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Rening av avfallsvatten från ett slakteri med fokus på bakteriella patogener

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SAMMANFATTNING

Denna studie genomfördes på ett slakteri i centrala Kampala, Uganda för att utvärdera reningsprocessen, med avseende på bakteriella patogener, av en integrerad bioprocess i pilotskala. Denna hade fyra reningssteg: anaeroba sekvensreaktorer; aeroba/anoxiska sekvensreaktorer; en högpresterande och hög temperaturbiogasanläggning och en konstruerad våtmark. Målsättningen med denna studie var att se ifall denna typ av rening på ett tillfredställande sätt kan rena avfallsvatten från slakterier.

Indikatororganismerna för fekal kontamination, *Escherichia coli* och *Enterococcus* spp. användes för att utvärdera i vilka nivåer bakterier fanns i det obehandlade vattnet och i vilken utsträckning nivåerna minskade i de olika reningsstegen. Vattenproverna analyserades för *Salmonella* för att se ifall humana patogener kunde överleva de olika reningsprocesserna. Antibiotikaresistensanalyser gjordes även på *E. coli*-stammarna för att se ifall det fanns någon antibiotikaresistens och ifall det fanns någon variation av denna parameter i de olika stegen av rening.

Fem prover av det obehandlade vattnet och från de olika reningsstegen samlades vid fem separata tillfällen. Resultaten från studien visade att ovanstående typ av rening verkar kunna minska mängden bakterier till en stor utsträckning, dock har inte mängden sjunkit tillräckligt för att man ska kunna använda vattnet för bevattning av ätbara grödor eller som dricksvatten. Det viktigaste reningssteget verkar vara de anaeroba sekvensreaktorerna.

Ingen *Salmonella* kunde påvisas men den humana patogenen *Shigella* kunde konstateras i flera prover, däribland från de sista reningsstegen. Endast i bakteriestammarna från den konstruerade våtmarken påvisades höga nivåer av antibiotikaresistens. Då våtmarken inte fungerat som den är konstruerad för under denna studie är det svårt att uttala sig om relevansen av detta resultat rörande antibiotikaresistens.

SUMMARY

This study was conducted at the City Abattoir in Kampala, Uganda to evaluate the cleaning process, with focus on bacterial pathogens, in a pilot scale integrated bioprocess. This consisted of four steps: anaerobic sequencing batch reactors; aerobic/anoxic sequencing batch reactors; a high performance temperature controlled methanogenesis digester and a constructed wetland. The objective was to determine if this type of cleaning process could be used to clean wastewater from abattoirs in a satisfactory way.

The indicator organisms for faecal contamination, *Escherichia coli* and *Enterococcus* spp. were used to investigate in what numbers bacteria could be found in the untreated wastewater and how much that was reduced in the different steps of the cleaning process. The water was analysed for *Salmonella* to see if human pathogens could be found and could survive the different steps of the treatment process. Antibiotic resistance analyses were also performed on the *E. coli* samples to see if any resistance could be found and if there was any variation of this parameter in the different treatment steps.

Five samples from the untreated water and from the different treatment steps were collected at five different occasions. The results from the study was that it seems that this type of cleaning plant can be used to decrease the numbers of bacteria in wastewater from abattoirs to a great extent though further decrease in bacterial numbers are needed before the water could be used for irrigation of edible crops or drinking. The most important cleaning step seems to be the anaerobic sequencing batch reactors.

No *Salmonella* was found in any samples but the human pathogen *Shigella* was found in several samples including from the final steps of cleaning. High levels of antibiotic resistance were only found in the isolates from the constructed wetland. It is hard to draw any conclusions from this result since the constructed wetland hadn’t been functioning as it was supposed to be when this study was conducted.

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NOTATION

BHI	Brain heart infusion
BOC	Biodegradable organic compounds
BOD	Biological oxygen demand
BPW	Buffered peptone water
CAMHB	Cation adjusted Mueller Hinton broth
CFU	Colony forming units
HPTC	High performance temperature controlled
HSSF	Horizontal subsurface flow
MIC	Minimal inhibitory concentration
MSRV	Modified semi-solid rappaport vassiliadis base
SBR	Sequencing batch reactor
SLABA	Slanetz & Barley medium
TSA	Trypsin soy agar
XLD	Xylose lysine deoxycholate

INTRODUCTION

A massive increase in demand for food of animal origin is taking place in developing countries. This is called “The Livestock Revolution”. The reasons for the increased demand are urbanization, income and population growth. (Delgado et al. 1999)

When the production of food of animal origin is increased, the health risks connected to livestock production are also increased. Some of the health risks connected to “The Livestock Revolution” are microbial contamination from foods handled in an unsafe way, animal-borne diseases, for example *Salmonella* and avian flu, and an increased concentration of antibiotics and pesticides higher up in the food chain. (Delgado et al. 1999)

Drinking water is a foodstuff that is at high risk for contamination and millions of people are drinking water with dangerous levels of chemical pollutants and biological contaminants due to unsatisfactory cleaning of wastewater of agriculture, urban or industrial origin (WHO 2013). Industries such as slaughterhouses, tanneries and dairy plants produce wastewater that has a great impact on the environment (Verheijen et al. 1996).

Nature can cope with small amount of waste that is produced in small-scale livestock production by different natural cleaning process mechanisms. But when the production is increased the natural cleaning processes is overburdened and problems with pollution start to occur. Methods to make better use of by-products and improve waste handling must be found. (Verheijen et al. 1996)

The objective of this study was to evaluate the treatment process in a pilot scale integrated bioprocess consisting of four different steps, at an abattoir in the centre of Kampala, Uganda. The focus was on bacterial pathogens and the indicator organisms for faecal contamination, *Escherichia coli* and *Enterococcus* spp., were used to investigate in which numbers bacteria can be found in the untreated wastewater and how much is reduced in the different steps of the treatment process. The water was also analysed for *Salmonella* to determine if human pathogens can be detected at different steps of the wastewater treatment process. Antibiotic resistance analysis were carried out on the *E. coli* isolates to see if any resistance could be found and if there was any variation of this parameter in the different treatment steps.

An overall question with this study was also if this type of water treatment process can be used in other developing countries to improve the water quality of abattoir wastewater in a robust and economically sustainable manner.

