreciate the changes during the filtering process.

The pH was measured using by a digital standard pH meter PHM210.

The EC was measured by Cond 340i.

Table 2. Characteristics of chemicals analyze kits used for the experiment.

Chemicals	Kit name	Measurement range	Unit	Standard method	Control solution name and value	Apparatus
COD (chemical oxygen demand)	Spectroquant COD Cell test (Hg-free)	500-10000	mg L^{-1}	No standard, but Hg-free	Potassium hydrogen phthalate solution 1.11769.0100, Merck 170 mg L^{-1}and Combi R1, combicheck 20 1.14675.0001, Merck 750 +-75 mg L^{-1}	Thermoreactor TR 420, Merck Germany, Spectroquant NOVA 60, Merck Germany pipettor,VWR, poland analog Vortex Mixer, VWR, USA
N (Total Nitrogen)	Spectroquant Nitrogen (total) Cell (test)	0.5-15 and 10-150	mg L^{-1}	EN ISO 11905-1 (digestion)	Nitrate standard solution 1.19811.05000, Merck 1000 mg L^{-1}	Spectroquant NOVA 60, Merck, Germany pipette, VWR, poland Analog Vortex Mixer, VWR, USA
NH$_4^+$ (Ammonia)	Spectroquant COD Cell test	0.5-16	mg L^{-1}	EPA 350.1,US Standard Methods 4500-NH3D, and ISO 7150/1	Combi R1, Combicheck 20 1.14675.0001, Merck 12+-1 mg L^{-1}	Spectroquant NOVA 60, Merck, Germany pipettor,VWR, poland Analog Vortex Mixer, VWR, USA
NO$_3^-$- (Nitrates)	Spectroquant COD Cell test (Hg-free)	1-50	mg L^{-1}		Nitrate standard solution 1.19811.05000, Merck 1000 mg L^{-1}	Spectroquant NOVA 60, Merck, Germany pipettor,VWR, poland Analog Vortex Mixer, VWR, USA

The concentrations of N-tot, TAN, NO$_3^-$ and COD were determined using the chemical kits by MERCK (Table 2). The sample was diluted if necessary when the chemical content exceeded the highest range of concentration of the kit, no values for chemical with lower concentration than that of the lowest range of the chemical kit.

The incubator Spectroquant TR 420 was used for the N-tot and the COD and the chemical contents was measured by the spectrometer Spectroquant NOVA 60.

The percentage reduction or changes in chemical content was estimated by the following equation

$$Reduction = 1 - \frac{C_{out}}{C_{in}} \times 100$$

Where C_{out} is the concentration of chemical in the effluent and C_{in} is the concentration in the artificial GW.

3.8 Microbiological parameters

3.8.1 Dilution series
Buffred NaCl Peptone water with Tween (pH 7), was used for the dilution which was made to the expected detection level of the microorganisms in the GW and the effluent from different material.

3.8.2 Bacterial analysis
Slanetz & Bartley agar (SlaBa) plates were used as cultivation subtract for *E. faecalis* (ATCC 29212) and were incubated at 44 °C for 48 hours.

Xylose lysine deoxycholate (XLD) plates were used to grow *Salmonella* spp and were incubated at 37 °C for 24 hours. Enumeration of the bacteria was performed by counting the colony forming units (CFU).

3.8.3 Bacteriophages analysis
The soft agar solution is melt in microwave and kept in 55 °C to avoid solidification.

Blood agar plates (BAB) were used as the cultivation subtract, the MS2 phage with host bacteria WG 49 (ATTC 700730), and PhiX-174 phage with host bacteria *Escherichia coli* (ATCC 13706). The bacteria were cultivated in nutrient broth during 3- 5 h at 37 °C before the sampling. One ml of the host strain, and 1 ml of the virus sample were added to 2 ml of the soft agar in an essay tube placed on the heating block to avoid the soft agar to solidify. The mixture was poured on the BAB plates and were incubated at 37 °C overnight. Enumeration of bacteriophages was performed by counting the plaques forming units (PFU) with their respective host bacteria.

Duplicate plating was applied to each microbial sample.

The remove of bacteria and virus was estimation by the following equation:

$$Log_{10}\ reduction = Log_{10}(C_{in}) - Log_{10}(C_{out}) \quad \text{(Eq. 1)}$$

Where C_{in} is the concentration of the artificial GW and C_{out} the concentration of the effluent.

3.9 Statistical analysis

Minitab 16 was the statistical software used in this study.

One-way ANOVA with 95% confidence interval were used to verify differences between filter materials. Tukey post-hoc method of grouping were used to find the differences. Graphical representations were conducted in Minitab 16.

4. Results

Table 3. Physical and chemical characteristics of the inflow GW.

