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Potential to inactivate microorganisms in sewage sludge by ammonia treatment

– Different temperatures and urea additions

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Abstract

Sewage sludge is rich in nutrients and organic matter, both of which can be useful in the agricultural system. By recycling the nutrients from our excreta, a more sustainable use of nutrients in agriculture can be achieved and the use of mineral fertilizer can thereby be decreased. There are however risks associated with the use of sewage sludge in agriculture, and these must be minimized to guarantee the safety of the environment and general public. The dispersion of heavy metals, organic pollutants and pathogens are the main risk factors generally considered. The focus of this study was on the reduction of pathogens in anaerobically digested, dewatered sewage sludge. Combinations of different temperatures (28.0, 33.0, 38.0, 41.5 and 44.0°C) and additions of urea (0.5, 1.0 and 1.5% wet weight) resulted in 20 treatments that were studied for seven days. The effect from added urea and temperature on the reduction of *Enterococcus* spp., thermo tolerant coliform bacteria (TTC), somatic coliphages and *Salmonella* spp. was analyzed. Additionally the treatments effect on pH, total nitrogen (N-tot) and total ammonia nitrogen (TAN) concentrations was also analyzed. The efficiency for treatments to reach threshold values proposed for Swedish future legislations concerning the studied organisms was assessed. Treatment temperature had a positive effect on reduction of all studied organisms and added urea had a positive effect on *Enterococcus* spp. and TTC reduction. Mean log10 reduction for *Enterococcus* spp. was 2.28 and five of the treatments (0.5, 1.0 and 1.5% urea at 44.0°C and 1.5% urea at 38 and 41.5°C) reached proposed (2010) legislative threshold values. Mean log10 reduction of TTC was 4.22 and proposed threshold concentrations were reached using most treatments, only control treatments (no added urea) at 28, 33 and 38°C, and 0.5 and 1.0% urea treatment at 28°C failed to reach proposed concentrations. Mean log10 reduction of somatic coliphages was 1.27 and none of the treatments reached proposed threshold reductions for viruses. The overall thresholds for 2010 years regulatory proposal was reached for most treatments. Complete compliance with the 2013 proposal could not be verified in this study since parasite reduction was not studied, and since the model organism for viruses used was conservative. The efficient reduction observed for most of the studied organisms lead to the conclusion that the method has great potential in this field of use. When also considering the added value of increased nitrogen content in the final product, to be used as closed-loop based fertilizer, the benefits using the method seem even greater. To further enhance the method, facilitating the fulfillment of future regulations, further calibrating is needed.

Sammanfattning

Avloppsslam innehåller näring och organiskt material som kan vara till nytta som gödselmedel och jordförbättrare i jordbruksmark. Ett mer cykliskt, hållbarare system för användande av växtnäring kan uppnås om andelen mineralgödsel som används inom jordbruket minskas och ersätts med avloppsslam. Det finns risker med användning av slam i jordbruket. Halter av tungmetaller, organiska föroreningar och patogena organismer är de tre huvudsakliga riskerna som rör slamhanteringen. För att slam ska kunna användas på mark där foder och livsmedel odlas måste dessa risker minimeras. Denna studie har undersökt möjligheterna att reducera oönskade organismer i avvattnat förbehandlat avloppslam från kommunala reningsverk. Försök gjordes i 20 kombinationer av olika temperaturer (28.0, 33.0, 38.0, 41.5 och 44.0°C) och tillsatser av urea (0.5, 1.0 and 1.5% våtvikt). Varje behandling pågick i sju dagar och behandlingarnas effekt på enterokocker, termotolleranta koliforma bakterier (TTC), somatiska kolifager och *Salmonella* spp. i slammet analyserades. Behandlingskombinationernas effekt på slammets pH, koncentration av totalkväve (N-tot) och koncentration av totalt ammoniumkväve (TAN = ammonium ($NH4^{+)}$ och ammoniak (NH3)) analyserades också. De studerade behandlingarnas förmåga att nå föreslagna framtida gränsvärden för de aktuella organismerna fastställdes. En hög temperatur gav ökad reducering av alla studerade organismer. Tillsats av urea hade en positiv effekt på reducering av enterokocker och TTC. Den genomsnittliga 10log-reduceringen av enterokocker var 2,28 och de föreslagna gränsvärdena för enterokocker nåddes i fem av behandlingarna. För TTC var den genomsnittliga 10log-reduceringen 4,22 och de föreslagna gränsvärdena uppfylldes med de flesta behandlingsmetoderna. Somatiska kolifager reducerades inte till föreslagna gränsvärden med någon av de använda behandlingarna, den genomsnittliga 10log-reduceringen var 1,27. Föreslagna gränsvärden från 2010 uppfylldes med de flesta av studiens behandlingar. Gränsvärden från 2013 års förslag, där andra organismer regleras, uppnåddes inte av någon av de använda behandlingarna. Detta berodde till stor del på att virus och parasiter tagits med som reglerade organismer. Inaktivering av parasiter studerades inte i denna studie och modellen för virus-inaktivering som använts var för konservativ för att uppfylla 2013 års lagförslag. Den effektiva reduktionen av de flesta av studerade organismer leder till slutsatsen att den studerade metoden lämpar sig bra för rening av avloppsslam. Tas även det ökade gödselvärdet, som skapas då kväve blandas i slammet, i åtanke verkar metoden lämpa sig speciellt bra för att skapa ett kretsloppsbaserat gödselmedel för användning i jordbruket. Fortsatta studier behövs dock för att optimera hygieniseringsprocessen så att den kan användas för att uppfylla framtida regleringskrav.

Contents

1 Introduction

The harvest of crops in an agricultural system inevitably leads to an export of nutrients from the fields where the crops are grown. Much of these nutrients are transported to urban areas for human consumption. At the end of the line in such a system a surplus of nutrients is created, with most of the nutrient ending up in the human excreta. To avoid nutrient depletion in the fields, a balance of nutrient flows in the cropping system need to be maintained by adding nutrients so that the field import and export are balanced. On Swedish crop production farms the import of nutrients commonly consists of mineral fertilizer. If the surplus of nutrient at the end of the line such as phosphorous (P) and nitrogen (N) is not taken advantage of by recycling to the field, a linear system depending on import of new nutrients to the system is maintained. The production of mineral fertilizer is dependent on energy and therefore exposed to uncertainties and fluctuations in price. Furthermore, the availability of easily extractable nutrient fractions as e.g. phosphorus is limited. By recycling, and thus to some extent replace the mineral fertilizer, a more cyclic system can be established. About 90% of Swedish households are connected to municipal sewage systems. As a result a majority of human excreta produced in Sweden is transported to, and treated at, municipal sewage treatment plants (STPs). Nutrient rich sewage sludge is produced at these STPs in the process of cleaning the waste water before it is allowed to be discharged into the natural water course.

The annual national sludge production 2012 was 207 Gg (TS). With a mean phosphorous concentration of 26.4 g/kg (TS) the total phosphorous in the produced sludge amounted to 5.5 Gg SCB (2014d). Of the produced sludge 83% (4547 Mg P) reached accepted threshold levels for use in agriculture, however, only 23% (1300 Mg P) was used for this purpose in 2012. According to the reported figures, the use of mineral fertilizer P could potentially be reduced by about 40% if all sludge that reach accepted threshold values were to be used to its potential in agriculture. In the current situation however, only approximately 12% of mineral fertilizer phosphorous application is substituted by the use of sludge. Differences in plant availability of phosphorus in sludge and inorganic phosphorous fertilizer as well as practical factors regarding logistics and distribution may cause practical problems for replacing mineral fertilizer with sewage sludge. Such factors are only briefly discussed in this study.

The use of sludge in agriculture is beneficial in many aspects. The most obvious benefit is perhaps the fertilizing effect of the sludge. Sewage sludge contains many plant nutrients, most importantly phosphorous and nitrogen, but also micronutrients. In a Swedish environmental perspective phosphorous is important as Swedish environmental objectives specifically focus on phosphorous recycling. However, it is important to remember that sludge also contains other important plant nutrients.

The soil phosphorous content is divided in to organic and inorganic phosphorous and the distribution between these pools depends on the properties of each soil. The inorganic pool can be further divided in to three fractions: directly plant available, indirectly plant available and non-plant available phosphorous. The inorganic non-plant available phosphorous consist of phosphorous absorbed to minerals. This stable phosphorous fraction is generally the largest in Swedish soils. The slow weathering of these minerals is the main source of nutrients in natural, undisturbed soils. The indirectly plant available phosphorous fraction consists of phosphates adsorbed to surfaces of iron and aluminum oxides and clay particles as well as bound to calcium (Djodjic, 2001). The phosphates in this fraction adsorb/desorb from the surfaces, thus becoming unavailable/available for plant uptake in a process regulated by equilibrium reactions (Sims and Sharpley, 2005).

The phosphorous available for plant uptake consists of mono- and di hydrogen phosphates present in the soil solution. Due to the low solubility of phosphates these available ions represents a very small part of the total soil phosphorous content and could easily be depleted through plant uptake. However, in Swedish agricultural soils total phosphorous is generally high, and the equilibrium between the fractions is restored as phosphates are desorbed from oxides and particle surfaces. The organic phosphorous exists in living organisms, in residues of dead organisms and as soil organic matter. The soil organic matter mainly consists of humic substances, stable organic compounds that originate from decomposed organisms. The availability of phosphorous in these compounds differs depending on degree of degradation (Nardi et al., 2002). Living organisms play a significant role of the phosphorous cycle. They take up and immobilize the available phosphates as well as mineralize and solubilize organic and inorganic compounds, enabling plant uptake. Additionally plant microbe interactions are also important to enhance plant phosphorous uptake (Turner et al., 2005).

The plant availability for phosphorous added to the soil with sludge is related to the methods used in the sewage treatment plant. In Sweden, sludge phosphorous is mostly bound to calcium, iron or aluminum and thus not immediately plant available. Fertilizing with such sludge increases the soil phosphorous reserve from which plant available phosphorous can be solubilized in a longer perspective. Due to the complexity of the phosphorous cycle, the plant availability cannot be easily assessed by only analyzing the source of phosphorous input. External factors such as climate and geography as well as the inherent properties of each soil (including chemical, physical and biological aspects) and farming operations (cultivation, crop rotation, liming etc.) are important in determining the fate of the applied fertilizer.

In addition to the fertilizing effect organic material in the sludge is also beneficial for the soil as it increases soil organic matter. This improves the soil aggregation and helps the maintenance of the soil structure (Six et al., 2000). This makes the soil less vulnerable to erosion and compaction while it becomes easier to cultivate and facilitates plant root growth. The improved soil structure also increases aeration, drainage and water and nutrient retention and microbial diversity (Joshua et al., 1998, Khaleel et al., 1981). These effects are especially beneficial for soils where organic fertilizer is not regularly applied, i.e. crop production farms where animal manure is not available and forage is not grown. Additionally, application of sludge to soil has proven an increase the activity of the enzymes urease and phosphatase in the soil which are important for increasing plant availability of nitrogen and phosphorous (Pascual et al., 2002).

By comparing the inputs and outputs of nutrients in an agricultural system, a balance can be made showing eventual surpluses. Of all the sewage sludge produced in Sweden in 2010, 25% was applied on arable land (SCB, 2012). Phosphorous is mainly applied in form of animal manure, and in 2013

72% of the phosphorous applied to arable land in Sweden was from manure (SCB, 2014a). The phosphorous used on Swedish arable land was 37 Gg 2010/11 and 37 Gg 2012/13 (SCB, 2014c, SCB, 2014b). Mineral fertilizer P application amounted to 9.9 Gg 2010/11. Removal of phosphorous by harvest of crops and crop residue year 2011 was 37 Gg and additional 1.6 Gg were reported as lost through leaching (SCB, 2014b).