LITERATURE REVIEW

Slaughter waste

The slaughter sites in developing countries are built in a wide variety of ways. They may vary from very modern slaughterhouses to simple slaughter slabs. The slaughter sites often lack the means to take care of the waste products; even the modern slaughterhouses have often improper waste treatment facilities. This means that many slaughterhouses pose threats to environmental health due to lack of proper sanitary techniques. The risk is especially significant if the slaughter site is in close proximity to highly populated areas. (Verheijen et al. 1996)

Different sources of waste in red meat slaughter can be categorized as animal pens; bleeding; carcass processing; offal and by-products processes and processing. The different types of waste can be categorized as solid, liquid and gaseous. (Chukwu 2008)

Solid waste is a by-product that can't be used in any way and must be dumped. Gaseous waste is air pollution such as methane gas or CO_2 and can have an impact on the environment as global warming, changes in the ozone layer or negative health conditions. (Verheijen et al. 1996)

Wastewater in the slaughter of pigs and cattle is produced at the washing of the carcasses when the hair or skin is removed and after the evisceration, washing of the slaughterhouse facilities and equipment. In excess of the wash water, liquid waste also includes remnants from the animal such as urine, faeces, viscera and blood. (Mittal 2004)

The quality and risk hazards of the wastewater depends on a number of factors (Massé & Masse 2000), among other things how much of the blood that is captured. Blood retention is considered the most important method to reduce biological oxygen demand (BOD) (Tritt & Schuchardt 1992). Of importance is also how much water that is used (if less water is used the pollution concentration is higher) (Massé & Masse 2000) and which type of animal that is slaughtered (BOD is higher in wastewater in the slaughter of cattle than of pigs) (Tritt & Schuchardt 1992).

The wastewater can affect the water quality in three ways: compounds that can be directly lethal to aquatic life, biodegradable organic compounds (BOC's) that can reduce the amount of dissolved oxygen and macro-nutrients such as nitrogen and phosphorus that can cause eutrophication (Verheijen et al. 1996). The wastewater may also include pathogenic microorganisms that normally exist on the hides or in the digestive tract of the animal (Mittal 2004).

Experiments performed at Bodija abattoir, Ibadan, Nigera, have shown that pathogenic organisms from the wastewater can find their way to dug out wells with drinking water in the vicinity of the abattoir. This shows that a satisfactory cleaning of wastewater must be

performed to avoid risks with drinking water and to secure that it is safe for human consumption. (Adeyemo et al. 2002)

Bacterial pathogens

When analysing for pathogens such as viruses, bacteria and parasites the direct detection is often a time-consuming business that requires lots of money and well-trained personnel. Therefore the use of indicator organisms that indicate faecal contamination is practiced. These are also being used to evaluate the effect of the cleaning process of contaminated water. (Bitton 2010)

An ideal indicator organism should be (Bitton 2010):

- non-pathogenic so that it can be handled by the analyst without the risk of sickness
- present in greater numbers than the pathogens
- present when the pathogen is present and absent when the pathogen is absent
- present in the intestines of warm-blooded animals
- at least as stable as the pathogen in the environment and cleaning process
- it should not multiply in the environment
- easy to detect

Some of the indicators that are commonly used are: Total coliforms, fecal coliforms, fecal streptococci, *Clostridium Perfringens, Salmonella, Shigella* and *Escherichia coli.* (Bitton 2010)

Faecal streptococci are a group of different streptococci types. They also have a subtype that is called *Enterococcus* spp. Faecal streptococci are often used to detect faecal contamination in water since they commonly inhabit the intestines of warm-blooded animals and humans. The subgroup *Enterococcus* spp. has been suggested to be a good indicator for the presence of viruses, especially in seawater and biosolids. (Bitton 2010)

Salmonella is a widely distributed Enterobacteriacae with over 2000 different serotypes (Bitton 2010). *Salmonella* can cause gastroenteritis, typhoid and paratyphoid fever (Bitton 2010). In the United States of America, people become sick from *Salmonella* mainly by food poisoning but the transmission to drinking water is still of major concern (Bitton 2010). *Salmonella* has been suggested to be one of the pathogens most likely to be spread with animal slurry (Jones 1979).

Escherichia coli is a facultative anaerobe that naturally occurs in the digestive tract of humans and warm-blooded animals (Bitton 2010). Many types of *E. coli* are non-pathogenic but there are several types that can be pathogenic to humans (Bitton 2010). Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a strain of *E. coli* that can cause severe cases of hemorrhagic colitis and can be associated with mortal systemic manifestations such as haemolytic uremic syndrome (HUS) (Ferens & Hovde 2011). The primary reservoir of *E. coli* O157 in the environment is cattle and possibly small domesticated ruminants (Ferens &

Hovde 2011). The ways of transmission to humans can be by dairy products and meat that is contaminated and not properly prepared, surface and drinking water, fruit juice and produce (Ferens & Hovde 2011). Contact with manure or animals and person-to-person contact can to a lesser extent also be ways of transmission (Ferens & Hovde 2011).

Listeria monocytogenes and *Campylobacter jejuni* are examples of other bacterial pathogens that can be found in the intestinal tract of animals and may cause sickness in humans due to ingestion (Mittal 2004).

The wastewater produced at abattoirs has been shown to contain several millions of coliform units (CFU)/100ml of fecal coliform, total coliform, and *Streptococcus* groups (Mittal 2004).

Different international standards and guidelines exist to give criteria of how much of different indicator bacteria that are allowed in irrigation water for crops that are likely to be eaten raw. The criteria for WHO is ≤ 1000 CFU/100ml of faecal coliforms (WHO 1989).

Antibiotic resistance

Antibiotics are the most important drugs in the treatment of bacterial infections. The increased usage of antibiotics in animal and human medicine, increased industrialisation and increased possibilities to travel around the world have resulted in that the threat from antibiotic resistance is worse than ever. (Hawkey & Jones 2009)

Bacteria with antibiotic resistance in the intestines of animals may after the slaughter of the animals be ingested by humans due to contamination of foodstuff (Van den Bogaard & Stobberingh 2000). These bacteria may cause sickness directly if they are pathogenic to humans or indirectly if they are a reservoir of antibiotic resistance with the possibility of transferring the resistance genes to possibly pathogenic bacteria (Van den Bogaard & Stobberingh 2000). Antibiotic resistance genes in water worldwide are a growing problem and the use of antibiotics in animal production is a big contributor (Zhang et al. 2009).

Water cleaning process

There are a number of different methods that can be used in the process of cleaning slaughterhouse wastewater (Johns 1995; Massé & Masse 2000).

Sewer discharge of the wastewater without any preliminary treatment can be used by small slaughterhouses that are in close proximity to a water cleaning plant that will process the sewage water (Massé & Masse 2000).

Primary treatment such as filtration, settling and dissolved air flotation are methods that are often used to remove fats, greases and suspended solids from the wastewater (Johns 1995).