Parameters	N	MIN	MAX	AVERAGE	SD
Total N (mg/L^{-1})	6	7.2	10.3	9.4	1.04
Ammonia(mg/L^{-1})	6	2.1	47.2	23.9	15.6
Nitrates (mg/L^{-1})	6	0.6	2.2	1.2	0.6
COD (mg/L^{-1})	6	4208	4895	4630.5	232.8
EC (mS/cm)	5	5.24	6.37	5.82	0.36
pH	6	7.77	8.45	8.04	0.23
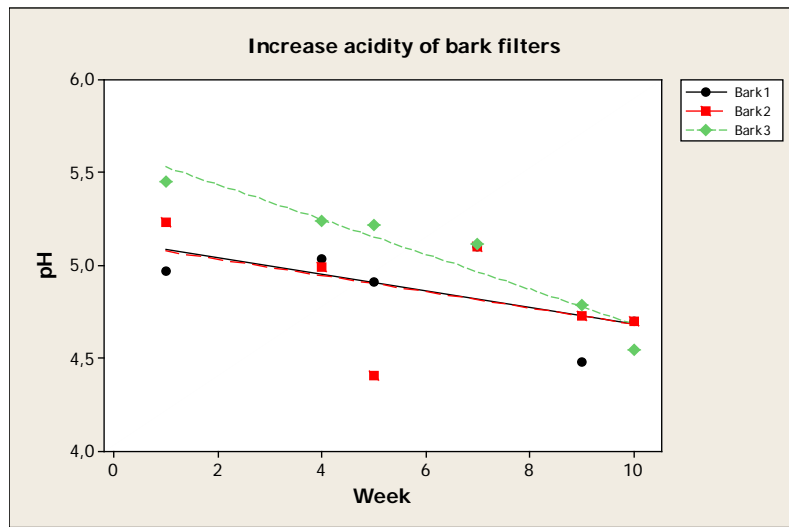
Bark1:
$R^2 = 40.68\%$
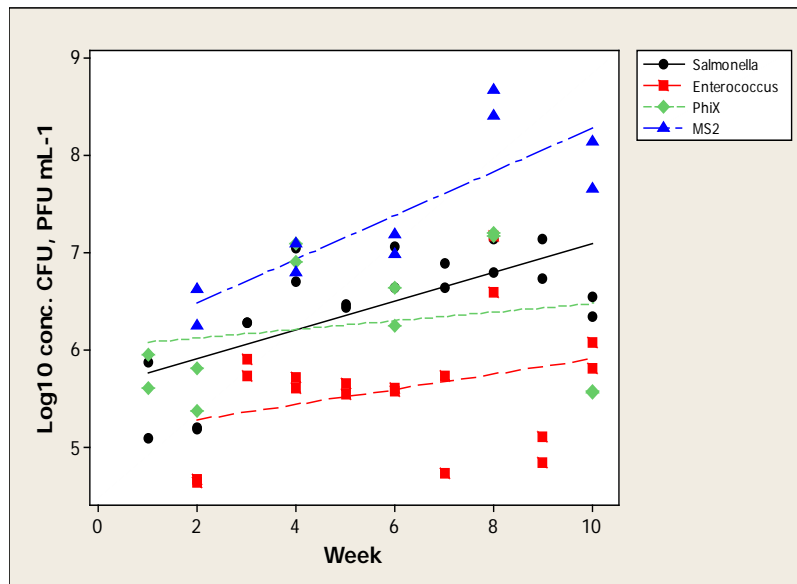$P = 0.173$

Bark2:
$R^2 = 23.49\%$
$P = 0.330$

Bark3:
$R^2 = 90.37\%$
$P = 0.004$

Figure 2. Decrease tendency of pH from the three bark filters effluents.

Salmonella **spp**
$R^2 = 47.4\%$
$P = 0.001$

Enterococcus **spp:**
$R^2 = 10.2\%$
$P = 0.197$

PhiX-174
$R^2 = 4.4\%$
$P = 0.514$

MS2
$R^2 = 69.6\%$
$P = 0.003$

Figure 2. Inflow microbial concentration characterized by a substantial increase of MS2 and *Salmonella* spp concentration in contrast with a slight increase of PhiX-174 and *E. faecalis* during the experiment.

The inflow viral concentration was increasing during the experiment. The highest concentration and the most significant increase was observed for MS2 phage. The number of PFU mL^{-1} varied between $3.07*10^6$ and $3.68*10^7$ for MS2 PHAGE; between $3.79*10^5$ and $1.57*10^7$ for PhiX-174.

The bacterial concentration in the inflow was also increasing with the highest concentration and significant increase observed for *Salmonella* spp. The concentration in inflow in *Salmonella* spp was in order of $1.59*10^5$ to $1.01*10^7$ CFU mL^{-1} and $4.68*10^4$ to $9.44*10^6$ CFU mL^{-1} for *Enterococcus* spp.