There are several concerns associated with the use of sewage sludge as fertilizer. One concern is the risk of spreading and accumulating heavy metals and organic contaminants in the fields used for food production, as this could potentially contaminate soil, ground water and crop. The content of unwanted contaminants in sewage sludge reflects the use of different products in the society, and sewage sludge has been indicated to be a suitable matrix for tracking the use of chemicals in society at large (Olofsson et al., 2012). The heavy metals found in sewage can, to varying degrees depending on the element, be traced back to households (food, pipes and taps, tooth fillings, laundry detergents, paint, drinking water) and industries (car washes, dentists, pipes and taps, drinking water) as well as storm water (runoff from roofs and roads) (Sorme and Lagerkvist, 2002). Organic contaminants reaching the STPs may originate from households , hospitals and industry (Olofsson et al., 2012). Other risks when using sludge as fertilizer include spreading microorganisms that may cause diseases, pathogens. Sewage sludge contains high concentrations of enteric and pathogenic organisms including *Listeria* spp, *Salmonella* spp., *Escherichia coli* among others as well as viruses (Dudley et al., 1980, Dumontet et al., 2001, Arthurson, 2008). Both the risk of spreading heavy metals and organic contaminants as well as unwanted organisms must be assessed and minimized before sludge can be used. The focus of this thesis is on minimizing the risk of spreading unwanted microorganisms by reducing their concentrations in the sewage sludge to a level considered safe for use.

Pathways for spread of pathogens can be divided in to direct and indirect contact (Kowal, 1985). Direct contact include touching the sludge or the soil where sludge has been applied as well as inhaling microbes that have become airborne during i.e. cultivation of the soil. Indirect contact can occur as a result of ingestion of water and food contaminated. Example of such ingestion can be consumption of contaminated food produced on fields where sludge has been applied or ingestion of contaminated drinking water, groundwater or recreational water. Another way of indirect contact can be through contact with pathogens or sludge transported through vectors, including insects, birds, rodents, grazing animals and pets. By inactivating the unwanted organisms at the STP site before transport and application, many of the above mentioned risks can be efficiently minimized at an early stage.

There are several methods for sanitational treatment of sludge and other fecal material. By adding ash or lime to the material, a high material pH can be accomplished. This high pH and the drying effect of the additive are both factors that help inactivation of organisms. Though, the high pH may also lead to significant losses of gaseous ammonia, leading to loss of nitrogen and consequently reduced fertilizer value of the material. Other treatment methods include different variations of heating the material to accomplish inactivation. Such methods include pasteurization, thermic hydrolysis, thermophilic composting, incineration and thermic dewatering (Vinnerås, 2013, Nordin, 2010).

Most of the sewage sludge used in Swedish agriculture is applied in the autumn since the nitrogen concentration of sludge is often not considered sufficient for spring application. When sludge is spread during spring farming operations, additional nitrogen fertilizer is often used to compensate for this lack of nitrogen. In addition to the mentioned sanitation methods there is sludge ammonia treatment. The treatment involves addition of nitrogen to the sludge in the form of urea or ammonium hydroxide

solution. This makes the ammonia method for sanitizing sludge interesting, as it increases nitrogen content of the sludge. Thus using this method, the need for compensatory nitrogen application could be avoided, saving time during spring cropping operations when timeliness is important. This thesis paper attempts to validate how ammonia treatment using different urea additions in combination with different temperatures could be used to sanitize anaerobically digested dewatered sewage sludge from two sewage treatment plants (STPs).

1.1 Aim

The general aim of this thesis was to determine the efficiency of different combinations of temperature and urea additions in reducing microorganisms in dewatered sewage sludge. Specifically the reduction of *Enterococcus* spp., thermo tolerant coliform bacteria (TTC), bacteriophages and *Salmonella* spp. was in focus of the study. The most relevant factors assumed related to the reduction were examined. Additionally the possibility of inactivating the studied organisms in compliance with future legislative thresholds was assessed. Four hypotheses were tested in this study:

1. An addition of urea-N leads to an increase of TAN concentration corresponding to the amount of added urea-N.
2. Increased temperature and additions of urea-N have a positive effect on reduction of the studied organisms.
3. The reduction of the studied organisms is mainly affected by the sludge NH_3-N concentration in combination with temperature.
4. Reduction of the studied organisms to levels complying with proposed future legislative thresholds is achievable using treatment combinations.

By finding the most efficient treatment combinations as well as the contributing factors for organism inactivation this study aim to serve as decision basis in further development of full scale treatment.

2 Background

2.1 Legislations

Current Swedish regulations do not prohibit the use of untreated sewage sludge on agricultural land as long as the soil is cultivated after application (Naturvårdsverket, 2010). However, the regulations regarding use of sludge in agriculture have been under revision for some years and in 2010 the Swedish environmental protection agency (SEPA) proposed regulations, as requested by the Swedish government, regarding the use of sludge on arable land. This proposal included threshold levels for concentrations of *Enterococcus* spp., *Escherichia coli* (*E. coli*) and *Salmonella* spp. The proposal was overruled by the government and did not take force. On request by the government, the SEPA proposed revised regulations again in 2013. In these latest proposals, treatment of sludge before application is required and the sludge quality is to be assessed by the degree of reduction achieved by treatment. One of the differences between the two proposals is that the 2010 proposal is based on the concentration of organisms while the 2013 proposal is based on the treatments efficiency in reducing organisms. Threshold values from both legislative proposals are presented in Table 1. Although no threshold values have been adopted, these proposed values give an indication of future possible restrictions and can be used as targets when developing models for future sludge sanitization.

Table *1*: Threshold levels and required reduction of microorganism content in sewage sludge for agricultural use have been proposed by the Swedish EPA at two times in recent years. Table 1 show the 2010 and 2013 legislation proposals.

	2010 proposal	2013 proposal
E. coli	<1000/g total solids	5 log10 reduction or <100 /g (ww)
Enterococcus spp.	<1000/g total solids	-
”Parasites”	-	3 log10 reduction
Salmonella spp.	0/25 g (ww)	0/50 g (ww)
Virus	-	3 log10 reduction

2.2 Ammonia treatment

The bactericidal mode of action of ammonia is not completely identified. Ammonia is highly volatile and soluble in water as well as lipids. It can pass easily through the bacterial cell membrane through diffusion at a speed 30 times faster than water (Walter and Gutknecht, 1986). Ammonia is a weak base and thus has an alkaline effect on its surroundings. Influx of ammonia to the bacteria cytoplasm leads to an increased ammonia concentration and consequently increased endocellular pH. A drastic influx of ammonia may lead to denaturation of proteins by hydrolysis, oxidation and attachment of atoms or chemical groups, breaking the protein hydrogen- and disulfide bonds (Bujoczek, 2001). Allievi et al.

(1994) concluded that using KOH and NH_4OH respectively to elevate pH resulted in a higher inactivation for the ammonium hydroxide treatment, even though the same pH was reached, a difference that was attributed to the ammonia content.

The virucidal mode of action of ammonia is also not completely known. Inactivation of poliovirus by ammonia is related to cleavage of RNA inside of the virus particle. Ammonia can freely diffuse through the virus coating and suggested inactivation mechanisms of action include stimulation of nuclease activity and alkaline hydrolysis as a result of increased pH. In turn this leads to irreversible degradation of the viral RNA (Ward, 1978, Ward and Ashley, 1977).

The ammonia (NH_3) concentration is largely controlled by three factors: temperature, pH and total ammonia nitrogen (TAN) concentration. These factors are combined in two equations (Equation 1 and Equation 2) that are used to model the NH_3 concentration (Emerson et al., 1975). As pH and temperature increase, so does the NH_3-N/TAN fraction (*f*). The addition of urea to the sludge increases the TAN concentration and, as a result, pH also increases. Measured values of temperature, pH and TAN can then be used to calculate the ammonia (NH_3) concentrations using Equation 1 and Equation 2.

Equation 1

$$f = 1/10\text{^}(pKa - pH) + 1$$

Equation 2

$$pK_a = 0.09018 + 2729.92/T$$

$$(T = absolute\ temperature)$$

Additionally, temperature affects the permeability of cell membrane (Booth et al., 1999). As higher temperature increase the permeability of the cell membrane, this may cause an increased influx of ammonia to the cell. Ammonia treatment of excreta based material has previously been proven successful by (Nordin et al. (2009), Mendez et al. (2008)) and Allievi et al. (1994) among others. The inactivation of *Salmonella* spp. and *Enterococcus* spp. in manure using combinations of temperature and ammonia content was examined by Ottoson et al. (2008). Both temperature, ammonia content and the combination of these two had a significant effect on inactivation of both *Salmonella* spp. and *Enterococcus* spp. Mendez et al. (2008) also confirmed that a higher temperature resulted in a significantly increased inactivation of both fecal coliforms and *Salmonella* spp. when treating with ammonia. When adding ammonia to the sludge, urea can be used as the ammonia source. When urea is added to sludge it is degrades to CO_2 and ammonia. This hydrolysis is catalyzed by the enzyme urease that abounds in fecal matter.

2.3 Model organisms

There is a wide range of microorganisms and potential pathogens in the fecal matter and it is not feasible to investigate the presence and inactivation of all of these. Fecal indicator organisms are organisms that can be used as indicators for fecal contamination. The presence and/or concentration of indicator organisms can be examined, giving indication of the extent of an eventual fecal contamination. Another use of the indicator organisms is to evaluate the treatment efficiency in relation to their concentrations in the treated material since they always are present at high

concentrations at start. Key fecal indicator organisms include thermo tolerant coliforms (TTC), *Enterococcus* spp. and bacteriophages. The following organisms were used in this study.

2.3.1 *Enterococcus* spp.

Enterococcus spp. are gram positive bacteria commonly used as indicator for fecal contamination. Due to its gram positive cell wall it is more resistant to external stress factors than the gram negative TTC and consequently it is a more conservative indicator for enteric pathogens. *Enterococcus* spp. bacteria are part of the genus *Enterococcus* and exist as commensal bacteria in relatively high numbers in the faeces of human and other warm blooded animals (Ashbolt et al., 2001, Zhang, 2012).They are opportunistic pathogens and are generally able to survive harsh conditions, including saline conditions as well as wide temperature (10-45° C) and pH (4.5-10) range. Optimal growth temperature is 35° C and the predominant species found in animal and human faeces is *E. faecalis* followed by *E. faecium* (Vinnerås, 2013). Due to its resilience to different conditions *Enterococcus* spp. is almost omnipresent in the environment. Antibiotic resistance is a concern regarding *Enterococcus* spp. as it has been found to develop resistance to several antibiotics (Bouki et al., 2013). The interest of *Enterococcus* spp. inactivation in sewage sludge is partly related to controlling spread of these resistant genes and bacteria in the environment (Hammack, 2012, Vinnerås, 2013).

2.3.2 Thermo tolerant coliform bacteria and *Escherichia coli*

Thermo tolerant coliform bacteria (TTC), also known as fecal coliforms, are gram negative bacteria that can produce acid and gas at 44.5°C. This group of bacteria has traditionally been used as a fecal indicator, especially for water quality. *Escherichia coli* are part of the TTC group and one of the most common enteric species in the human intestine, as a part of the normal intestinal flora. It is the most appropriate group to use for detection of fecal contamination from warm blooded animals, i.e. humans (Ashbolt et al., 2001). The majority of *E. coli* does not cause disease whilst in the gut, however there are some pathogenic strains causing gastroenteritis. Though *E. coli* mostly is harmless while in the gut, it a great cause of foodborne illness when ingested as a result of contamination (Feng, 2012). In this study it is used as a model for gram negative enteric bacteria.

2.3.3 Bacteriophages

Bacteriophages (phages) are viruses that infect bacteria, but apart from that are physiologically similar to human viruses. In this thesis they were used as a model organism for enteric virus inactivation.

2.3.4 *Salmonella* spp.

Salmonella spp. is a gram negative bacterium in the family *Enterobacteriacea* and in the genus *Salmonellae*. It is one of the most prevalent bacterial pathogens and it is problematic for use of bio solid fertilizer due to its zoonotic properties and its capability to survive and re-grow in the environment. *Salmonella* spp. can cause two types of illness, gastrointestinal illness and typhoid fever and the species of greatest concern for public health is *S. enterica* (Hammack, 2012). Presence and inactivation of *Salmonella* spp. was analyzed in this study. *Salmonella* spp. is an important organism to study as it is a pathogen that is commonly found in treated sewage sludge and it is often considered in legislations regarding use of organic waste.