Post-primary treatment can be performed in a number of ways. Which way often depends on the location (Johns 1995). Post-primary treatment can be categorized in secondary treatment where organic materials are removed in biological processes and tertiary treatment where suspended or dissolved substances such as phosphorus and nitrogen are removed (Mittal 2006). The different treatment steps can overlap depending on how the cleaning plants are designed. In the case of secondary treatment using aerobic/anoxic reactors removal of nitrogen and phosphorus takes place before the tertiary treatment (Odong et al. 2012).

Aerobic treatment performed by microorganisms, leads to an aerobic digestion and organics are degraded in the presence of oxygen (Massé & Masse 2000). Aerobic biological-treatment systems can be used in different ways (Johns 1995; Massé & Masse 2000). Constructed wetlands consist of different chosen soils and plants that are used to clean the wastewater. They often have the advantages of low operational cost, simplicity and low energy requirements (Johns 1995). Aerobic lagoons are large shallow basins that use algae for wastewater treatment in combination with other microorganisms (Massé & Masse 2000). Activated sludge systems are systems where a constant recycling of a small part of the sludge is put back into the aeration basin, examples of such systems are sequencing batch reactors and oxidation ditches (Massé & Masse 2000). Trickling filters use a bed of highly permeable media with an attached microbial flora. Wastewater is poured over the bed system (Massé & Masse 2000).

Anaerobic treatment is performed by microbes but without the presence of oxygen and degrades organics into methane (Massé & Masse 2000). Different anaerobic systems exist and some examples are anaerobic lagoons, where wastewater flows in near the bottom of the lagoon with a scum on top to secure the anaerobic conditions and high-rate anaerobic reactors that reduce the required area and accelerate the treatment time (Massé & Masse 2000).

Concerning the bacterial load in wastewater, there are a number of factors that influence the decay and potential for growth of viable bacteria: temperature, pH, volatile fatty acids, treatment time, nutrients available and bacterial species (Sahlström 2003).

MATERIALS AND METHODS

The City Abattoir in Kampala

The City Abattoir in Kampala is situated near the centre of the town in the eastern industrial part of the city. The slaughterhouse was built in 1935 by the British Colonial Government and has not been modified much since. About 200 goats and sheep, 500-700 cattle and also a number of chickens are slaughtered daily. The killing and bleeding takes place outside on a concrete platform and the carcasses are then moved inside for processing. None of the blood is collected and all the wastewater from the slaughter site is washed down into Nakivubo channel that eventually drain into the lake Victoria at the inner Murchison Bay. Lake Victoria is the second largest freshwater lake in the world and is an important source of food (fish), irrigation and drinking water but is also a common place to dump wastes from industries, agriculture and domestic sources (Kayombo & Salaam 2005). Studies have shown that abattoirs are one of the major pollutants of wastewater into Lake Victoria (Kyambadde et al. 2006).

The water cleaning plant

The water cleaning process at the City Abattoir in Kampala is a pilot scale integrated bioprocess and has been developed by the Department of Biochemistry and Sports Science (Makerere University) under Bio-Innovate Africa Project. The Swedish International Development Agency (SIDA) funds the project. It consists of four different steps. The first two steps include a number of sequencing batch reactors (SBRs). The first SBRs have an anaerobic process and the second an aerobic/anoxic process. The third step consists of a constructed wetland planted with *Cyperus papyrus.* After the wetland, the treated water is released into the channel. An alternative route for the water includes a High performance temperature controlled (HPTC) methanogenesis digester producing biogas. The digester collects water from the anaerobic SBRs and releases the treated water into the channel without further processing either in the aerobic/anoxic SBRs or the wetland.

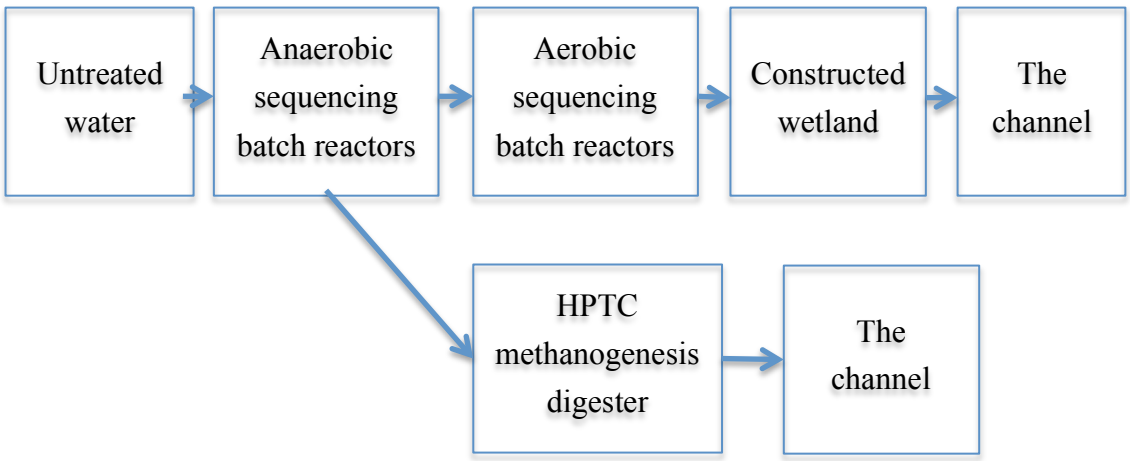

Figure 1. Flowchart over the water flow in the cleaning plant.

Untreated wastewater from the abattoir is pumped from a collection chamber first to the anaerobic SBRs. Anaerobic treatment is performed by microbes in the absence of oxygen and degrades organic matter into methane and other by products. The residence time in the tank is 48 hours before the water is pumped to the aerobic/anoxic SBRs.

In the aerobic/anoxic SBRs organic matter are removed by aerobic degradation; nitrogen removed by nitrification-denitrification processes and phosphorus removed by phosphorus accumulating microorganisms and sludge wastage. The treatment time is 32 hours.

Both the anaerobic and aerobic/anoxic SBRs were inoculated with sludge from reactors treating breweries wastewater at the start of the experiments in April 2011 (Odong et al. 2012). The sludge is used to get the correct microbial communities for the microbial process. The sludge retention time is 60 days in the anaerobic SBRs and 7 days in the aerobic/anoxic SBRs. The anaerobic and aerobic/anoxic SBRs can be seen in Figure 2.

Figure 2 (Johan Thorell 2013). The anaerobic sequencing batch reactors (on top) and aerobic/anoxic sequencing batch reactors (below).