Salmonella **spp:**
$R^2 = 0.3\%$
$P = 0.841$

Enterococcus **spp:**
$R^2 = 47.5\%$
$P = 0.028$

PhiX-174
$R^2 = 4.4\%$
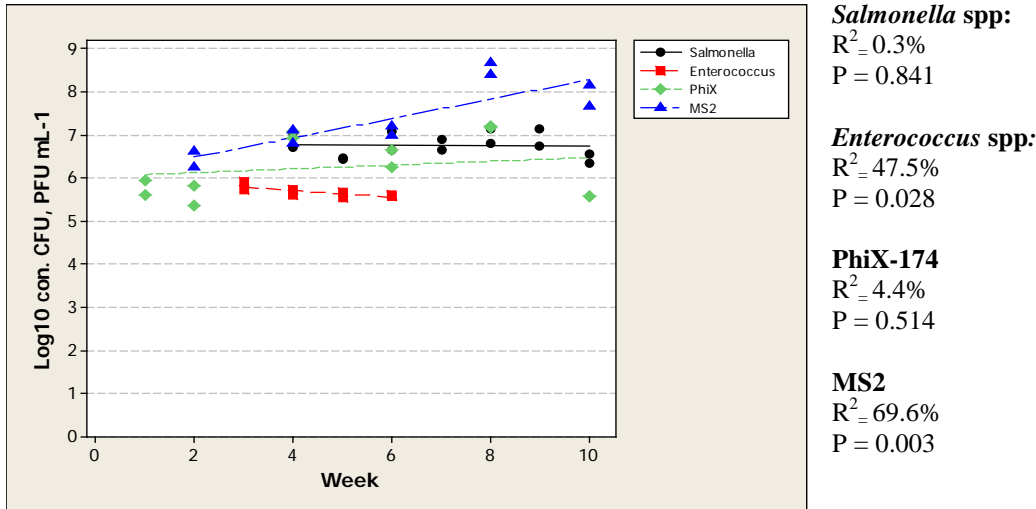$P = 0.514$

MS2
$R^2 = 69.6\%$
$P = 0.003$

Figure 3. Period of stable inflow concentration for *Salmonella* spp. and *Enterococcus* spp.

The concentration in *Salmonella* spp was more or less stable during six consecutive weeks in the experiment, fluctuating between 6.4 log$_{10}$ CFU mL^{-1} and 7.2 log$_{10}$. The same stabilization was observed with *Enterococcus* spp during four weeks, fluctuation between 5.6 log$_{10}$ CFU mL^{-1} and 5.9 log$_{10}$ CFU mL^{-1}, which is within the error margin of the analysis method.

Acti-char:
$R^2 = 35.06\%$
$P = 0.000$

Bark:
$R^2 = 34.42\%$
$P = 0.000$
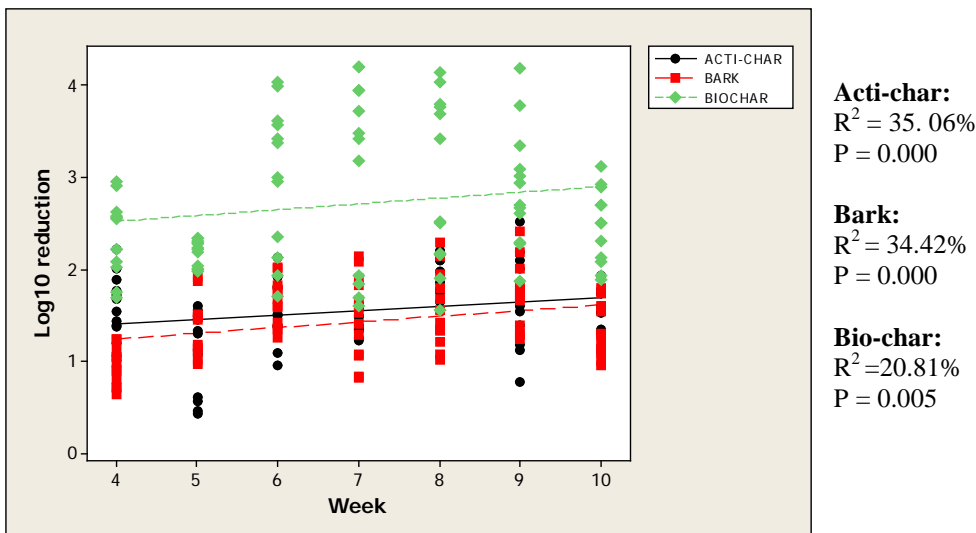
Bio-char:
$R^2 = 20.81\%$
$P = 0.005$

Figure 4. Log$_{10}$ reduction of *Salmonella* spp. over time.