3 Materials and methods

3.1 The sludge

The sludge used in this study was collected at Kungsängen STP, Uppsala and at Bromma STP, Stockholm. Kungsängen STP treat 120 500 population equivalents and 166 000 people live in its catchment area. Corresponding figures for Bromma STP are 184 000 and 328 000 respectively (Vatten, vatten, 2013, Lindh, 2013). In both STPs the sludge was stabilized and used for methane production using mesophilic anaerobic digestion. The sludge mean retention time in the digesters was 20 days at Kungsängen and 31 days at Bromma. Kungsängen STP used ferric chloride as a flocculent while Bromma STP used ferric sulfate.

Collection of sludge for the study took place during a period of eight weeks between March and May 2013. Six batches of sludge were collected, two from Kungsängen STP and four from Bromma STP. The sludge from Kungsängen was collected from the dryer centrifuge and the sludge from Bromma was on occasions collected from the centrifuge dryer and on occasions from the storage silo, as shown in Table 2. One of the centrifuges in Kungsängen was undergoing repairs during the time for sample collection. As a result the total solids (TS) were lower than normally for dewatered sludge from this STP.

Table 2: *The sewage treatment plant (STP) and site of collection for the specific sewage sludge batches are presented.*

Batch nr	STP	Collection site
1	Kungsängen	Centrifuge dryer
2	Kungsängen	Centrifuge dryer
3	Bromma	Silo
4	Bromma	Silo
5	Bromma	Centrifuge dryer
6	Bromma	Centrifuge dryer

3.2 The experimental set up

Twenty different combinations of temperature and urea concentration (treatments) were used for assessing inactivation of *Enterococcus* spp., TTC, bacteriophages and salmonella. Temperatures of: 28, 33, 38, 41.5 and 44°C were used in combinations with urea added in ratios of: 0.5, 1.0, and 1.5% (wet weight). Additionally, for each temperature a control treatment with no added urea was studied. The batches used for the different treatment replicates and their treatment codes are shown in Table 3.

Table 3: *The different combinations of added urea and temperatures used to treat the sludge. The sludge batches used for the specific treatments are presented (given in brackets) as well as the treatment code (in bold).*

urea (% weight)	Temp (°C)				
	28 (°C)	33 (°C)	38 (°C)	41.5 (°C)	44 (°C)
0	**00@28**	**00@33**	**00@38**	**00@415**	**00@44**
	(1, 3, 6)	(2, 3, 5)	(1, 4, 6)	(2, 4, 5)	(1, 3, 6)
0.5	**05@28**	**05@33**	**05@38**	**05@415**	**05@44**
	(2, 4, 6)	(1, 4, 5)	(2, 3, 6)	(1, 3, 5)	(2, 4, 5)
1.0	**10@28**	**10@33**	**10@38**	**10@415**	**10@44**
	(1, 3, 5)	(2, 3, 6)	(1, 4, 5)	(2, 4, 6)	(1, 3, 6)
1.5	**15@28**	**15@33**	**15@38**	**15@415**	**15@44**
	(2, 4, 5)	(1, 4, 6)	(2, 3, 5)	(1, 3, 6)	(2, 4, 5)

The sludge used for treatment was collected as six batches, and each of the six sludge batches was used for one incubation session consisting of ten different treatments (Table 3).The urea and temperature combinations. Each of the ten urea and temperature combinations (treatments) was tested in three replications, using different batches for each replication to cover eventual temporal and spatial variations of the sludge.

During startup approximately 110 g of wet sludge per treatment replicate was placed in a Stomacher bag® (Seward, UK). The bags were then shaken manually for homogenization and to break up larger chunks of sludge. Urea was added to the bags according to the treatment combinations given in Table 3. The bags were then shaken manually to maximize incorporation of urea into the sludge. After shaking the bags, the mix of sludge and urea was emptied into 250 ml plastic cups. The cups were closed with a lid and, additionally, silicone was used to seal the lids to prevent loss of ammonia gas. The sealed cups were put in heat incubators according to Table 3 for one week (7 days).

Four of the sealed cup lids broke during treatment due to high pressure in the cup when using sludge from batch 5. These treatment replicates were canceled and repeated using sludge from batch 6 instead. Consequently only six treatment replicates were performed using batch 5 and 14 treatment replicates were performed using batch 6. For treatments using sludge from batch 6 a special nozzle was made using a syringe and a balloon to minimize the pressure on the plastic cups and avoid leakage of ammonia gas.

3.3 Sampling

Analysis of total solids (TS), pH, total nitrogen (N-tot), total phosphorous (P-tot), total ammonia nitrogen (TAN), *Salmonella* spp., thermo tolerant coliform bacteria (TTC), *Enterococcus* spp., somatic coliphages and f-RNA phages was performed on the sludge before and after each treatment. Before treatment, sludge analysis was performed using three samples per batch. After one week of sludge treatment the analyses were repeated, except for total solids and total phosphorus. The post- treatment sludge was analyzed using one sample per treatment replicate, resulting in three analyzed samples per treatment.

Before sampling, the sludge was mixed manually to minimize effects of eventual sludge heterogeneity. The sludge was scooped up from the batch container/treatment-cup and transferred to sample container using plastic spoons. Approximately 10 g of sludge per sample was separated was used for pH and nutrient analysis. For TS analysis 25-35 g of sludge per sample was collected. To prepare dilution series for analysis of TTC, *Enterococcus* spp. and bacteriophages, 10 g of sludge per sample was used. For *Salmonella* spp. analysis 25 g samples of sludge were used.

3.4 Analyses

3.4.1 Total solids

Samples were put in a cold oven that was heated up to 105°C for 24 h. After heating, samples were left to cool in the oven for 14h. After cooling the weight change was used to calculate the total solids content.

3.4.2 pH and nutrient analysis

The samples used for pH and nutrient analysis were diluted 1:4 (weight based) using deionized water in a Stomacher bag ® (Seward, UK) mixed by hand and subsequently transferred to and left to rest in a sealed container for 1h. After resting, pH was measured using a standard pH meter (PHM 210, Radiometer, Copenhagen) and electrode (PHC 2051, Radiometer, Copenhagen). For analysis of nutrient content, volume based dilution series were produced from the initial 1:4 slurry to obtain concentrations in accordance with Spectroquant cell test range. TAN analysis was performed using the indophenol blue method (Spectroquant 1.14559.0001; Merck, Darmstadt). Total nitrogen was analyzed using oxidation and 2.6-Dimethylphenol method (Spectroquant 1.14763.0001; Merck, Darmstadt). Total phosphorus was analyzed using oxidation and phosphomolybdenum blue method (Spectroquant 1.14729.0001; Merck, Darmstadt). Instruments used for nutrient analysis were Spectroquant NOVA 60 spectrophotometer (Merck, Darmstadt) and Thermoreactor TR 420 (Merck, Darmstadt) for oxidation/digestion of N-tot and P-tot samples.

The ammonia-N (NH_3-N) concentration was obtained by multiplying measured TAN concentrations with the fraction of NH_3, calculated using Equation 1 and Equation 2 (Emerson et al., 1975).

3.4.3 Microbiological analyses

For microbial analyses, except *Salmonella* spp., the sludge samples were diluted 1:9 in Stomacher bags using buffered NaCl peptone solution (pH 7.2) with tween (SVA, Sweden). The bag was massaged to obtain a homogenous slurry. These first dilutions were then further 10-fold diluted up to 3

times using the same dilution solution. The prepared dilution series were left to rest for 20-30 minutes before plating.

Thermo tolerant coliform bacteria were enumerated with Violet red bile agar (VRG) pour plate method using 1 ml of 1 and 2 log10 diluted sludge, resulting in a detection limit of 10 CFU/g wet sludge. The plates were incubated in 44 ±1°C for 24 ±3 hours before typical colonies were counted. In the startup analysis for each batch a representative selection of ten typical TTC colonies from each plate were further analyzed in lactose tryptone lauryl sulphate broth (LTLSB) tubes with Durham tubes to analyze if the colonies were *E. coli*. Gas in Durham tubes after 24 h in 44°C was considered a confirmation off general TTC presence. These TTC positive tubes were added 0.3-0.5 ml of Kovacs reagent to detect *E. coli* presence. Positive reaction, indicating presence of E coli, was confirmed by a change of color to red-ish.

Two plating approaches were used for *Enterococcus* spp. enumeration on Slanetz-Bartley (SlaBa) plates. Sludge treated with a combination of high urea and temperature were plated using 0.2 ml of 1 log10 diluted sludge on five plates per replicate. This method was used to push the detection limit for the most efficient treatments, enabling CFU detection at a lower dilution, and resulted in a detection level of 10 CFU/g (ww) sludge. Treatments predicted to be less efficient, due to treatment using lower temperature and urea addition, were plated using 0.1 ml of 2 and 3 log10 diluted sludge on one plate per dilution and replicate. This resulted in a detection limit of 100 CFU / g wet sludge. The plates were then incubated for 48 ±2 h in 44°C ± 0.2. After incubation, all red, maroon and pink colonies were presumed to be *Enterococcus* spp.

The host bacteria used for detection of f-RNA phages and somatic coliphages was *S. typhimurium* WG 49 (ATCC® 700730™) and *E. coli* 13706 (ATCC® 13706™), respectively. Host solutions were cultivated in nutrient broth (SVA, Sweden) at 38°C ±1 during shaking and used within approximately 3 h and while still in log growth phase. One (1) ml of sample and 1 ml of host solution was added to tubes containing 2 ml of melted soft agar in (46°C) heat blocks (Grant Instruments Ltd, England). After vortexing a tube, the content was spread on blood base agar (BAB) plates (SVA, Sweden). The plates were incubated at 38°C ±1 for 11 h. Plaque forming units (PFU) were counted and used to calculate phage concentrations. The plating was performed using 10log dilutions of 1 and 2, resulting in a detection limit of 10 CFU / g of wet sludge.

Salmonella

Salmonella spp. was enriched giving the presence or absence in 25 g of wet sludge. In the enrichment procedure 25 g of sludge was diluted 1:9 using buffered peptone water (BPW) (SVA, Sweden). Resulting slurry was incubated for 18 h in 38°C ±1. Three drops of the incubated slurry was applied on to modified semisolid Rappaport Vassiliadis semisolid plates (MSRV) incubated 24 h at 41.5°C. After incubation, positive samples showed loss of color in the agar around the drops, indicating presence of flagellated salmonella. Plates with negative results were returned to the incubator for 24 more hours. If no positive results were seen after 48 hours the samples were considered free from salmonella. If positive samples appeared during the first or second incubation time the droplet sites were further examined for *Salmonella* confirmation. To confirm *Salmonella* spp. presence, colorless agar from the positive droplet sites on the MSRV plates were spread on Xylose Lysine Deoxycholat agar (XLD) and Brilliant Green agar (BG) plates. Xylose Lysine Deoxycholat and BG plates were incubated for 24 h at 38°C. After incubation, typical *Salmonella* spp. looking colonies were further confirmed using triple sugar iron tubes and urea broth.

3.5 Statistical analysis

The average start concentrations in different batches of sludge used in the experiment was compared by single factor analysis of variance (Anova) followed by post hoc analysis with Tukey's Honestly Significant Difference (HSD) test (at family rate 5). Correlation between the analyzed sludge factors was performed using Pearson product moment correlation coefficient. General regression analysis was performed for bacteria and coliphage inactivation using temperature vs added urea-N and post treatment NH_3-N concentrations. If not indicated other, all statistical analysis has alpha levels ≤ 0.05. All statistical analyses were performed in Minitab 16 (Minitab Inc., USA).

4 Results

4.1 Initial sludge

In the initial sludge, TS was significantly lower for sludge collected at Kungsängen STP (mean 20.8%) than sludge collected at Bromma STP (mean 31.5%). Mean TS from all batches was 28.0%. As seen in Table 4, TS was positively correlated with TAN, P-tot, TTC and *Enterococcus* spp. concentrations and negatively correlated to N-tot concentrations.