After the aerobic/anoxic SBRs the water is pumped to a constructed wetland with a horizontal subsurface flow (HSSF) planted with *Cyperus papyrus* in gravel. The wetland is supposed to remove residual organic and inorganic matters through different processes including filtration, adsorption and absorption, precipitation and sedimentation. The idea is that the water flow should be constant through the wetland but during this study the drainage pipe from the wetland had been closed since the *Cyperus papyrus* had started to wither due to lack of water. The water had been stagnant for some weeks when this study was conducted. The constructed wetland can be seen in Figure 3.

The High performance temperature controlled (HPTC) methanogenesis digester was when this study started just recently installed. It had been fed with water from the anaerobic SBRs for two months and none of the water had been regularly released back into the channel. To increase the production of biogas anaerobic bacteria were added regularly into the digester. The biogas produced in the digester is stored in a big gas bag on the cleaning plant premises. The biogas bag can be seen in Figure 4.

Figure 3 (Johan Thorell 2013). The constructed wetland with Cyperus papyrus and Hydroponic system planted with Collard Greens.

Figure 4 (Johan Thorell 2013). The biogas bag.

Collecting samples

Water samples were collected at five different occasions. All samples were collected in the morning hours around 08.00-10.00 when the slaughter was finished and the slaughter site, and processing areas were being washed.

The samples were collected in sterilized glass sample bottles. The untreated water was collected from the channel prior to the site where water was being pumped to the cleaning plant. Water from the anaerobic SBRs was collected from the effluent water that was being released into the aerobic/anoxic batches. The samples from the aerobic/anoxic SBRs were collected directly from the open batches. The samples from the wetland and the digester were collected separately from the effluent treated water that was released into the channel.

No consideration of the processing time in the different steps in the cleaning process was taken when collecting the samples. No new untreated water had been refilled in the anaerobic SBRs for at least 24 hours when the samples were collected.

Choices of indicator organisms

In this study *Escherichia coli* and *Enterococcus* spp. were chosen to be used as indicators of faecal contamination and to evaluate the effect of the cleaning process by quantifying the bacterial pressure.

Methods for detection of *Salmonella* were used to show if bacteria that can be directly pathogenic to humans could be found in the untreated water and if these pathogens also could be found in the treated water. The objective was to determine if the cleaning plant effectively cleans the water from these pathogens.

Dilution and filtration

After the water samples had been collected they were brought to the laboratory for analysis the same day. The samples were transported in a cooling bag. For the analysis of *E. coli* and *Enterococcus* spp., dilution series were performed in duplicate and 10 ml of the samples were mixed in 90 ml dilution liquid, consisting of 8.5 g NaCl and 1 g peptone per litre of distilled water. The dilution series were made in a number of steps. The first series dilutions were made from 1/10 to 1/100 000 for the untreated, anaerobic, aerobic/anoxic and digester samples and 1/10 to 1/1000 for the wetland sample. The whole volumes of four of the dilutions for each sample were then filtrated through filters with a pore size of 0.45 µm. The filtration equipment created a negative pressure with a hand pump. The choices of four dilution steps to be used in the first series were based on earlier studies done locally with the same equipment. The dilution steps 1/100 to 1/100 000 for the untreated, anaerobic, aerobic/anoxic and digester samples and the undiluted to 1/1000 for the wetland sample were filtered and the filtration papers were put on agar plates. For the following four series the dilution steps were modified based on the results from the first series. Only three dilutions were then filtered.

Analysis of *Escherichia coli*

After the filtrations, one filtration paper per plate was put on plates with Lactose TTC agar with Tergitol-7. The plates were then incubated at 44°C for 24±2 hours. After the incubation the plate with the easiest countable amount of colonies were selected and the number of colonies with a typical *E. coli* appearance (yellow or orange) were counted. The numbers were then transformed into colony forming units/100ml (CFU/100ml). Five colonies with a typical appearance were picked with 1 µl loops and streaked on Trypsin Soy Agar (TSA) plates divided into five fields. The plates were then incubated at 37°C over night. Oxidase- and Indol-tests were then performed on colonies from the TSA-plate to confirm that the colonies were truly *E. coli*, with a negative oxidase reaction and a positive indol reaction.

Colony materials from the TSA-plate were collected with 10 µl loops and mixed with 1.5 ml BHI-freezing broth in cryo tubes for the transport to Sweden for the antibiotic resistance tests. The cryo tubes were vortexed and stored in a -20°C freezer in waiting for the transport. The BHI-freezing broth consisted of 830 ml Brain heart infusion (BHI) solution and 170 ml Glycerol per litre finished broth.

Analysis of *Enterococcus* spp.

After the filtrations, one filtration paper per plate was put on plates with Slanetz & Barley medium (SLABA). The plates were then incubated at 44°C for 48±4 hours. After the

incubation the plate with the easiest countable amount of colonies was selected and the filtration papers were moved to Bile Esculin acid agar plates. The plates were incubated for 2 hours at 44°C. The colonies that changed colour to a black appearance were then counted and the numbers were transformed into CFU/100ml.

Analysis of *Salmonella*

As much as possible of the undiluted water samples were filtered through a filter with a pore size of 0.45 µl. The plan was to filtrate 300 ml of the undiluted water but the total amount that could be filtered before the filters were clogged ranged from 10-40 ml. The filters were then moved into bottles with 50 ml of Buffered peptone water (BPW) for pre-enrichment and incubated at 37°C for 18±2 hours. After the incubation, 100 µl from the broth was distributed into three droplets on a modified semi-solid rappaport vassiliadis base (MSRV) agar plate and incubated for 24±2 hours at 41.5°C. If there was a suspected growth of *Salmonella* with an opaque swarming around the colony, 1 µl loops were dipped in the outer rim of the swarmings and streaked on Xylose lysine deoxycholate (XLD) agar plates with 10 µl loops. The XLD-plates were then incubated at 37°C for 24±3 hours. After the incubation, if there was growth of colonies with a cerise or black colour, five of them were picked with 1 µl loops and re-streaked on purple agar plates divided into five fields and incubated for 24 hours at 37°C. If there was no yellow colour change on the agar, which meant that the colony was negative to lactose-fermentation, a colony was picked with a 1 µl loop and mixed with 5 ml distilled water in a test tube. The mixture was then vortexed and used in an API 10S test. In series 1-4 two API tests were used on each purple agar plate but in serie 5 only one API test was used per plate. The tests were incubated for 18-24 hours at 37°C, the last reagents were then added and the results were noted.