The bio-char filter was more effective in the reduction of *Salmonella* spp during the period of stable inflow concentration and all filters materials present a slight trend to improvement during this period.
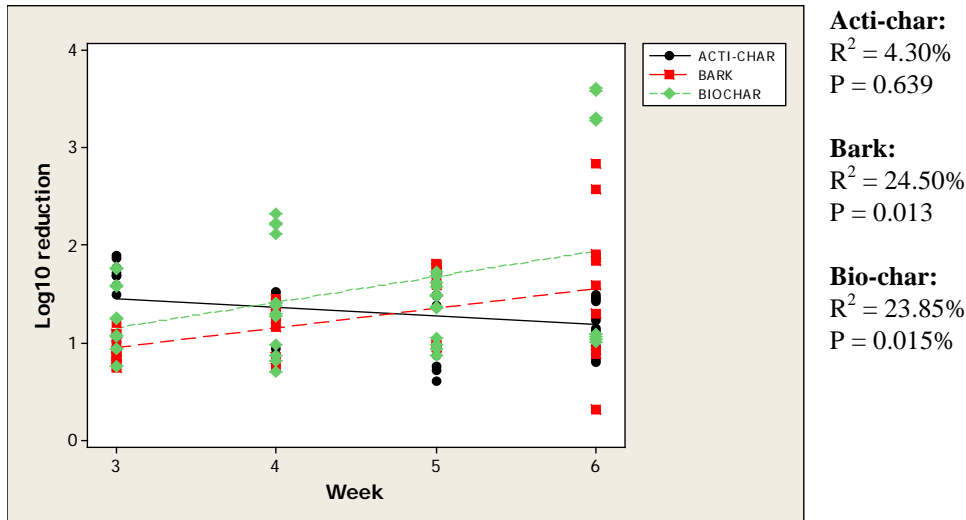

Acti-char:
$R^2 = 4.30\%$
$P = 0.639$

Bark:
$R^2 = 24.50\%$
$P = 0.013$

Bio-char:
$R^2 = 23.85\%$
$P = 0.015\%$

Figure 5. Log_{10} Reduction of *E. faecalis* over time.