Table 4: *Levels of Total solids (TS), pH, total N (N-tot) total ammonia nitrogen (TAN), total P (P-tot) thermo tolerant Coliform bacteria (TTC) Enterococcus spp., and somatic coliphages were examined in the initial sludge before treatment. The mean results are presented below. Standard deviation (SD) and coefficient of variation is also presented as well as range and correlations between the different factors.*

Factor (unit)	Mean (SD)	CV	Min-max	Correlations
TS (% of ww)	28.0 (5.57)	0.19	19.6 - 34.6	TAN^{+}, TTC^{+}, $P\text{-}tot^{+}$, *Enterococcus* $spp.^{+}$, $Ntot^{-}$
pH	7.90 (0.25)	0.030	7.5 - 8.2	TAN^{-}
N-tot (mg / g)	4.41 (1.85)	0.42	1.50 - 9.00	$P\text{-}tot^{-}$, TTC^{-}, TS^{-}
TAN (mg / g)	1.44 (0.50)	0.35	0.79 - 2.20	TS^{+}, TTC^{+}, $Ptot^{+}$, *Enterococcus* $spp.^{+}$, pH^{-}
P-tot (mg / g)	6.30 (2.68)	0.42	2.85 - 11.70	TS^{+}, TAN^{+}, TTC^{+}, *Enterococcus* $spp.^{+}$, $Ntot^{-}$
TTC (CFU / g ww)	7.68×10^{6} (1.49×10^{7})	1.94	1.70×10^{4} - 4.70×10^{7}	TS^{+}, TAN^{+}, $P\text{-}tot^{+}$, *Enterococcus* $spp.^{+}$, $Ntot^{-}$
Enterococcus spp. (CFU / g ww)	1.74×10^{6} (2.47×10^{5})	1.41	1.82×10^{4} - 7.00×10^{6}	TS^{+}, TAN^{+}, $P\text{-}tot^{+}$, TTC^{+}
Somatic coliphages (CFU / g ww)	2.69×10^{3} (1.89×10^{3})	0.70	5.45×10^{2} - 8.27×10^{3}	-

Initial sludge mean pH was 7.9 with a standard deviation of 0.25. The initial pH differed between batches (figure 1) but there was no difference in pH between sludge collected from the different treatment plants. However, there were differences in batch pH related to the batch collection site (Table 2). Sludge collected from the centrifuge dryer (batch 1, 2, 5 and 6) had a higher mean pH than sludge collected from the silo (batch 3 and 4) 8.1 and 7.6 respectively. The pH had a negative correlation with TAN, but did not correlate with other factors.

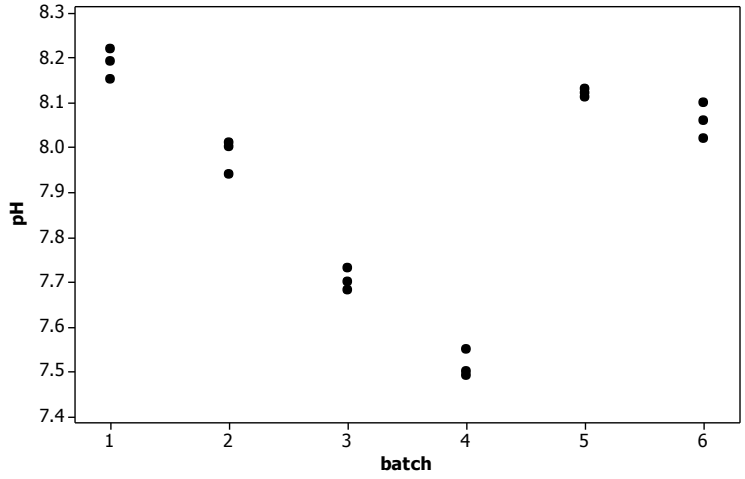

Figure 1: The measured pH in the sludge before treatment of the different batches, see table 2 for explanation of batches.

The nutrient content varied greatly between sludge batches, as seen in Figure 2 and Figure 3. Mean N-tot concentration was 4.41 mg/g and the mean coefficient of variation within batches sample replicates was 0.22. The corresponding values for P-tot were 6.30 mg/g and 0.12. Total ammonia nitrogen mean value was 1.44 mg/g and the mean coefficient of variation within batches was 0.042, substantially lower than for both N-tot and P-tot. N-tot correlated negatively with P-tot, TTC and TS, but no correlation was found with TAN. A positive correlation was seen between TAN and P-tot and both of these independently showed positive correlations with *Enterococcus* spp., TTC and TS. Mean N-tot and P-tot concentrations were not different for sludge collected at the two STPs. The mean TAN concentration was higher in sludge collected at Bromma compared to Kungsängen (1.71 and 0.90 respectively). The different collection sites used (centrifuge and silo) did not result in different concentrations of N-tot, TAN or P-tot.

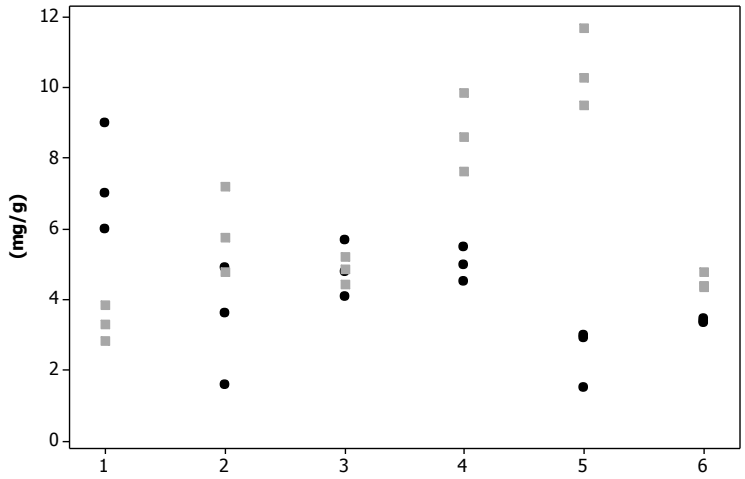

Figure 2: Total N (N-tot)(●) and total P (P-tot)(■) concentrations (mg/g) measured before treatment of the different batches, see table 2 for explanation of batches.

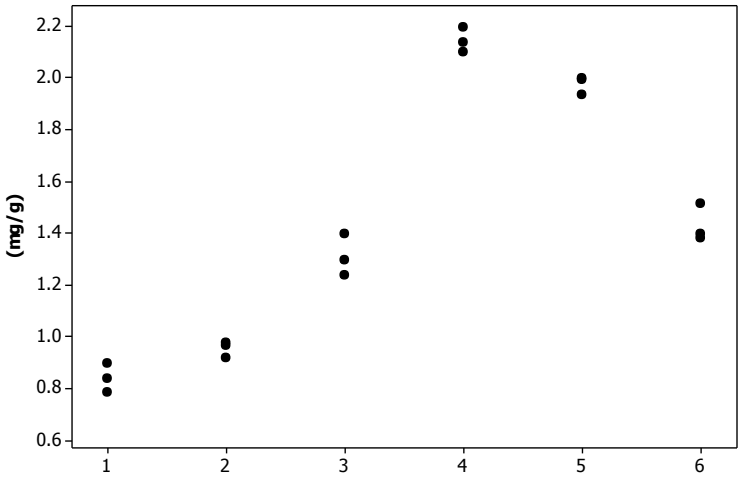

Figure 3: measured total ammonia nitrogen (TAN) (mg/g) of the different batches before treatment, see table 2 for explanation of batches.

Sludge collected at Bromma STP had higher *Enterococcus* spp. and TTC mean concentrations than Kungsängen sludge: 6.12 vs 4.51 log10 CFU/g for *Enterococcus* spp. and 6.43 vs 4.47 log10 CFU/g for TTC. However, large variations of TTC concentration were observed for batches of Bromma sludge. The different collection sites (silo or dryer) did not influence *Enterococcus* spp. or TTC concentrations significantly. *Enterococcus* spp. and TTC concentrations were in the same range as one another in the individual batches (Figure 4), and the concentrations were also correlated to each other. The analysis of presumed TTC colonies on the pre-treatment plates confirmed that 86% of colonies were general TTC and 66% of colonies were confirmed as *E. coli*. *Salmonella* spp. presence per 25 g wet sludge was confirmed in all batches of sewage sludge used.
Before treatment, only low concentrations of f-RNA phages were found in sludge from batch 2 and 5. The analysis of f-RNA phages was subsequently excluded from the study. The mean concentration of somatic coliphages in initial sludge was 2.7×10^3 PFU/g ww and levels did not differ significantly between Bromma and Kungsängen STPs. The concentrations of somatic coli phages varied less than the other studied organisms and no significant correlations were found with any of the other examined factors of the initial sludge.

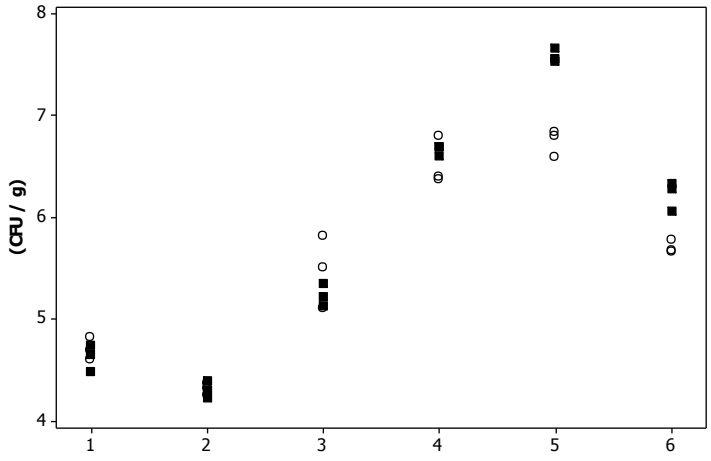

Figure 4: Concentrations (cfu g^{-1}) of *Enterococcus* spp. (○), thermo tolerant coliform bacteria (■) in the sludge of the different batches pre-treatment, see table 2 for explanation of batches.

4.2 After treatment

4.2.1 Sludge chemical properties

Regression analyses showed that an increased addition of urea had a positive correlation with post treatment pH, N-tot and TAN after the one week incubation (Figure 5 a-c) whereas no significant effect from temperature was observed on the mentioned parameters. (Table 5). Even though no urea was added, mean values for pH, N-tot and TAN increased also in the control treatments (Table 5).

Table 5: Mean pH and mean concentrations of N-tot, TAN and calculated NH_3-N (mg/g wet weight) after treatment is presented for the different urea-N additions. The increase of these parameters during treatment is also presented. Coefficient of variation (CV) is presented within parenthesis.

Added urea (% ww)	Added urea-N (mg/g ww)	pH_{END} (CV)	TAN_{END} (mg/g ww) (CV)	Increase of TAN (mg/g ww) (CV)	$Ntot_{END}$ (mg/g ww) (CV)	Increase of Ntot (mg/g ww) (CV)	NH_3-N (mg/g ww) (CV)
0.0	0.0	8.0 (2.8)	4.00 (37.8)	2.61 (44.3)	7.85 (38.3)	3.29 (111)	0.44 (55)
0.5	2.3	8.2 (1.6)	6.81 (21.7)	5.40 (23.5)	11.60 (17.9)	7.23 (40.4)	1.16 (60.3)
1.0*	4.7	8.4 (1.4)	8.24 (14.7)	6.85 (14.2)	13.7 (24.0)	9.17 (48.3)	1.92 (42.5)
1.5*	7.0	8.5 (1.1)	11.0 (15.0)	9.61 (16.8)	17.3 (17.4)	12.9 (31.6)	3.17 (25.1)

**Values for TAN and increase of TAN have been adjusted with respect to three outliers in replicates of treatments 15@28 and 10@38 of batch 5 and 10@44 of batch 6.*