Analysis of Antibiotic resistance

The *E. coli* isolates were transported to Sweden in an unbroken cold chain. With 1 µl loops material from the still frozen isolates were collected and streaked on cattle blood agar plates with 10 µl loops. The plates were incubated at 37°C for 24±2 hours. One colony from each isolate on the blood agars were collected with 1 µl loops and re-streaked on new cattle blood agar plates with 10 µl loops. The plates were then once again incubated at 37°C for 24±2 hours.

The method used to perform the antibiotic resistance analysis was the VETMIC™ GN-mo panels. They are MIC-based (Minimal Inhibitory Concentration) broth micro dilution panels for antimicrobial susceptibility testing of Gram-negative bacteria.

The technique of direct colony suspension inoculum was used. Five colonies from the cattle blood agar plates were collected with 1 µl loops and suspended in 4 ml sterile 0.9% NaCl. The suspensions were vortexed and 20 µl of the suspensions were transferred to 10 ml of cation adjusted Mueller Hinton broth (CAMHB). The new solutions were vortexed and then poured out in empty petri dishes. With a multi-pipette the solutions were portioned out with 50 µl in each well of the VETMIC™ GN-mo panels. The wells were sealed with transparent

covering tape and the panels were incubated at 35-37°C for 18 hours. For each panel, 10 µl of the inoculum were streaked on cattle blood agar plates for purity control. The agar plates were incubated at 37°C for 18 hours.

To verify the density of the inoculum, 10 µl of the inoculum from one of the isolates halfway through the analysis and 10 µl of the inoculum from the last sample were diluted in two separate test tubes with 10 ml 0.9% NaCl. 100 µl from each of the vortexed solutions were spread on cattle blood agar plates and were incubated at 37°C for 18 hours together with the other agar plates. After the incubation a viable count was made.

To control the function of the test a quality control organism was included in the analysis. The *E. coli* control with known MIC-values was analysed the same way as the other isolates and the results from the resistance pattern were compared and confirmed with the known pattern.

The reading of the results was done by placing the panels on a viewing device with a light source and enlargement mirror placed so that the under side of the panel could be seen. Bacterial growth was detected as a pellet in the bottom of the well or increased turbidity.

The MIC was read in the well with lowest concentration with no visible growth. According to recommendations from the manufacturer of the test was the MIC read in the well with lowest concentration with less than 20% growths in the wells with sulphonamides. The judgement of which wells that had no growth or growth with less than 20% in the sulphonamides wells were done with ocular estimation.

RESULTS

Escherichia coli

The numbers of *E. coli* in the water samples from the different steps is presented in Table 1. The sample amount was too small to make any meaningful statistical analysis. Only values from sampling occasions 1-4 are accounted for concerning the aerobic samples. In sampling occasion 5 of the aerobic samples, all the filtration papers were too thick with colonies to be able to be counted.

Table 1. Escherichia coli numbers. Values in CFU/100ml

	Untreated	Anaerobic	Aerobic/anoxic	Wetland	Digester
Sampling occasion 1	$6.6x10^6$	$1.8x10^5$	$8.9x10^3$	$8.9x10^3$	$1.8x10^6$
Sampling occasion 2	$1.0x10^8$	$1.2x10^5$	$6.0x10^4$	$1.1x10^5$	$1.0x10^7$
Sampling occasion 3	$9.0x10^7$	$4.7x10^6$	$1.0x10^4$	$7.8x10^4$	$1.2x10^6$
Sampling occasion 4	$3.3x10^7$	$1.1x10^4$	$5.0x10^4$	$5.6x10^4$	$1.8x10^6$
Sampling occasion 5	$6.0x10^7$	$1.0x10^7$	$>10^6$ (Removed from median and mean values)	$1.2x10^4$	$2.7x10^6$
Median	$6.0x10^7$	$1.8x10^5$	$3.0x10^4$	$5.6x10^4$	$1.8x10^6$
Mean value	5.8×10^7	$3.0x10^6$	$3.2x10^4$	$5.3x10^4$	$3.5x10^6$

Table 2 shows the log reduction of *E. coli* based on the median and mean values of the water samples. Note that sampling occasion 5 from the aerobic/anoxic SBR:s is removed from the calculations of the median and mean values. The log reduction shows how much of the total numbers of *E. coli* that are removed in the different cleaning steps. The removal is high in both the anaerobic and aerobic/anoxic steps but in the effluent water from the digester an increase of *E. coli* can be seen instead of a decrease based on the median values.

Table 2. Log reduction of Escherichia coli based on median and mean value.

	Anaerobic	Aerobic/anoxic	Wetland	Digester
Log reduction (based on median)	2-log reduction	1-log reduction (without sampling occasion 5)	0-log reduction/increase	1-log increase
Log reduction (based on mean value)	1-log reduction	2-log reduction (without sampling occasion 5)	0-log reduction/increase	0-log reduction/increase

In total 121 out of 122 colonies tested for the indol reaction were positive and 117 out of 122 colonies tested for the oxidase reaction were negative which shows that the colonies that were counted on the Lactose TTA-plates with a high security most likely was *E. coli*.

Enterococcus spp.

The numbers of *Enterococcus* spp. in the water samples from the different steps are shown in table 3. The sample amount was too small to make any meaningful statistical analysis. Only values from sampling occasions 1-4 are accounted for concerning the anaerobic samples. Confirmations of the suspected *Enterococcus* spp. colonies were not possible due to lack of Bile Esculin agar plates.

Table 3. Enterococcus spp. numbers. Values in CFU/100ml

	Untreated	Anaerobic	Aerobic/anoxic	Wetland	Digester
Sampling occasion 1	$5.2x10^6$	$1.1x10^5$	$1.4x10^4$	$3.2x10^3$	$6.7x10^4$
Sampling occasion 2	$4.4x10^6$	$1.1x10^3$	$1.7x10^4$	$2.0x10^4$	$2.1x10^4$
Sampling occasion 3	$2.0x10^6$	$1.6x10^5$	$8.9x10^3$	$1.8x10^4$	$1.6x10^4$
Sampling occasion 4	$2.6x10^6$	$3.3x10^4$	$1.0x10^4$	$1.2x10^4$	$1.7x10^4$
Sampling occasion 5	$2.4x10^7$	$>10^5$ (Removed from median and mean values)	$4.8x10^5$	$7.0x10^3$	$1.1x10^5$
Median	$4.4x10^6$	$7.2x10^4$	$1.4x10^4$	$1.2x10^4$	$2.1x10^4$
Mean value	$7.6x10^6$	$7.6x10^4$	$1.1x10^5$	$1.2x10^4$	$4.6x10^4$

Table 4 shows the log reduction of *Enterococcus* spp. based on the median and mean values of the water samples. Note that sampling occasion 5 from the anaerobic SBR:s is removed from the calculations of the median and mean values. The log reduction shows how much of the total numbers of *Enterococcus* spp. that is removed in the different cleaning steps. The reduction is highest in the anaerobic step with a 2-log reduction based both on median and mean values. The log reduction based on the mean values in the aerobic/anoxic step showed on an increase in numbers.