The performance of the filter materials in the reduction of *E. faecalis* was in general lower that of *Salmonella* spp. Some sign of improvement with bio-char and bark was observed, unlike the activated charcoal which decreased in performance. The reduction in bio-char and bark were lower than that observed with activated charcoal at the beginning of this period of stable inflow concentration of *Enterococcus* spp, but became higher at the end.
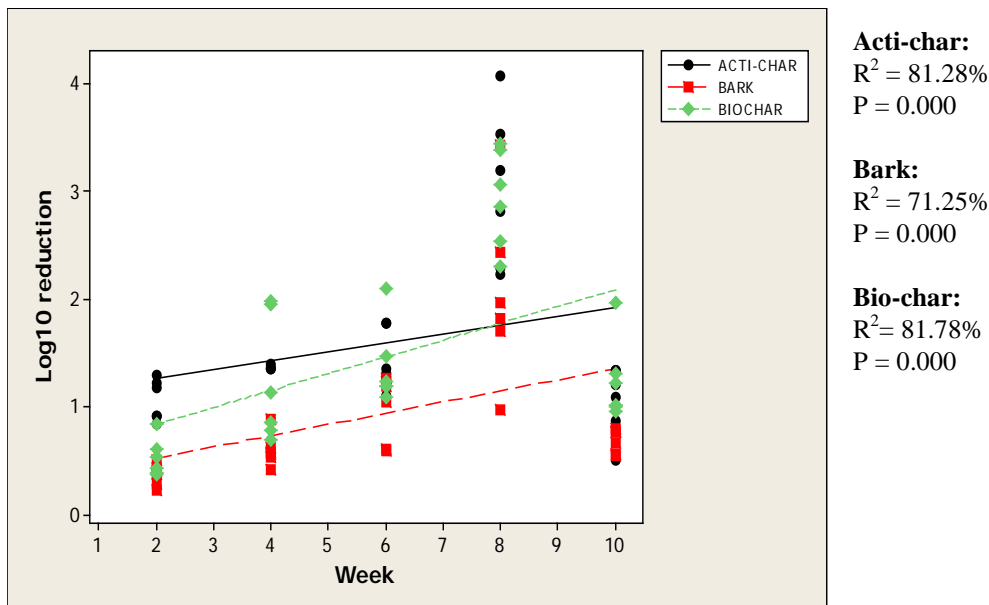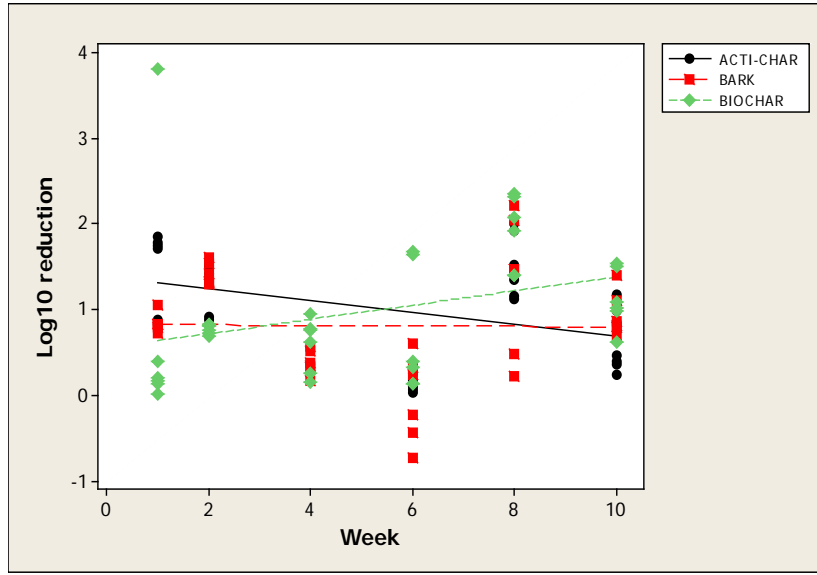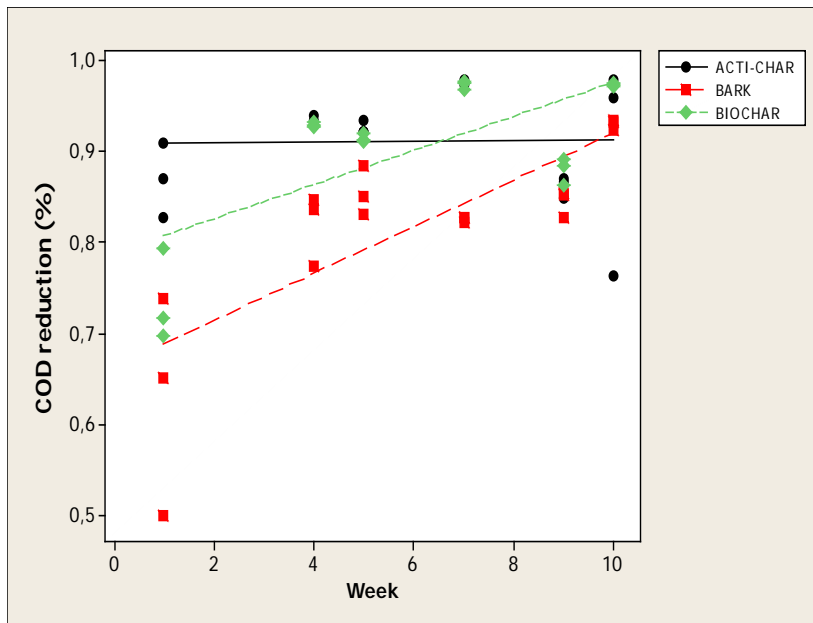

Acti-char:
$R^2 = 81.28\%$
$P = 0.000$

Bark:
$R^2 = 71.25\%$
$P = 0.000$

Bio-char:
$R^2 = 81.78\%$
$P = 0.000$

Figure 6. Log_{10} Reduction of MS2 over time.

Figure 7. Log_{10} Reduction PhiX174 over time.

Figure 8. Percentage reduction COD over time.

The reduction of COD in activated charcoal filter was high from the beginning of the experiment and did not particularly change during the course of the experiment. The smallest reduction observed was 76% and the greatest 98%, yielding an average of 96%. For the bark and the bio-char filters the lowest reduction rate were observed at the beginning, 50% and 70% respectively and the highest at the end , 94% and 98%, respectively through a constant increase during the experiment with an average of 82% and 90%, respectively.

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Table 4. Mean value of pH and EC in the inflow GW and effluent from the filters.

	pH Mean	SD	EC mean (mS/cm)	SD
Inflow	8.04	0.23	5.82	0.36
Bark	4.93	0.28	5.26	1.38
Bio-char	8.13	0.15	4.10	1.20
Acti-char	8.05	0.19	4.70	0.74

The pH of the effluent from the bio-char and activated charcoal filters was as alkaline as the pH of the inflow. The pH values in bark filters effluents were approximately three pH units lower than in the other filters.

The bark filters perform the lowest reduction in EC, with high variation. No of the filter materials present a particular effectiveness in reduction of the electric conductivity, but a reduction was observed in effluent from the bio-char and the activated charcoal filters.