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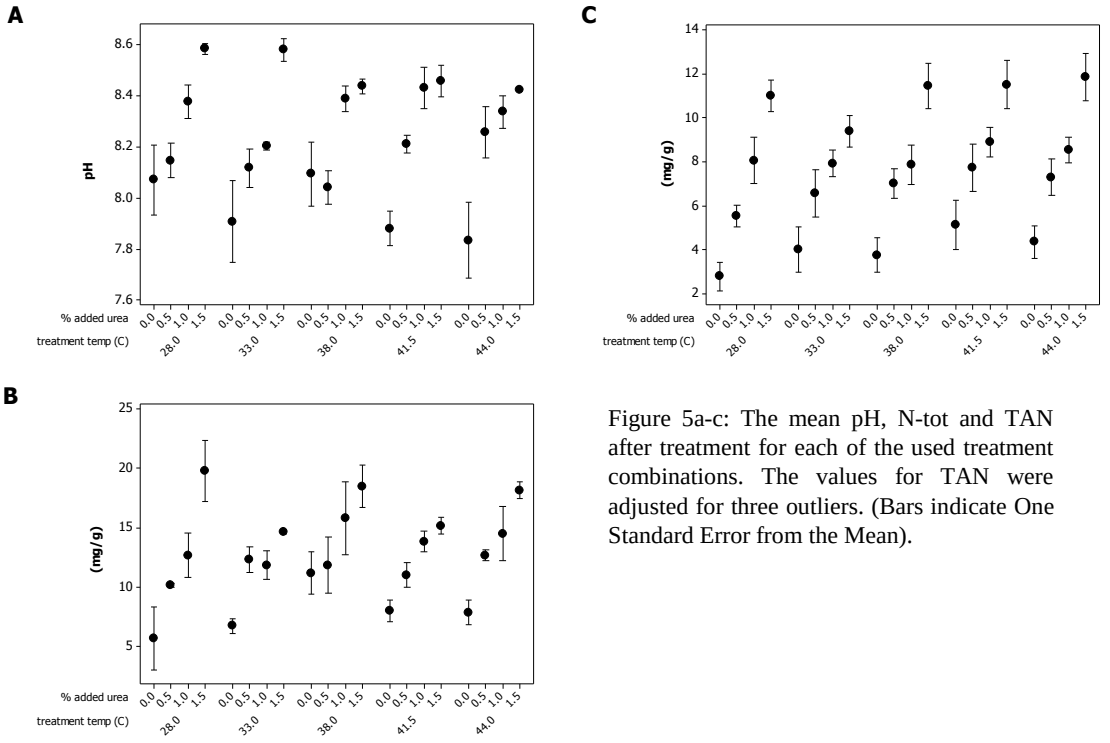

Figure 5a-c: The mean pH, N-tot and TAN after treatment for each of the used treatment combinations. The values for TAN were adjusted for three outliers. (Bars indicate One Standard Error from the Mean).

Three outliers were identified when plotting the increase in TAN (mg/g) against added urea-N (mg/g), 15@28 and 10@38 of batch 5 and 10@44 of batch 6 (Figure 6). These treatments were prepared with urea-N additions of 7.0 (15@28), 4.7 (10@38) and 4.7 (10@44) mg/g, but the measured TAN increase was 15.23, 15.18 and 11.47 respectively resulting in post treatment TAN values of 17.20, 17.15 and 12.90 mg/g. This was higher than expected based added urea, especially in the 10@38 replicate (the third largest increase of all examined replicates). The validity of these unproportionally high TAN values was not confirmed by a corresponding increase in pH or in reduction of any of the studied organisms. Two of these replicates (15@28 and 10@38) also showed an unexpectedly high increase of N-tot. This indicated that a sampling error may have occurred, as both TAN and N-tot samples were performed using the same dilution series. Subsequently the post treatment TAN values for these replicates were considered flawed and substituted with the mean TAN of the remaining two replicates of the same treatment. This affects the independence of the results for these treatments and reduces the variation, which should be considered when analyzing the results.

The measured mean increase of both TAN and N-tot was high compared to the expected increase based on the added urea in the treatments, up to two times higher (Table 6). These increases of TAN in the control treatments that could not be attributed to addition of urea were used as an indicator of the normal conversion of non-TAN nitrogen to TAN during the treatment process. When the mean increases of N-tot and TAN in the controls (representing the "natural" increase at each temperature) were subtracted from the total increase of the treatments using added urea, more likely values for the N-increase from the addition of urea was obtained. As seen in Figure 7, especially the values for TAN increase are close to the expected values, represented by the black thick line.

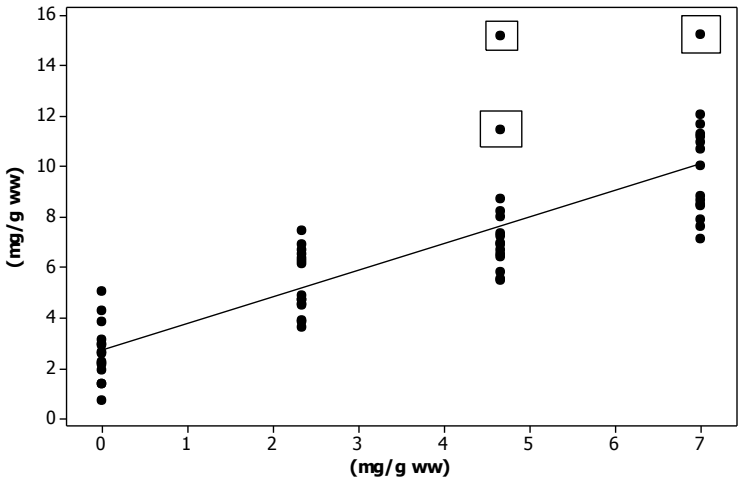

Figure 6: The relation between added urea and increase in TAN (total ammonia nitrogen) concentration (r2= 69.6 and regression coefficient = 1.1). The original values for outliers 15@28, 10@38 of batch 4 and 10@44 of batch 5 are shown in squares. These were replaced with mean of the remaining two treatment replicates in further analyses.

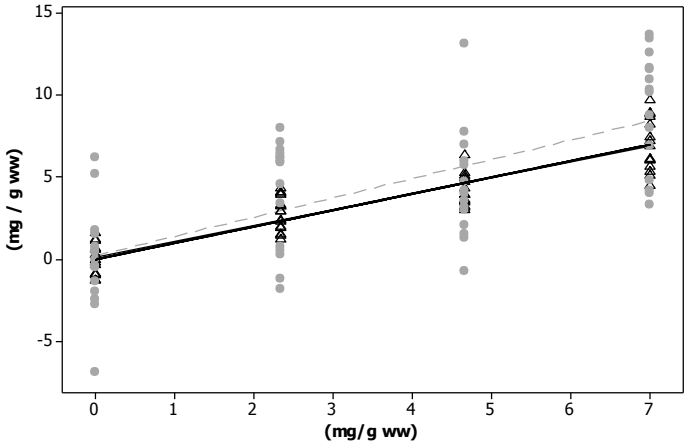

Figure 7: Mean increase in total ammonia nitrogen (TAN) (Δ and ⎯) as well as total nitrogen (N-tot) (● and - - -) (mg/g wet weight) by the addition of urea nitrogen. Values adjusted for "natural" increase in concentrations obtained from control treatments are plotted. A bold black line is representing the expected increase of TAN.

Table 6: The change in total ammonia nitrogen (TAN) as well as total nitrogen (N-tot) in control treatments (no urea added) for the different treatment temperatures.

Temp (°C)	Increase of TAN in control treatment (mg/g ww)	Increase of N-tot in control treatment (mg/g ww)
28	1.58	0.420
33	2.58	3.12
38	2.28	5.92
41.5	3.42	4.34
44	3.16	2.63

The NH_3-N concentrations of the treated sludge, as calculated using Equation 1 and Equation 2, increased with increased addition of urea and temperature, as expected. This increase is seen in Figure 8, where the mean NH_3-N concentration for each treatment combination is presented. Post treatment mean NH_3-N concentrations for the different urea additions are presented in Table 5.

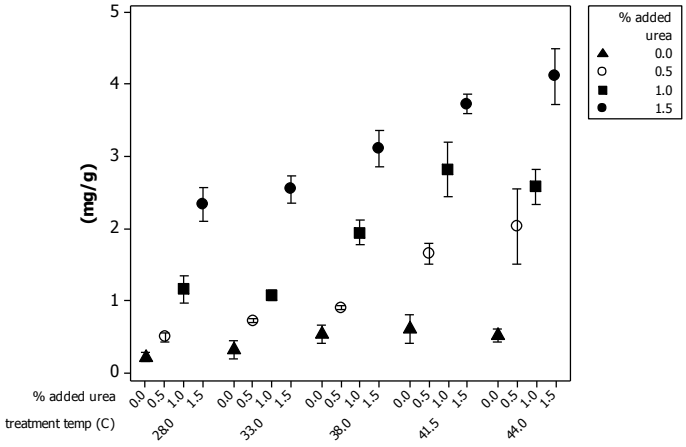

Figure 8: The effect on NH_3-N concentration by the different treatment combinations. (Bars indicate One Standard Error from the Mean)

4.2.2 *Enterococcus* spp. inactivation

The mean log10 reduction of *Enterococcus* spp. for all treatment replicates at all temperatures was 2.3 ranging from -0.06 (growth in one replicate of 10@28 treatment) to 5.6. A 3 log10 reduction was reached with 1.0 and 1.5% urea at 38°C and for all the treatments (including controls) at 41.5 and 44.0°C (Table 8 and Figure 9). Temperature and post treatment NH_3-N concentrations were significant factors for the *Enterococcus* spp. log10 reduction (Pearson correlation). Regression analyses showed that temperature and NH_3-N concentration combined were the most suitable factors for modeling the inactivation of *Enterococcus* spp.. At 38°C the *Enterococcus* spp. reduction was related to added urea and NH_3-N concentrations (Figure 9 and Figure 10) with treatments with 1.0 and 1.5% added urea, reaching noticeably higher reduction than control and 0.5% urea treatments. All treatments at 44.0°C, except the control, reached concentrations below 1000 CFU / g (TS), the threshold concentration in the 2010 legislation proposal whereas at 38 and 41.5°C only the highest urea addition of 1.5% reached the proposed level (Table 7).

Treatment replicates using batch 5 resulted in higher mean post treatment concentrations than the other replicates despite same reduction, this due to the high initial concentration in this batch. Sludge from Bromma STP did for the same reason result in a higher post treatment *Enterococcus* spp. concentration than Kungsängen.

Table *7*: The mean concentration of thermo tolerant coliform bacteria (TTC) and *Enterococcus* spp. after the different treatments. Standard error of the mean is also presented.

Mean post-treatment concentrations	TTC (CFU/g ww) (100 = threshold -2013 proposal)		TTC (CFU/g TS) (1000 = threshold -2010 proposal)		*Enterococcus* spp. (CFU/g TS) (1000 = threshold -2010 proposal)	
Treatment code	Mean	SE Mean	Mean	SE Mean	Mean	SE Mean
00@28	3.5×10^{4} Ö	1.9×10^{4}	1.3×10^{5} Ö	6.6×10^{4}	2.5×10^{5} Ö	1.3×10^{5}
05@28	1.2×10^{3}* Ö	1.1×10^{3}	3.7×10^{3}* Ö	3.5×10^{3}	1.0×10^{6} Ö	9.5×10^{5}
10@28	3.2×10^{2}* Ö	3.2×10^{2}	9.2×10^{2}*	9.2×10^{2}	1.9×10^{6} Ö	1.2×10^{6}
15@28	<100 CFU/ g	-	<1 CFU /10 g	-	1.3×10^{6} Ö	6.5×10^{5}
00@33	3.4×10^{4} Ö	2.1×10^{4}	1.1×10^{5} Ö	5.7×10^{4}	9.1×10^{5} Ö	7.2×10^{5}
05@33	<100 CFU/ g	-	<1 CFU /10 g	-	1.1×10^{6} Ö	8.9×10^{5}
10@33	<100 CFU/ g	-	<1 CFU /10 g	-	9.9×10^{4} Ö	5.8×10^{4}
15@33	<100 CFU/ g	-	<1 CFU /10 g	-	2.7×10^{5} Ö	2.3×10^{5}
00@38	4.4×10^{2}* Ö	4.4×10^{2}	2.2×10^{3}* Ö	2.2×10^{3}	1.4×10^{5} Ö	1.1×10^{5}
05@38	3.3×10^{1}*	3.3×10^{1}	1.5×10^{2}*	1.5×10^{2}	4.7×10^{4} Ö	2.3×10^{4}
10@38	<100 CFU/ g	-	<1 CFU /10 g	-	7.2×10^{4}* Ö	4.9×10^{4}
15@38	<100 CFU/ g	-	<1 CFU /10 g	-	5.2×10^{2}*	5.2×10^{2}
00@415	<10 CFU/ g	-	<1 CFU /10 g	-	3.9×10^{4}* Ö	2.0×10^{4}
05@415	<10 CFU/ g	-	<1 CFU /10 g	-	1.7×10^{4}* Ö	1.6×10^{4}
10@415	<10 CFU/ g	-	<1 CFU /10 g	-	6.4×10^{3}* Ö	6.4×10^{3}
15@415	<10 CFU/ g	-	<1 CFU /10 g	-	5.7×10^{2}*	3.7×10^{2}
00@44	<10 CFU/ g	-	<1 CFU /10 g	-	4.0×10^{3}* Ö	3.8×10^{3}
05@44	<10 CFU/ g	-	<1 CFU /10 g	-	1.0×10^{1}*	1.1×10^{1}
10@44	<10 CFU/ g	-	<1 CFU /10 g	-	<10 CFU/ g	-
15@44	<10 CFU/ g	-	<1 CFU /10 g	-	<10 CFU/ g	-

** At least one of the underlying treatment replications failed to detect the level of reduction.*

Ö Over proposed threshold value.