Table 4 Log reduction of Enterococcus spp. based on median and mean value.

	Anaerobic	Aerobic/anoxic	Wetland	Digester
Log reduction (based on median)	2-log reduction (without sampling occasion 5)	0-log reduction/increase	0-log reduction/increase	0-log reduction/increase
Log reduction (based on mean value)	2-log reduction (without sampling occasion 5)	1-log increase	1-log reduction	0-log reduction/increase

Salmonella

All samples that were analysed on MSRV-agar grew with an appearance possible for *Salmonella* and were streaked on XLD-agar plates. Table 5 shows how many of the samples that had *Salmonella*-like colonies on the XLD-agar. It also shows the results on the purple agar and which results that were presented on the API 10S tests. A percentage of 50% certainty was decided to be the lowest to confirm a bacterium in the API 10S test.

None of the results from the API 10S tests showed on *Salmonella*. The most common result from all the steps of the cleaning process, in total of 16 samples, was *Citrobacter freundii*. Four samples from the untreated water, anaerobic SBRs, aerobic/anoxic SBRs and digester water were identified as *Shigella spp*.

Table 5. Results from XLD, purple agar and API 10S

	Number of samples with *Salmonella*-like appearance on XLD-agar	Number of samples with no colour change on purple agar	API 10S results Number of samples and percentage of certainty for the test result
Untreated water	4/5	4/4	1 *Shigella* spp (63.5%) 6 *Citrobacter freundii* (54.9%)
Anaerobic	1/5	1/1	2 *Citrobacter freundii* (54.9%)
Aerobic/anoxic	2/5	2/2	1 *Shigella* spp *(87.1%)* 2 *Citrobacter freundii* (54.9%)
Wetland	3/5	2/3	1 *Citrobacter freundii* (54.9%) 1 *Shigella* spp (63.5%)
Digester	4/5	4/4	1 *Shigella* spp *(87.1%)* 5 *Citrobacter freundii* (54.9%)

Antibiotic resistance

In Table 6 the results from the VETMIC™ GN-mo antibiotic resistance panels can be seen. The cut-off (ECOFF) values were taken from the EUCAST MIC distribution website (European Committee on Antimicrobial Susceptibility Testing, last accessed 2013-11-11. http://www.eucast.org). Values over the cut-off were considered resistant against the antibiotic type.

The results from the viable count were that 60 Colony Forming Units (CFU) could be counted on the isolate halfway through and 35 CFU could be counted on the plate from the last isolate. The correct density of the inoculum should give 10-50 CFU which means that the density of our isolates were acceptable although a little high on the first control.

In total six isolates showed results of resistance against any type of antibiotics. One isolate from the untreated water showed resistance against Ampicillin and Ciprofloxacin. Two isolates from the anaerobic SBRs showed resistance, one against Tetracycline and the other against Kanamycin and Cefotaxime. In the three resistant isolates from the wetland, resistance against two, five and six antibiotic types were found. All three were resistant against Tetracycline and Sulfamethoxazole. Two were also resistant against Ampicillin and Trimethoprim. One was additionally resistant against Ciprofloxacin.

Table 6. Results from VETMIC ™ GN-mo panel. Values of MIC in µl/ml (S)=Sensitive (R)=Resistant

Sample	Am	Ci	Nal	Gm	Sm	Tc	Ff	Cs
Cut-off value	>8	>0,06	>16	>2	>16	>8	>16	>2
Control *E. coli*	4 (S)	0,03 (S)	4 (S)	1 (S)	8 (S)	<1 (S)	<4 (S)	1 (S)
Water 1 Untreated	4 (S)	0,06 (S)	2 (S)	0,5 (S)	4 (S)	<1 (S)	<4 (S)	<0,5 (S)
Water 1 Anaerobic	2 (S)	0,06 (S)	2 (S)	1 (S)	8 (S)	2 (S)	8 (S)	1 (S)
Water 1 Aerobic	2 (S)	0,06 (S)	4 (S)	1 (S)	8 (S)	2 (S)	8 (S)	<0,5 (S)
Water 1 Wetland	**>128 (R)**	**0,5 (R)**	16 (S)	0,5 (S)	**256 (R)**	**64 (R)**	8 (S)	<0,5 (S)
Water 1 Biogas	2 (S)	0,03 (S)	2 (S)	1 (S)	8 (S)	<1 (S)	8 (S)	<0,5 (S)
Water 2 Untreated	4 (S)	0,06 (S)	4 (S)	1 (S)	16 (S)	<1 (S)	8 (S)	<0,5 (S)
Water 2 Anaerobic	2 (S)	0,06 (S)	2 (S)	0,5 (S)	8 (S)	<1 (S)	8 (S)	1 (S)
Water 2 Aerobic	4 (S)	0,06 (S)	2 (S)	0,5 (S)	4 (S)	<1 (S)	<4 (S)	<0,5 (S)
Water 2 Wetland	<1 (S)	0,03 (S)	2 (S)	1 (S)	4 (S)	32 (R)	<4 (S)	<0,5 (S)
Water 2 Biogas	4 (S)	0,06 (S)	4 (S)	0,5 (S)	8 (S)	<1 (S)	8 (S)	<0,5 (S)
Water 3 Untreated	**32 (R)**	**0,12 (R)**	4 (S)	0,5 (S)	4 (S)	<1 (S)	<4 (S)	<0,5 (S)
Water 3 Anaerobic	<1 (S)	0,06 (S)	4 (S)	0,5 (S)	8 (S)	**32 (R)**	<4 (S)	<0,5 (S)
Water 3 Aerobic	2 (S)	0,06 (S)	2 (S)	1 (S)	8 (S)	8 (S)	8 (S)	<0,5 (S)
Water 3 Wetland	2 (S)	0,06 (S)	4 (S)	0,5 (S)	8 (S)	<1 (S)	8 (S)	<0,5 (S)
Water 3 Biogas	8 (S)	0,06 (S)	4 (S)	1 (S)	8 (S)	2 (S)	8 (S)	<0,5 (S)
Water 4 Untreated	2 (S)	0,06 (S)	2 (S)	0,5 (S)	16 (S)	<1 (S)	16 (S)	<0,5 (S)
Water 4 Anaerobic	8 (S)	0,06 (S)	8 (S)	0,5 (S)	8 (S)	<1 (S)	16 (S)	<0,5 (S)
Water 4 Aerobic	<1 (S)	0,03 (S)	2 (S)	1 (S)	8 (S)	<1 (S)	<4 (S)	<0,5 (S)
Water 4 Wetland	2 (S)	0,06 (S)	2 (S)	0,5 (S)	32 (R)	**64 (R)**	<4 (S)	<0,5 (S)
Water 4 Biogas	2 (S)	0,03 (S)	2 (S)	0,5 (S)	4 (S)	<1 (S)	<4 (S)	<0,5 (S)
Water 5 Untreated	2 (S)	0,06 (S)	4 (S)	1 (S)	8 (S)	2 (S)	8 (S)	<0,5 (S)
Water 5 Anaerobic	8 (S)	0,03 (S)	8 (S)	0,5 (S)	4 (S)	<1 (S)	8 (S)	<0,5 (S)
Water 5 Aerobic	2 (S)	0,06 (S)	2 (S)	0,5 (S)	8 (S)	<1 (S)	<4 (S)	1 (S)
Water 5 Wetland	**>128 (R)**	0,06 (S)	8 (S)	0,5 (S)	**>256 (R)**	**64 (R)**	8 (S)	<0,5 (S)
Water 5 Biogas	4 (S)	0,016 (S)	2 (S)	1 (S)	4 (S)	<1 (S)	<4 (S)	<0,5 (S)