Table 5. Nitrogen Changes (mg L^{-1}) (±SD)

		Week					
		1	**4**	**5**	**7**	**9**	**10**
Tot-N	*Inflow*	*9.1*	*9.6*	*7.2*	*10.3*	*10*	*10*
	Bark	51±10	163±25	151±38	205±27	207±18	204±15
	Bio-char	53±16	128±13	161±19	216±18	208 ± 3	235±15
	Activated-char	1 ± 0.1	1± 0.3	0.8±0.2	0.9±0.5	0.8±0.4	1.1±0.9
NH_4^+	*Inflow*	*2.1*	*5.41*	*29*	*27.6*	*31.8*	*47.2*
	Bark	46 ± 12	13 ± 8	9 ± 7	16 ± 3	43 ± 13	67 ± 15
	Bio-char	1 ± 0	0.6± 0	*	*	*	*
	Activated-char	*	*	*	*	*	*
NO_3^-	*Inflow*	*1.5*	*0.6*	*0.7*	*0.6*	*1.6*	*2.2*
	Bark	145±51	68 ± 37	100±52	172±27	168±19	162±23
	Bio-char	116±22	153 ± 9	153 ± 6	107±35	179±12	204±15
	Activated-char	*	*	*	*	*	1 ± 0

*below detection level 0.5 mg L^{-1}.

A steep increase in the N-tot was observed in the effluent from the bark and bio-char filters compare to the inflow, in which the nitrogen content was quite stable during the experiment. The nitrogen content of the effluent was low at the beginning but increase by time with some fluctuations and the highest value was observed in the late period of the experiment. No reduction of N-tot or NO_3^- content was observed with bark and bio-char filters. All nitrogen in the inflow (N-tot, TAN, and NO3⁻) were lost during the passage through the activated charcoal filters.

Activated charcoal was very efficient in reduction of N-tot.
The highest nitrogen content has been observed in bio-char filters effluent.

The TAN content of the inflow was not stable during the experiment and was steady increasing, expect the week 7. The bark filters was not stable in the reduction of TAN, they exhibited a rise or decrease during the experiment.
The totality of ammonia was removed during the passage in bio-char and activated charcoal filters.

The inflow concentration of nitrate was very low and stable during the experiment (0.6-2.2 mg/L^{-1}). The nitrates content observed in the effluents from bark and bio-char was extremely high and fluctuated greatly.

5. Discussion

5.1 pH changes

The mean pH-value of 8.04 (Table 4) of the GW used in this study fit in the range of pH -values 8 -10 has been reported by Eriksson et al. (2002).

The decline of pH in the bark filters (Figure 1) was probably due to the combined effect of the release of humic acid (HA) when the bark is decomposing (Unsal & Sozudogru Ok, 2001) and the production of hydrogen ions by nitrification of NH_4-N to NO_3-N (Dalahmeh et al., 2012). The acidic environment in bark filters was not sufficiently low to reach lethal levels for *Salmonella* spp. and *Enterococcus* spp. The pH in bark filter never reached a neutral state during the experiment (Table 4) as observed by Ramiyeh (2013) with the same filter, the continue decomposition of bark producing more HA conducing to a steady drop of pH in bark filter. The use of the effluent from bark filters in places with sufficient water can accelerate the process of acidification. The degradation of bark filter can lead to a loss of mass and changes in porosity of the filter media, as observed by Saliling et al. (2007) with wood chips and wheat straw.

5.2 Removal of COD and nutrients

The high reduction of COD observed in the activated charcoal filters (Figure 9) can be the result of adsorption to the surface of the filter media, as the specific surface area of activated charcoal filter was high (>1000 m^2 g^{-1}). A high and constant reduction of COD in activated charcoal filters was experienced, a god efficacy of activated charcoal in removal of COD have also observed by Chaillou et al. (2011). In bark and bio-char filters, the removal of COD was increasing during all the experiment period. The continuous increase was probably due to the stimulation of biodegradation by the abundance of nitrogen in those filters.

Concerning nitrogen, only the activated charcoal filter could reduce nitrogen, unlike the bark and bio-char filters, where an increase of total nitrogen in the effluent and a change of nitrogen by ammonification and nitrification occurred. The nitrogen generated in the GW as a result of the passage through those filters, increases the fertilizer value of the treated GW. On the other hand, extra care has to be taken so that the effluent from those filters does not end up in water bodies, as it could contribute to eutrophication and nitrate pollution of drinking water.

This abundance of nitrogen in those filters can enhance the biological treatment since studies conducted by Jefferson et al. (2001) showed an improvement of biological treatment of GW after addition micronutrients (Zn) and macronutrient (N, P), improvements that they attribute to the stimulation of microbiological activity resulting in an increase of COD removal and oxygen uptake. Thus, with the nutrient rich filters a combined effect of the physical (mechanical) filtration and the microbiological processes can be achieved.