Tukey analysis showed that the mean log10 reduction of *Enterococcus* spp. was not affected by initial TS, STP, batch, pH, added urea-N, initial TAN or STP collection site. Treatment temperatures however did result in different grouping of the mean log10 reductions with a higher reduction at higher temperatures.

For nine of the treatments at temperatures 38.0, 41.5 and 44.0 ° C the detection limit was reached in at least one replicate (Table 8 and Figure 9). In the two lower temperatures where the *Enterococcus* spp. reduction limit was not reached and the actual reduction could be analyzed, it was increased by both higher temperature and more added urea (Figure 10). As expressed in Equation 1, the NH_3-N concentration increases with increasing temperature; however the reduction was further affected by temperature (Figure 10).

Table 8: Mean log10 reduction for thermo tolerant coliform bacteria (TTC), *Enterococcus* spp. and somatic coliphages is presented for each of the used treatment combinations. Standard error of the mean is also presented.

Log10 red.	Thermo tolerant coliform bacteria (TTC)		*Enterococcus* spp.		Somatic coliphages	
Treatment	Mean	SE Mean	Mean	SE Mean	Mean	SE Mean
00@28	1.0	0.61	0.63	0.27	1.1	0.30
05@28	>3.6	0.87	0.76	0.43	0.93	0.50
10@28	>4.2	0.29	0.52	0.31	0.99	0.31
15@28	>5.2[£]	2.0	0.96	0.12	0.80	0.41
00@33	2.3	1.1	0.77	0.11	0.46	0.26
05@33	>4.7[£]	1.0	0.71	0.054	1.0	0.26
10@33	>4.3	0.56	1.1	0.15	0.91	0.42
15@33	>4.9[£]	0.62	2.0	0.72	1.1	0.31
00@38	>4.2	1.3	1.5	0.057	1.4	0.39
05@38	>4.0	0.86	1.2	0.31	1.3	0.45
10@38	>5.3[£]	0.87	>2.7	0.52	1.6	0.48
15@38	>4.3	0.56	>3.6	0.48	1.2	0.38
00@415	>5.2[£]	0.98	2.7	0.31	>1.5	0.65
05@415	>4.8[£]	0.90	>3.1	0.34	1.5	0.31
10@415	>4.7[£]	0.72	>3.5	0.97	1.4	0.45
15@415	>4.4	0.47	>3.4	0.18	>1.7	0.54
00@44	>4.4	0.47	>3.1	0.49	1.3	0.32
05@44	>4.7[£]	0.72	> 4.5	0.66	1.2	0.27
10@44	>4.4	0.47	> 4.3	0.31	2.0	0.43
15@44	>4.7[£]	0.72	> 4.5	0.66	>2.0	0.43

> reduction beyond detection limit in at least one treatment replicate

[£] *log10 reduction is in compliance with the proposed legislation threshold.*

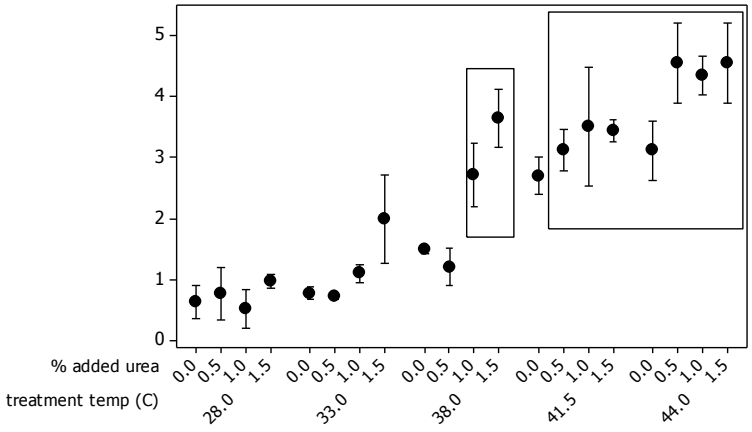

Figure 9: The mean log 10 reduction of *Enterococcus* spp. resulting from the different treatments combinations of added urea-N and temperature. The figures in boxes are underestimated due reduction beyond detection limit.

Due to that the reduction able to study in relation to initial concentration was met by most of the treatments at 41.5°C and above, the effect of NH_3-N concentration could not be determined. However, at 38°C extrapolating from the regression based on the actual reduction indicate that the potential reduction with 1.5% urea is higher than what could be measured (Figure 11). In the 28°C treatments NH_3-N concentration had a smaller effect on reduction compared to 33 and 38°C treatments (Figure 10). The *Enterococcus* spp. reduction was plotted against temperature and added urea-N (mg/g ww) (Figure 11). The figure shows that increasing temperature and added urea-N had a positive effect on reduction. The figure also confirms what was seen in Figure 9Figure 10; that the added urea gave the strongest effect at 38°C whereas at the higher temperatures the temperature alone is sufficient to achieve reduction in 1 week.

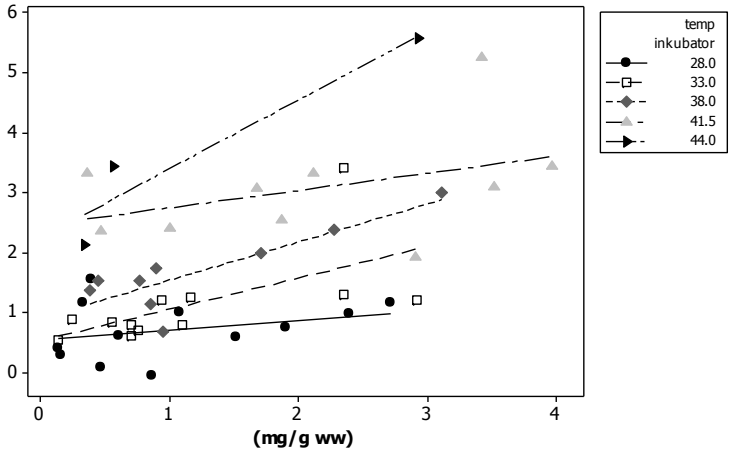

Figure 10: The *Enterococcus* spp. log 10 reductions plotted against post treatment NH3-N concentrations plotted separately for each of the used temperatures. This figure is based only on the treatment replicates that resulted in an observation of the actual log 10 reduction, treatment replications resulting in reduction beyond detection limit are not represented.

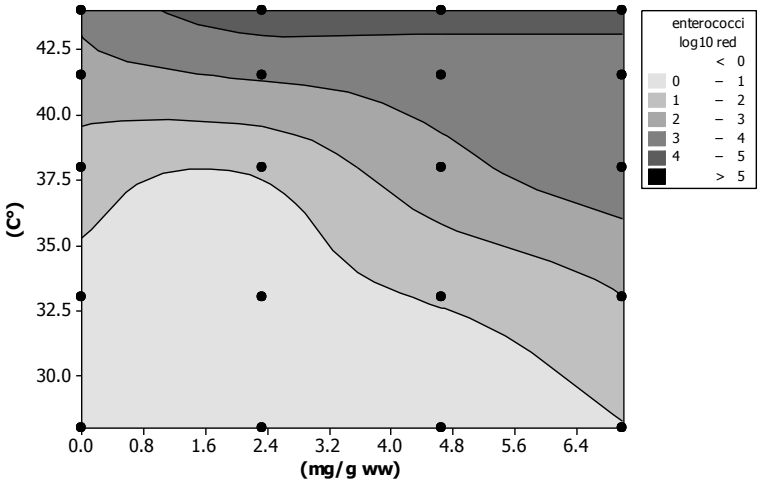

Figure 11 The effect of temperature and added urea-N on the log 10 reduction of *Enterococcus* spp.. The plot shows the individual replications and their resulting log 10 reductions.

4.2.3 TTC inactivation

Post treatment TTC log10 reduction was correlated to added urea-N, NH_3-N concentration and treatment temperature (Pearson correlation). The Mean TTC log10 reduction for all treatments and temperatures was 4.22 and this reduction had a stronger relation to NH_3-N concentration than to added urea. General regression analysis showed that treatment temperature and added urea-N in combination were the most suitable factors to model post treatment log10 reduction. However, these models were poor in predicting the treatment outcome (with r^2 of 19.8 and 20.5 respectively). TTC concentrations were reduced to less than 1000 CFU /g TS (legislative proposal 2010) for all treatments with urea except 0.5% urea treatment in 28°C. Control treatments (no urea added) at 41°C and above did also reach this threshold. Less than 100 CFU /g ww (legislative proposal 2013) was reached for all treatments with urea except with 0.5 and 1.0% urea treatment in 28°C as did controls (no added urea-N) at 41°C and above (Table 7). The initial concentration in most sludge from Kungsängen was too low to confirm the 5 log 10 reductions required for validation of treatments in the year 2013 proposal (Figure 12). A mean log 10 reduction meeting this threshold was only reached for nine of the used treatment combinations, mainly when using sludge form Bromma (Table 8).

The Bromma sludge reached a significantly higher mean log10 reduction (4.84) than the Kungsängen sludge (2.96) (Figure 12). For 49 out of 60 (82%) the detection limit was reached after 1 weeks treatment resulting in mainly censored values for the reduction. A positive effect of NH_3-N concentration on the log 10 reductions was only seen in the lower temperatures, 28.0 and 33.0°C (Figure 12). These combinations were also the only ones where the level of reduction was detected for all replicates. The control treatments at 38°C and above all resulted in a reduction beyond detection limit. As shown in Figure 8, the NH_3-N concentration in the three control treatments at 38°C and above was not higher than NH_3-N concentrations in the controls treatments of the two lower temperatures. Due to problems detecting the reduction level, the real TTC reduction as a function of temperature and NH_3-N could not be properly modeled using this data.

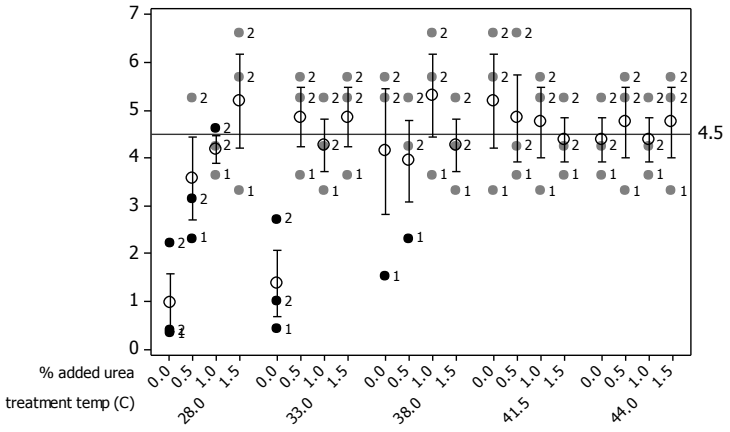

Figure 12: The effect of the used treatment combinations on the mean log10 reduction (y-axis) of thermo tolerant coliform bacteria (TTC). The reduction for each treatment replication is also showing as a dot. A black dot (●) indicates that that the detection limit was not reached, while a gray dot (●) indicates that the detection limit was reached. The number in connection to each dot represents the STP from which the sludge was collected for each replicate. (1 = Kungsängen STP and 2 = Bromma STP). The horizontal line represents the threshold limit for the 2013 regulation proposal. (Bars are One Standard Error from the Mean)

4.2.4 Somatic coliphage inactivation

The mean log 10 reduction of somatic coliphages was 1.27 and ranged between -0.02 and 2.64. This reduction was positively correlated to both treatment temperature and post treatment NH_3-N concentration, but not to added urea-N. However, no difference in reduction was observed between the different treatment combinations. When modeling the relations between log10 reduction, temperature and NH_3-N concentration, using general regression, temperature had a positive effect but NH_3-N concentration did not affect the reduction significantly. This model was poor in predicting the reductions (r^2=21.6). Three of the treatments (control in 41.5°C and 1.5% urea in 41.5°C and 44.0°C) resulted in reduction beyond detection limit in one of the three replicates, as presented in Table 8. Noticeably the 0.5% urea treatments failed to produce a higher mean 10 log reduction than the controls in all temperatures except 33.0°C. This is illustrated in Figure 13. The only difference of mean log 10 reductions based on treatment temperature was fund between treatments of 33.0 and 44.0°C (Tukey grouping). No other difference was found based on treatment temperature. As seen in Figure 13 and Figure 14, the additions of urea lead to increased reduction in all temperatures except for the 28°C treatments. In the 28°C treatments the added urea seems to have had a negative impact on the log10 reduction. This impact was not statistically significant and it indicates a pattern that was not expected, as the NH_3-N concentration in these treatments increased with increased addition of urea (Figure 8). The greatest positive effect of added urea was seen in 33.0 and 44.0°C treatments. Between these temperatures the effect of added urea only slightly increased log10 reduction (Figure 13 and Figure 14).