Am	Ampicillin	Sm	Streptomycin
Ci	Ciprofloxacin	Tc	Tetracycline
Nal	Nalidixic acid	Ff	Florfenicol
Gm	Gentamicin	Cs	Colistin

Table 6. Continue. Results from VETMIC™ GN-mo panel. Values of MIC in µl/ml (S)=Sensitive (R)=Resistant

Sample	Su	Tm	Cm	Km	Ctx	Caz
Cut-off value	>64	>2	>16	>8	>0,25	>0,5
Control *E. coli*	<8 (S)	1 (S)	4 (S)	<8 (S)	0,12 (S)	0,5 (S)
Water 1 Untreated	16 (S)	1 (S)	4 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 1 Anaerobic	32 (S)	1 (S)	8 (S)	**16 (R)**	**1 (R)**	<0,25 (S)
Water 1 Aerobic	16 (S)	1 (S)	8 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 1 Wetland	**>1024 (R)**	**>16 (R)**	4 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 1 Biogas	64 (S)	1 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 2 Untreated	16 (S)	1 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 2 Anaerobic	16 (S)	1 (S)	4 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 2 Aerobic	32 (S)	1 (S)	4 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 2 Wetland	16 (S)	0,25 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 2 Biogas	32 (S)	0,5 (S)	4(S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 3 Untreated	32 (S)	0,5 (S)	4 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 3 Anaerobic	16 (S)	0,25 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 3 Aerobic	16 (S)	0,5 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 3 Wetland	16 (S)	0,5 (S)	8 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 3 Biogas	<8 (S)	0,5 (S)	8 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 4 Untreated	16 (S)	0,5 (S)	8 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 4 Anaerobic	16 (S)	0,5 (S)	8 (S)	<8 (S)	0,12 (S)	0,5 (S)
Water 4 Aerobic	<8 (S)	0,5 (S)	<2 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 4 Wetland	**>1024 (R)**	0,5 (S)	4 (S)	<8 (S)	0,06 (S)	<0,25 (S)
Water 4 Biogas	32 (S)	1 (S)	4 (S)	<8 (S)	0,12 (S)	<0,25 (S)
Water 5 Untreated	<8 (S)	0,25 (S)	4 (S)	<8	0,12 (S)	<0,25 (S)
Water 5 Anaerobic	64 (S)	2 (S)	4 (S)	<8	0,12 (S)	<0,25 (S)
Water 5 Aerobic	<8 (S)	0,25 (S)	8 (S)	<8	0,06 (S)	<0,25 (S)
Water 5 Wetland	**>1024 (R)**	**>16 (R)**	8 (S)	<8	0,12 (S)	<0,25 (S)
Water 5 Biogas	32 (S)	0,5 (S)	4 (S)	<8	0,06 (S)	<0,25 (S)

Su	Sulfamethoxazole	Km	Kanamycin
Tm	Trimethoprim	Ctx	Cefotaxime
Cm	Chloramphenicol	Caz	Ceftazidime

DISCUSSION

Due to limited time, equipment and funding, the study was limited to five samples from each treatment step, and therefore the results are relatively few to be statistically analysed. The results from the study was used to draw conclusions about this cleaning plant but can not be used to draw general conclusions due to the limited amount of samples.

Laboratory methods

The precision of the dilution series could have been improved if this study could have been continued. More dilution steps should have been done and more of the dilutions should have been filtrated. The counting of CFU in sampling occasion 5 was not possible because there were too many colonies to be able to count. Since the number of bacteria seemed to vary to a great extent more dilutions should have been filtrated to be able to get a plate with countable CFU.

The counting of CFU was based on subjective ocular judgement of the appearance of the colonies. This means that the numbers cannot considered to be highly accurate. That the plates with easiest countable numbers were chosen based on subjective judgement means that the CFU could have been different if another plate would have been chosen. Because of these possible errors the margins of error could be large. With the use of log reduction the chance of true divergence is bigger than with comparison of exact numbers.

Escherichia coli and *Enterococcus* spp

The results from the untreated water samples showed high concentrations of both *E. coli* and *Enterococcus* spp. Wastewater from abattoirs has been shown to contain high levels of different bacteria with animal origin (Mittal 2004). One out of five isolates from the untreated water showed resistance against antibiotics and it was only resistant against two types of antibiotics. This indicates that the bacteria from the slaughtered animals do not seem to be a large source of antibiotic resistance.

The results from both the *E. coli* and *Enterococcus* spp. analysis indicate that the anaerobic sequencing batch reactors decrease the number of *Enterococcus* spp. with a 2-log reduction and of *E. coli* with a 2-log reduction based on median and 1-log reduction based on mean value from the untreated water. It can thereby be concluded that the sequencing batch step are the most important one considering the water cleaning process based on bacterial content. Note that sampling occasion 5 from the anaerobic SBR:s is removed from the calculations of the median and mean values of *Enterococcus* spp.