The low nitrogen content in activated charcoal (Table 5) can be unfavorable for the survival of microorganisms and exclude those filters as media in biological treatment. The removal of nitrogen is probably the result of adsorption onto the activated charcoal surface.

5.3 Microbial Removal

5.3.1 Removal of *Salmonella* spp. and *E. faecalis*

The properties of the bacteria, the features of the biofilm-supporting materials (filters media and the columns wall in this study), environmental factors as pH and temperature, the presence of other bacteria, the nutrients (quality and quantity) in the media, together play a role in the adherence of bacteria to surfaces and thus the biofilm formation (Van Houdt & Michiels, 2010).

In this study the temperature was 25°C in the laboratory whilst in reality there is a w fluctuation of temperature, an average of temperature quite below or higher in different regions around the world than that in the laboratory. The temperature is supposed to have an influence on the reduction of bacteria, a low temperature preventing the adsorption of bacteria by enhancing the viscosity of the bacterial surface polymer and of the liquid, by reducing the chemisorption and certain types of physical adsorption and by changing the physiology of bacteria (Kristian Stevik et al., 2004). The results of this study will probably not be valuable in areas with temperature that significantly deviate from 25°C.

The fragmentation of raw pine bark in the preparation of the bark filter medium increases the contact surface and thereby enhance the degradation of bark, followed by a mass loss of the filtering media that hinder the formation of biofilm because of the instability of the microbiologically active surface. According to Dalahmeh et al. (2012), the formation of biofilm is the main mechanism in the removal of bacteria. The bark, unlike the bio-char and the activated charcoal, is not processed, thus can have more microbiologically active surface leading to rapid degradation of the filter media.

The flow through the filter media in this experiment was unsaturated as the feeding was intermittent and the hydraulic load low. In unsaturated flow the macrospore are filled with air and the flow occur only in microspores, the transport of microorganisms therefore should have occurred mostly in the smaller pores in this study (Kristian Stevik et al., 2004).

The bio-char filters were better and significantly different from the activated charcoal and the bark filters in the reduction of *Salmonella* spp. However, no significant difference between activated charcoal and bark was observed. The average *Salmonella* spp $\log_{10}$ reduction for bio-char, activated charcoal and bark were 2.72 ±0.74; 1.55±0.4; 1.43±0.4, respectively, those results are similar to the performances realized by the different filters materials at the end of the experience conducted by Ramiyeh (2013).

The relative high reduction of *Salmonella* spp in bio-char filter can be attributed to the small porosity of those filters combined with the relative big size of *Salmonella* spp., along with their motility, which facilitate the finding of potentially adhesive surfaces (Kristian Stevik et al., 2004) . Furthermore, the high concentration of *Salmonella* spp. can ease the contact with the filters surface thus lead to more adhesion and thereby higher reduction. The motility of *Salmonella* spp. can facilitate them withstanding the repulsive forces of the filter media surfaces and thereby their movement along the surfaces, which ease the growth and spreading of the biofilm. The presence

of Fimbriae, thread-like structure that protrude from *Salmonella* spp. cells surface, plays a central role in the establishment of stable adherence to the surfaces (Van Houdt & Michiels, 2010).

The difference in hydrophobicity of the surface of bacteria can influence the degree of affinity to the filter media surface. In two different studies on bacterial adhesion to rubber surface, *Salmonella* spp. was demonstrated to have a lower contact angle compared to *E. faecalis* (Sinde & Carballo, 2000; Teixeira et al., 2005). *Salmonella* spp. are hence more hydrophobic than *E. faecalis,* and thus their adhesion to hydrophobic bio-char filter surface can be favored and stronger. There was no significant difference between the bio-char, the activated charcoal and the bark filters in removal of *E. faecalis*. The average *E. faecalis* $\log_{10}$ reduction was 1.51±0.73; 1.32±0.35; 1.26±0.51 respectively.

One of the characteristics of bark filter that stand out from the others filters is the acidic condition (low pH) (Table 4). In this study it appeared that the degree of acidity of bark filters did not have a great impact on the *E. faecalis*. The capacity of adhesion to surfaces of some bacteria have been observed to reduce in acidic environment (Tresse et al., 2006). Svensäter et al. (1997) has established the killing pH of several bacteria between 4.5 and 2.5. The killing pH of *E. faecalis* was found to be 2.5.

5.3.2 Bacteriophages
Bark filters were significantly less effective in removal of MS2 than the bio-char and activated charcoal filters with the $\log_{10}$ reduction of 0.94, 1.47 and 1.61 respectively. The low pH in bark filters was close to the isoelectric point (EIP) of MS2 (3.5-3.9) and should have decreased the negative charge of MS2, reducing the repulsion between the negatively charged HA and promote the transport of MS2 (Bradley et al., 2011). This result is in agreement with that of Zhuang and Jin (2003), where the presence of HA had significantly promoted the transport of MS2 and by Bradley et al. (2011) where the adsorption of MS2 have been reduced in the presence of natural organic matter. A strong electrostatic adsorption of MS2 to HA was observed even at low concentration (Zhuang & Jin, 2003).