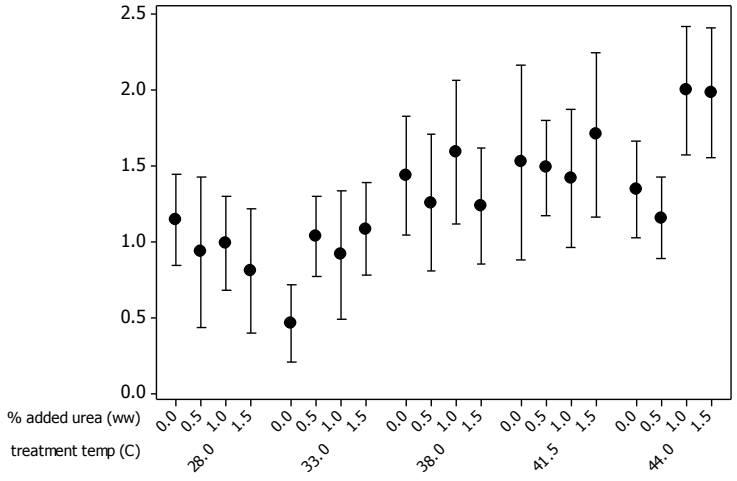

Figure 13. The mean log 10 reductions of coliphages for each of the studied treatment. (Bars are One Standard Error from the Mean)

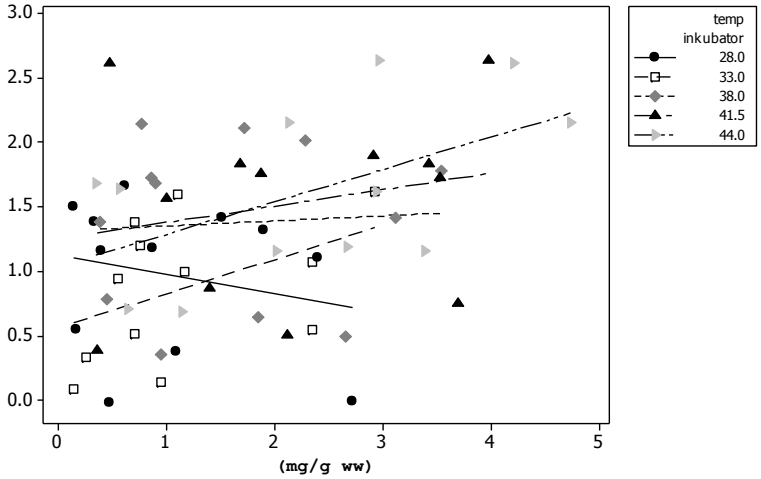

Figure 14. Log 10 reductions of coliphages plotted against post treatment NH3-N concentrations, here plotted separately for each of the used temperatures.

4.2.5 *Salmonella* spp. inactivation

Salmonella spp. presence was confirmed in post treatment sludge of two replicates from controls treated at 28°C. All other treatments managed to reduce *Salmonella* spp. beyond detection level (>1 CFU /25g).

5 Discussion

5.1 Reduction

5.1.1 *Enterococcus* spp.

The positive impact of both temperature and NH_3-N concentration on *Enterococcus* spp. 10log reduction (as presented in Table 8 and figures 9, 10 and 11) was in accordance with previous results (Ottoson et al., 2008, Allievi et al., 1994). However, the *Enterococcus* spp. log 10 reductions were lower than the suggested by the D-values reported by Nordin et al. (2009) in comparable treatment combinations. Reported D-value times for 34°C in the mentioned study were 4.7, 3.7, 2.6, and 2.1 days for 0.0, 0.5, 1.0, and 1.5% of added urea respectively. The corresponding treatment combinations in the present study (33.0°C treatments) did not reach 1 log10 reduction for the control and the 0.5% urea combinations. Only 1.0 and 1.5% urea treatments in this temperature reached 1 log10 reduction (mean log10 reductions of 1.1 and 2.0 respectively). The previous study used non processed human feces with added *Enterococcus* spp., differing from the sludge used in the present study in aspects of TS and pH among others. As the sludge used in the present study had previously been through mesophilic treatment, it is possible that weaker strains of *Enterococcus* spp. could already have been inactivated. This would explain the difference in results from such similar treatment methods.

The effect from the added urea and subsequently the NH_3-N concentration on log10 reduction was most pronounced in the 38°C treatments. This is shown in Figure 9 and Figure 9 where the reduction is almost 2 log10 higher for 1.0% urea than for 0.5% urea. The effect of urea and NH_3-N concentration at the higher temperatures could not be established since detection limit was reached at time for sampling and the reduction level could not be determined. In the lower temperatures (28 and 33°C) however, as seen in Figure 9 and Figure 10, the effect of added urea and increased NH_3-N concentration was not as great as in the 38°C treatments, still there was an effect. The effect on inactivation from ammonia at 38°C, in comparison to the lower and higher temperatures, is further confirmed in figure 11. In this figure the 38°C treatments show a greater response in log10 reduction vs. added urea-N. Due to the rapid reduction in the higher temperatures, the effect of adding urea in these temperatures was not verified (Figure 9). The relatively smaller effect of ammonia concentration on reduction in the higher temperatures (41.5 and 44°C) can be partly explained by a relatively efficient inactivation even using low ammonia concentrations. This in combination with reduction beyond detection limit in the higher temperatures results in a less steep slope for this temperature in Figure 10.

5.1.2 *TTC*

The observed positive effect on reduction based of added urea-N in the two lowest temperatures is a pattern that would also be expected in higher temperatures. Because no TTC was found in most of the sludge treated with the higher temperatures, it is difficult to draw conclusions regarding the differences in efficiency of these individual treatment combinations. As seen in Figure 12 the addition of urea in the three highest temperatures did not cause a higher reduction than the control treatment. This does not mean that higher temperature treatments were not more efficient than the lower temperature treatments, it simply reflects that TTC was completely reduced in many of these higher temperature treatments. For the treatment time studied here (7 days) temperature alone above 38°C was sufficient to reach the detection limit and thus

Due to the high efficiency of most of the treatment combinations using the higher temperatures, an effect of temperature on the TTC log10 reduction was only observed in the lower temperatures, as seen in Figure 12. The missing effect of increased additions of urea and temperature in the higher temperatures is misleading and can be explained by the problems detecting levels of reduction. For TTC in contrast to *Enterococcus* spp. the log10 reduction was mainly affected by added urea-N and treatment temperature in combination. The results show that TTC reduction was affected more by NH_3-N concentration than by temperature. This differs from the results concluded by Mendez et al. (2008) showing that temperature was the significant factor for fecal coliforms, and that NH_3-N concentration was not. The reduction of TTC differed depending on the STP from which the sludge originated (Figure 12). This difference in reduction based on STP can be explained by the quality of the initial sludge. Sludge from Bromma had a higher initial TTC concentration than Kungsängen sludge. In the cases where the reduction level was not detected, meaning that no TTC was found after treatment, the initial bacterial concentration was the limiting factor in deciding the treatment efficiency. As the reduction cannot be larger than the initial concentration, a larger initial concentration resulted in a larger reduction in these cases. The perceived reduction thus became tainted by the initial concentration, a factor that itself varied between batches (Figure 4). Bromma had a higher initial TTC concentration and as a result the efficiency in treatment of Bromma sludge seems more efficient. The problems detecting the level of reduction in the higher temperatures and the resulting lack of data makes it difficult to draw any conclusions for these treatments. The results for log10 reduction of TTC in this study are not suitable to base conclusions regarding exact levels of reduction to expect from the treatments. This is due to the problems determining the real reduction. However, what the results do show is the level of reduction that at least can be reached using the treatment combinations. The failure in detecting levels of reduction was partly caused by an underestimation of the treatment efficiencies in reducing TTC. To examine if there is a difference in reduction between the treatments using the higher spectrum of NH_3-N concentrations and temperatures, these combinations of could be studied using a shorter treatment period.

5.1.3 Somatic coliphages

The results showed that temperature was the most important factor to reduce the somatic coliphages in this study. However, the different treatment combinations had no significant relevance to the resulting reduction of coliphage and the resulting reductions did not differ significantly between the used treatments. This result coincides with the results of (Emmoth et al., 2008, Emmoth et al., 2007), in which increased NH_3-N concentration had no effect on the reduction of somatic coliphages. In the mentioned study, the treatment consisted in treating hatchery waste spiked with somatic coliphages using temperatures of 14°C in combination with NH_3-N concentrations in the range of 0.0 – 0.75%

(w/w). In the present study, treatments used higher temperature and NH_3-N concentrations. Consequently a greater reduction was achieved, as expected. Increasing the NH_3-N concentration by addition of urea-N cannot be motivated based on the present results. Increased NH_3-N concentration seemed, however, to have a slightly greater effect at temperatures of 33 and 44°C compared to the other temperatures used (Figure 13 and Figure 14), motivating further investigation using these specific temperatures. As these were the temperatures where the added urea had greatest effect, a greater amount of added urea using the same temperatures may lead to a more distinct reduction. The over-all results, considering all used temperatures, supports results from (Emmoth et al., 2008, Emmoth et al., 2007) concluding that somatic coliphage seem resistant to ammonia treatment. As illustrated in previous studies (Emmoth et al., 2008, Emmoth et al., 2007), and confirmed in this study, somatic coliphages can be seen as a conservative indicator for virus inactivation. Thus, the reduction of the complete sewage sludge viral flora cannot be directly interpolated from the results of this study. None of the treatments in the present study were able to reduce the somatic coliphages to levels complying with proposed thresholds for viruses. Furthermore, the concentration of somatic coliphages in the initial sludge was lower than the log 10 reduction required in the proposed legislation. Therefore, compliance with these proposals would have been impossible to prove, even at complete reduction, using the sludge of this study.

5.1.4 *Salmonella* spp.

As *Salmonella* spp. was only found in the control after treatment in 28°C, the necessity of adding urea as a mean of reducing *Salmonella* spp. in the higher temperatures is not considered necessary. With the exception of the 28°C control treatment, all other treatments complied with the proposed accepted reduction threshold value for 2010. Absence of *Salmonella* spp. per 50 g material, in accordance with the 2013 proposal, could not be verified in this study since 25 g samples was used. Ammonia treatment has previously been shown to reduce *Salmonella* spp. to the same degree as TTC (Mendez et al., 2008) and to higher degree than (Nordin et al., 2009). This indicates that the reduction of *Salmonella* spp. was more efficient than could be verified with the method used for analysis in the present study.