The aerobic/anoxic step gave a decrease in the *E. coli* content. Log reduction based on the median values was 1-log reduction and 2-log reduction based on mean values. In the *Enterococcus* spp. analysis the results based on the median values showed no log reduction or increase but a 1-log increase based on the mean values. Note that sampling occasion 5 from

the aerobic/anoxic SBR:s is removed from the calculations of the median and mean values of *E. coli*.
Sample number five was collected 24 hours after the aerobic/anoxic SBRs had been refilled and thereby had not completed the cleaning process. This is a possible reason why there is an increase of *Enterococcus* spp. in the results because of a much higher concentration of bacteria than any of the other samples taken from that site. This perhaps makes the mean value unreasonably high. A larger study is needed to be able to get statistically secured results.

The wetland samples in the *E. coli* analysis showed approximately the same numbers as in the prior treatment step but in the *Enterococcus* spp. analysis there was a 1-log reduction based on mean values. The results indicate that there are no apparent decrease of *E. coli* but a reduction of *Enterococcus* spp. in the wetland area. The results from the antibiotic resistance analysis show that the *E. coli* isolates from the wetland were the most resistant. They were even more resistant than the isolates from the untreated water. Three out of five isolates were resistant against any type of antibiotics and they were resistant against multiple types of antibiotics. The fact that the water had been stagnant instead of a constant flow, with the possibility for bacteria to multiply could be a likely explanation for the increased number of resistant *E. coli* isolates. It is hard to know where the resistant strains come from but it is possible that they are contaminated from the environment. Since there is no roof over the wetland it is possible for birds to defecate into the wetland water. Weaver birds actually build their nests and roots on the papyrus inflorescence.

The samples collected from the high performance temperature controlled (HPTC) methanogenesis digester water showed a 1-log increase in *E. coli* based on median values but no reduction or increase in *Enterococcus* spp. It indicated that there is an increase of *E. coli* in the digester system. Possible explanations for the increase of bacteria are that the temperature is higher in the digester than in the environment. The temperature in the digester varies between 25-30° C and gives better conditions for the bacteria to grow. The pH in the digester is closer to neutral than in the anaerobic sequencing batch reactor and thereby most likely gives a better environment for the bacteria to grow. That there is an addition of bacteria to boost the biogas production could also be a possible explanation to the increase of *E. coli*. The bacteria that are added come from rumens of cattle and no tests have been done to see exactly which bacteria they contain. It is possible that some *E. coli* is added during the handling and feeding into the digester.

One problem in this study was the possibility to collect samples. To save time and minimize transport costs, all the samples from the different steps were collected at the same time without consideration of when the batches were fed with fresh wastewater and when they were being discharged. This could have been a major source of error in this study. The risk is that the results from this study show false high numbers of bacteria in the samples from the anaerobic and aerobic/anoxic sequencing batch reactors. A larger number of samples collected when the different batches were emptied would show a more accurate result with

smaller variations. The time when the samples were collected was based on the time when the maximum of wastewater were being released into the channel, during the morning hours when the slaughter-area was being cleaned. So at least the untreated water can be considered to show accurate values.

Salmonella

No samples in any step of the cleaning process showed positive results on the final *Salmonella* tests. It is unlikely that no *Salmonella* is present in the untreated wastewater since the water is very contaminated with faecal content from the slaughtered animals. The reason that no *Salmonella* could be found is most likely due to the ineffective filtration equipment. The plan was to filtrate 300 ml of undiluted water but only 10-40 ml could be filtrated before the filter clogged up. It is possible that *Salmonella* would be found if larger amount of water could be filtrated. Other methods exist to cultivate *Salmonella* in smaller amount of water and should be considered to be of use if the study should be redone or continued unless better filtration equipment could be available.

Although no *Salmonella* was found, other bacteria such as *Citrobacter freundii* were proven to exist in every step of the cleaning process. This is a possible indicator that the same bacteria can survive through the cleaning process. Since *C. freundii* is an environmental bacterium it is hard to draw any good conclusions as to whether it is the same bacteria that follow the water from the untreated wastewater through the cleaning process or if the bacteria is contaminated into the different batches. Both the aerobic/anoxic sequencing batch reactors and the wetland are open systems were contamination from for example birds are possible. It is more unlikely that contamination of the anaerobic SBRs and HPTC digester system are contaminated, since they are closed systems.

Shigella was the most likely bacteria in four samples with 87.1% certainty in two samples and 63.5% in the other two. There is a relatively high probability that *Shigella*, which is a human pathogen that can cause shigellosis or bacterial dysentery (Bitton 2010), can survive the cleaning process. The host animals of *Shigella* are primates including humans, which means that the bacteria most likely has been transmitted to the water with human faeces. The bacteria are thereby unlikely to have been transmitted to the water from the slaughter of animals. It is possible that workers at the abattoir or people living close to the channel defecate into the channel with untreated water and thereby transmits *Shigella.* It is although alarming that two of the samples came from the water that is released back into the channel, one from the wetland and one from the digester. This means that water contaminated with human pathogens is being released into the channel.

Conclusion

The conclusion from this study is that it seems that this type of cleaning plant can be used to decrease the number of bacteria in wastewater from abattoirs to a great extent. Although the decrease is high, the water that is released back into the channel don't reach WHO's criteria for approved irrigation water of ≤1000 CFU/100ml coliform bacteria (WHO 1989). The

numbers of coliform bacteria are most likely even higher than the numbers of *E. coli* since *E. coli* is part of the group coliform bacteria. This means that the water isn't fit for irrigation and thereby even less suited for drinking. Further cleaning steps are needed before the water could be used for irrigation or consumption.

The most important cleaning step seems to be the anaerobic sequencing batch reactors. According to Johns (1996) anaerobic systems are well suited for the treatment of slaughterhouse wastewater. The advantages over the aerobic systems according to Johns (1996) are that they produce a smaller amount of sludge, a higher degree of biological oxygen demand is removed at a lower cost and the methane-gas that is produced can also be used as fuel.

The use of biogas production is a good way to make some profit out of the cleaning process. It can otherwise be hard to motivate the management of abattoirs to build and maintain a cleaning plant. Since there is an increase of *E. coli* in the digester step perhaps the possibility to reroute the water flow so that the aerobic/anoxic and wetland comes after the HPTC methanogenesis digester. This is theoretical possible and the plans exist to reroute the water flow at this cleaning plant.

When this study was conducted the wetland was not functioning as it was supposed to do. It would be interesting to do the same analyses when the wetland has a constant flow since there is hard to draw conclusions of the importance of the results from this study.

Although bacterial content is an important parameter when evaluating a water cleaning plant, there are a number of other parameters that should be evaluated as well. Some of them are turbidity, amount of nitrogen and phosphorus and chemical oxygen demand. To make a complete evaluation of the cleaning process these and other parameters should also be evaluated.

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