In comparison, there was no significant difference between the filters materials in the removal of PhiX-174. In the study by Zhuang and Jin (2003), the transport of PhiX-174was only minimally affected by the presence of HA at different concentration . PhiX-174 is less negatively charged when compared to MS2, since the surface charges of viruses plays a considerable role in their sorption aptitude (Michen & Graule, 2010).

5.4 Filters features that promote the removal
As the bark and activated charcoal filters were more porous than bio-char filter (Table 1), the water velocity should be lower and consequently the fractional reduction of motile bacteria as *Salmonella* spp. should decrease, which has been observed by Camesano and Logan (1998). At low velocity the motile bacteria can avoid attachment to filtration media. In the less porous bio-char filter, the velocity will take over the motility of the bacteria and enhance the collision of those bacteria with the filtration media.

The fact that the bio-char filter was significantly more effective in the reduction of *Salmonella* spp. indicate that the properties of the bacteria had weighed more in the removal than that of filters materials in this study.

5.5 Filters performance with time

The filters were run for twenty weeks, ten weeks without added wastewater and ten weeks with added wastewater. The filters likely need to be in use for more time to see whether the introduction of antagonist organisms by the addition of real wastewater to the synthetic GW will improve their performance. The active bio-control have been experienced by Leverentz et al. (2006) with food-borne pathogens on apple, the population of *Salmonella enterica* serovar Poona have reduced by 2 log units after five days storage at 25 °C. A high inoculum level of cultivable native antagonist organisms in the filters is a path that could be explored for improved pathogen reduction.

The addition of natural wastewater to the artificial GW had probably promoted the formation of biofilm in all filters and even out the performance of the filters in reduction of *E. faecalis* and make the BC filters more effective in reduction of *Salmonella* spp compared to the experiment conducted by Ramiyeh (2013).

5.6 Possible improvements to the system

Because of the difficult to find a single filter that is effective in reduction of all chemicals or/and all microorganisms present in GW, the set-up of the upgrading system should be revised by introducing a sequential or step-by-step filtration by different materials for different microorganisms. For example, the iron oxide amended bio-sand filter has performed a high remove of MS2 observed by Bradley et al. (2011). The most physically effective filters could be used in sequence with the most biologically ones, to have a high overall upgrading status a sequential filtration starting with activated charcoal filters followed by bio-char filters can probably result in an enhanced removal rates compared to using a single filter media.

Two parallel and comparative studies on upgrading of real and synthetic GW from the same place (same country for example) and different regions (arid and water ample areas) can elucidate the features of GW that promote or impede its upgrading.

The systematic use of tracers in the process of filtration is recommendable because it can facilitate finding the exact connection between the inflow and the effluent in terms of time; i.e. whether there is a definitive microbial removal or just a delay in release to the effluent.

6. Conclusion

This study was set up to investigate the upgrading potential of onsite bio-filtration. It emerged that there is a varying efficacy of filters media according to pathogen and microbial indicators and macro and micro nutrients present in the artificial GW.

The bark filter was relatively good in the removal of *Salmonella* spp, with more than 2 $\log_{10}$ reduction, no significant difference between filters in removal of *E .faecalis* was established, but the bark filters perform less in removal of MS2.

All the filters have been effective in the reduction of COD, in particular the activated charcoal filters with an average of 96% reduction. The effectiveness of the bark and bio-char filters in COD reduction improved during the experiment.

Some filters media can contribute to decrease the quality of GW in certain aspects; the acidifying effect of bark filters is an illustration.

The results of this study indicate that the optimal reduction can be reach by the filter material having the physical and environmental characteristics that promote the adhesion of the microorganisms to surfaces. In view of the diversity of common pathogens found in GW, it can be difficult to find a single filter material that is effective in the reduction of all those pathogens. More studies are necessary to investigate the fate of the biofilm, whether there is a definitive degradation of microorganisms in the filters or just a delay of the release but also the long term performance of the filters.

The designing of a filtration set-up should start by the understanding of the features of the pathogens of interest that promote the biofilm formation and then find the filter material that avail those features. One possibility to explore is the establishment of natural antagonist microorganisms existing in GW. Those organisms can, by competition for nutrients and place, by inhibition or drastic changes of the environment (pH for example) reduce the pathogenic population.

A close collaboration with the food industry can be a huge source of inspiration regarding the attachment conditions of bacteria on surfaces because of their long experience in research for avoiding biofilm formation.

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