5.1.5 Over-all compliance with proposed regulations

The treatment compliance with the threshold values differed between 2010 and 2013 legislative proposals. The threshold concentrations of the 2010 proposal (Table *1)* were met by five of the treatments (15@38, 15@415, 05@44, 10@44 and 15@44) (Table *7*). An insufficient observed reduction of *Enterococcus* spp. was the main reason for the relatively low compliance to these threshold values. However, as previously discussed, this observed reduction was misrepresentative due to very efficient reduction in relation to the initial concentration.. The reduction of TTC and *Salmonella* spp. complied with the 2010 proposed threshold to a higher degree. The threshold values from the 2013 year proposal were not reached regarding the suggested 3 log10 reduction of viruses in any of the treatments in this study, as shown in Table *7* and Table 8. However, the somatic coliphages studied in the present work can be considered a conservative model for viruses. This means that that a reduction in general viral flora is expected to be higher than the observed phage reduction in this study. Additionally “parasites” was also a criteria in the 2013 proposal, but not included in this study, restricting confirmation of over-all compliance to the suggested levels. The presence of *Salmonella* spp. was studied per 25 g of sludge in comparison to in 50 g of sludge, as proposed in the 2013 proposal. Thus fulfillment of this criterion cannot be concluded for any of the treatments. When excluding the criteria for virus reduction and *Salmonella* spp. absence, most treatment reached compliance with the proposed thresholds (year 2013).

5.2 Treatment effect on sludge chemical properties

Treatment temperature had no effect on pH, N-tot and TAN concentrations, as seen in Figure 5 a-c. The concentrations of N-tot and TAN after treatment was higher than expected based on amount of added urea (Figure 6). The unexpectedly high levels of post treatment TAN concentration could possibly be explained by conversion of non-TAN N sources to TAN. Concerning N-tot however, the unexpected increase could not be attributed to such conversions between different N-sources. An alternative explanation is that water may have condensed on the cup walls. This would have increased the sludge TS, and thus the sludge TAN and N-tot concentration. The adjustments made to the post treatment N-tot and TAN concentrations (based on the calculated "natural" increase) resulted in values much closer to expected (Figure 7). Although such an adjustment does not affect the measured post treatment concentrations, it can be used as an indicator of the effect of the added urea. The deviation between added with urea and measured post treatment TAN lead to the possibility to use either of these factors when analyzing the reduction. As NH_3-N is believed the most important factor for inactivation, it is central to understand the effect added urea has on the final TAN, and thereby NH_3-N, concentration. Also, this deviation exposes the importance of calibrating the amount of urea needed for any specific NH_3-N concentration. As the NH_3-N concentration (Figure 8) is calculated using the measured TAN, understanding of this factor is important.

5.3 Initial sludge quality

Mean sludge total solids (TS), as presented in the environmental reports for the two STPs 2013, was 34.2 and 26.3 for Bromma and Kungsängen respectively. The measured TS of Bromma sludge matched the reported values well while Kungsängen sludge mean TS was lower than presented values. This difference can be explained by the repair service of one centrifuge during sampling. The fraction of TS can be seen as a degree of dilution of the non-water soluble components in the sludge. Meaning that as water, and water soluble components, are extracted by the centrifuge dryer – the concentrations of less, water soluble components becomes higher. Thus, a negative correlation between TS and water soluble sludge components can be expected. The positive correlation between TS and bacteria concentration can be explained by the above mentioned relationship. As bacteria is to a large degree bound to particles, their concentration increased with increased TS.

The mean pH in the studied sludge was higher than reported by the both STPs, 8.1 and 7.8 respectively for Bromma and Kungsängen. Reported values were 7.5 and 7.4 respectively. When looking at the different collection sites (centrifuge and silo) individually the batches collected in the silo matched the reported pH, while the sludge from the centrifuge was higher in pH, pushing up the mean. This indicates that the values in the environmental report could be based on samples collected from the silo. The lower pH in the sludge collected in from the silo may be explained by leakage of ammonia gas from the sludge when stored in the silo. As ammonia is a weak base, the loss of TAN in the sludge would then cause a lower sludge pH, as seen in the results for batches collected from the silo (Figure 1). As the pH is a factor involved in the regulation of NH_3/NH_4^+ ratio, this is an important factor to consider.

Mean N-tot, TAN and P-tot concentration (mg/g ww) in the sludge was considerably lower than the reported values from both STPs. Reported values were slightly higher for Bromma sludge as compared to Kungsängen. The measured concentrations of N-tot were 14.66 and 13.15 for Bromma and Kungsängen respectively. Corresponding values for TAN were 3.43 and 2.89 and for P-tot 9.98 and 7.89 (Lindh, 2013, vatten, 2013). The big variations in the initial sludge batches between N-tot

and P-tot concentrations seemed too large to be caused by normal temporal variations in the sludge. Instead, they may in part be explained by the sampling- and analysis method. The methods for N-tot and P-tot analysis is originally intended for waste water, a homogenous solution, whereas the slurry analyzed in this study was not homogenous but contained solid particles not evenly distributed. This may have caused the relatively large variation between replicates from the same batch (Figure 2). The method used for TAN analysis included a filtering phase, resulting in a homogenous solution for analysis. This homogeneity is reflected by the smaller variation between the replicates of same batch TAN samples (Figure 3). The deviation of the measured results and the results reported from the STPs brings further suspicions over the reliability of the performed measurements. However, the increase in TAN in the treatment samples corresponded well to the addition of urea-N, indicating that, at least, TAN analysis was functioning as expected.

5.3.1 Recommendations

One problem during this study had to do with detecting the treatment reduction level, especially for TTC. As a result, no substantial relationship could be established between treatment factors and TTC reduction. To avoid such problems, proper planning and continuous adjustments between treatment replicates should be performed. Future studies using similar treatment combinations should pay close, continuous, attention to dilution series and the duration of the treatments. Both of these factors are important to optimize treatments to achieve that reductions of organisms does not reach beyond the detection limit.

6 Conclusions

The observed increase of sludge mean TAN concentration was greater than added urea-N. An increase of TAN in control treatments, where no urea was added, suggests that there was a "natural" convesion between the sludge nitrogen fractions during treatment. This is an important factor to consider, as the concentration of NH_3-N (the bactericidal and virucidal component) is directly dependent on the TAN concentration. The first null hypothesis – that that an addition of urea-N leads to an increase of TAN concentration corresponding to the amount of added urea-N – can be rejected, as the increase of TAN was greater than the added urea-N.

A general conclusion regarding the effect of added urea-N and temperature on reduction of the studied organisms could not be made based on the obtained results. Added urea-N did not correlate with 10 log reduction of any of the studied organisms. The lack of correlation between reductions and added urea-N may be explained by the discrepancy between measured increase of TAN concentration and the added urea-N. Treatment temperature correlated with 10 log reduction of *Enterococcus* spp. and somatic coliphages. Due to the fast inactivation of TTC in relation to the treatment time temperature alone at temperatures of 38 and above resulted in end concentrations below the detection limit, so did urea addition at all temperature. A correlation between 10 log reduction of TTC and temperature could not be confirmed in this study. This lack of correlation is not credible and does not correspond well to results from previous studies. The cause of this dissonance is probably related to the problems finding the real level of reduction of TTC and should be seen as a reflection of the high treatment efficiencies. The treatment period was too long to observe the expected correlation; a shorter treatment period could most likely be used to observe such a correlation. The second null hypothesis cannot be rejected.
The NH_3-N concentration, calculated using equation 1 and 2 combining treatment temperature and measured post treatment TAN concentrations, was the most suitable predictor for 10 log reduction of the organisms in this study. It correlated positively to 10 log reductions of all of the studied organisms except *Salmonella* spp.. Thus, the third null hypothesis can be rejected. The observed disagreement between added urea-N and measured increase of TAN underlines the importance understanding the flows of nitrogen between different fractions in the sludge during treatment. Finding the factors controlling these internal flows during treatment may be important in development of an efficient process of treatment.

The treatments used in this study were insufficient in reaching some of the proposed future threshold levels of reduction. Compliance to the legislative proposal from year 2010 was reached using five of the treatments in the higher range of temperature and added urea. The thresholds of the 2013 year proposal could not be met by the treatments in this study. The fourth null hypothesis can be rejected.

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9 Appendix

9.1 Populärvetenskaplig sammanfattning

När vi äter mat så är det dels för att få energi och dels för att få i oss näring. Maten vi äter innehåller många näringsämnen som vi måste få i oss för att kunna överleva. När barn och fram till att vi är fullvuxna går en del av näringsämnen åt som byggstenar för vår kropp. Efter tillväxtfasen i början av livet infinner sig en balans av den mängd näringsämnen som vi tar in (som mat och dryck) och den mängd som lämnar oss (som kiss och bajs).

Samma näringsämnen som är livsviktiga för oss är också livsviktiga för andra djur, men även för växter. Till exempel har de växter som vi äter mycket av – så som vete och ris – behov av samma näringsämnen som vi människor. Till stor del används idag mineralgödsel för att växterna vi odlar ska få sina näringsbehov uppfyllda. Detta är till viss del problematiskt, dels eftersom vissa av näringsämnena är ändliga, och dels för att det krävs energi för att utvinna och förädla näringsämnen för användning som gödselmedel. En större återanvändning av den näringen vi får i oss skulle kunna leda till en mer cyklisk växtnäringsförsörjning. Detta skulle innebära ett mer hållbart system för användande av växtnäring där andelen mineralgödsel som används inom jordbruket minskas till fördel för användning av avloppsslam.

Det finns dock risker med användning av slam i jordbruket. Tungmetaller, organiska föroreningar och sjukdomsframkallande mikroorganismer är de tre huvudsakliga riskerna med slamanvändningen. För att slam ska kunna användas på mark där foder och livsmedel odlas måste dessa risker minimeras. Denna studie har undersökt möjligheterna att reducera olika oönskade bakterier som finns i det slam som produceras när vårt avloppsvatten renas. I studien gjordes försök där slam blandades med olika tillsatser av urea och fick stå i olika temperaturer. Tanken var att testa hur detta påverkar avdödningen av oönskade organismer. Eftersom att urea innehåller kväve, ett viktigt näringsämne inom växtproduktion, så kan man säga att slammets växtnäringsvärde ökar när urea tillsätts. Totalt testades 20 olika behandlingar som kombinerade olika urea-tillsatser och temperaturer. Varje behandling pågick i sju dagar och därefter studerades behandlingarnas effekt på olika bakterier och virus. Dels analyserades vilka faktorer som var viktiga för att döda de olika organismerna, och dels studerades vilka olika behandlings-metoder som var bäst på att döda organismerna. För att veta om de olika metoderna skulle kunna användas i verkligheten i framtiden så jämfördes de mot förslagna framtida gränsvärden för hur mycket av dessa bakterier och virus som skulle kunna accepteras i slam för användning i växtproduktion. Två föreslag fanns för reglering av olika organismers förekomst i slammet, ett från 2010 och ett från 2013.

Det visade sig att hög temperatur gav ökad reducering av alla studerade bakterier och virus. Tillsats av urea hade en positiv effekt på reducering av vissa bakterier (enterokocker och termotoleranta koliformer (TTC)). De föreslagna gränsvärdena för enterokocker nåddes i fem av behandlingarna. För TTC uppfylldes de föreslagna gränsvärdena med de flesta behandlingsmetoderna. Somatiska kolifager reducerades inte till föreslagna gränsvärden med någon av de använda behandlingarna. Föreslagna gränsvärden från 2010 uppfylldes med de flesta av studiens behandlingar. Gränsvärden från 2013 års förslag, där andra organismer (än de som var med i denna studie) regleras, uppnåddes inte av någon av de använda behandlingarna, och modellen för virus-inaktivering som använts i denna studie var relativt konservativ.

Den effektiva reduktionen av de flesta av studerade bakterier och virus ledde slutsatsen att den studerade metoden lämpar sig bra för rening av avloppsslam. Tas även det ökade gödselvärdet, som skapas då kväve blandas i slammet, i åtanke verkar metoden lämpa sig speciellt bra för att skapa ett kretsloppsbaserat gödselmedel för användning i jordbruket. Innan denna metod kan börja användas i stor skala så behövs dock en fortsatt kalibrering av vilka temperaturer och tillsatser av urea som passar bäst för att uppnå olika reduceringsgrader för specifika bakterier och virus. Resultatet av denna studie kan förhoppningsvis komma till användning för att fullfölja ett sådant kalibreringsprojekt.

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