Fate of estrogens and illicit drugs during urine separation and treatment

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1. ABSTRACT

The presence and their subsequent treatment of nutrient in municipal wastewater is still at the front end of environmental engineering despite several decades of successful research and applications. In recent times, the source separation followed by the treatment of urine or resource recovery from human urine has evolved as emerging management strategies albeit mostly at research stage. Human contributes about 80% of the nitrogen, 70% of the potassium and up to 50% of the total although the urine only contributes 1% of the total volume of the wastewater. It is also a known fact that many of the micropollulatnts get into municipal wastewater through urine excretion. The information on the fate of micropollutants during urine treatment and resource recovery is scarce and this study will focus on this emerging area of research. This is one of few studies to evaluate the feasibility of simultaneous P recovery and N removal using combination of chemical and biological processes. The primary results showed above 95% P recovery via struvite precipitation. This study also investigated the possibility of using deammonificaiton processes in lab scale reactors feed with diluted urine after struvite precipitation. The serum bottles tests showed the deammonificaiton reactors performances were not affected by up to 100ug/L E2.

2. INTRODUCTION AND OBJECTIVES

The world population has tripled in the last decade, and it is expected to reach 9.6 billion by 2050. Increased urbanization and industrialization, coupled with clime change related issues, has led to the increase in the consumption of water. Providing adequate water has become a major challenge (Wutich et al., 2014). Despite decade long efforts, the presence of nutrients in point and non-point source waters still threatened the long term sustainability of surface water. Surface water resources contribute significantly to the global water sustainability. Roughly 2.5% of the earth's water is considered fresh water with roughly 0.03% being surface water and only 0.007% considered easily collectable surface waters.

As stated above, the presence and their management of nutrients in the municipal wastewater is still a challenge and, environmental engineers and scientists are constantly developing new methodologies to achieve low levels of nutrients in the final treated wastewater (Guest et al., 2009). The main nutrients of concern are nitrogen and phosphorus. In recent years, realizing the importance of nitrogen and phosphorus as useful resources, the focus of environmental engineers and water practitioners has shifted from removing these nutrients from wastewater to recovering them. However, given the volume of wastewater generated on the daily basis, this approach of managing nutrients seems challenging. One alternate approach is to separate the nutrients at the source before they mix with the rest of the wastewater and become diluted and recover them. This requires a careful consideration about the sources contributing nutrients to municipal wastewater. In this regard, urine separation at source followed either treatment or nutrient recovery have received lots of attention in recent times (Ronteltap et al., 2010; Udert et al., 2003).

Human urine is the most nutrient-abundant part among the domestic waste components. About 80% of the nitrogen (N), 70% of the potassium (K) and up to 50% of the total phosphate (P) in domestic wastewater come from urine, which only contributes 1% of the total volume of the wastewater (Larsen and Gujer 1996, Larsen and Lienert 2004). As a result, urine separation at source and its treatment or resource recovery from it is a path forward in water reuse and nutrient management. In fact, urine separation and its management is being regarded as a viable alternative to meet grant challenges related to nutrient management in municipal wastewater (Maurer et al., 2006). The separate collection and treatment of urine has attracted considerable attention in the wastewater community in the last few years. So far, several main processes have been proposed to remove/recovery nitrogen and phosphorus from urine and incudes volume reduction (evaporation (Wieland, 1994), freeze-thaw (Lind et al., 2001), reverse osmosis (Dalhammar, 1997)), P-recovery (struvite formation (Johnston and Richards, 2003)), N-recovery (ion-exchange (Jorgensen and Weatherley, 2003), ammonia stripping (Behrendt et al., 2001)), N-removal (nitrification (Johansson et al., 1999; Udert et al., 2003c), anammox (Udert et al., 2003c)).

The main nutrients of concern are nitrogen and phosphorus, the presence of both in surface waters causes eutrophication problems. As noted above, previous research efforts have employed phosphorus recovery primarily through struvite, a white crystal, precipitation. Ion exchange and biological processes have been used for nitrogen removal/recovery from urine. However, there have very few efforts which focused on both phosphorus and nitrogen removal/recovery simultaneously. In this applied research, we combined the chemical recovery of phosphorus through struvite precipitation followed by the treatment of nitrogen containing urine using deammonification process. **The first goal of this research was to evaluate the feasibility of simultaneous P recovery and N removal using a combination of chemical and biological processes.**

Unfortunately, apart from nutrients, urine also contains many trace organics (micropollutants) such as illicit drugs and estrogens in concentrations much higher than those generally present in municipal wastewater (Xiao and McCelly, 2000). These trace organics may range from estrogens to illicit drugs such as heroine and cocaine or their derivatives. (Wolgast, 1993) These trace organics, e.g. hormones and pharmaceuticals, can transport into the aquatic ecosystems via urine (Ritschel and Kearns, 1999). As much as 80% of the natural estrogens and 67% of the artificial hormone 17β-ethinyl estradiol (E2) are excreted via urine (review in Christiansen et al., 2002). Beside estrogen, the illicit drugs have become novel environmental contaminants through human consumption and excretion in recent years (Richardson, 2009; Zuccato and Castiglioni, 2009). The presence of illicit drugs might affect aquatic organisms, biota and the ecosystem as therapeutic drugs (van Nuijs et al., 2011b).

The fate of these micropollutants, particularly illicit drugs and estrogens, has not been given much attention during the aforementioned urine treatment/management strategies. For example, what is the fate of estrogens (or other micropollutants) during struvite precipitation and nutrient recovery from urine? Most of these micropollutants are hydrophobic in nature and hence, they may partition into sturvite crystals during the chemical precipitation process. This poses an important environmental concern because in this case, struvite coming from urine, will simply serve as transport medium for these estrogens when it will be used as a fertilizer. **The second goal of this research proposal is to investigate the fate of selected illicit drugs and estrogens during simultaneous P recovery and N removal processes.**

3. MATERIALS AND METHOD

3.1 nutrients recovery and removal from urine

In this applied research, we used the chemical recovery of phosphorus through struvite precipitation, and the treatment of nitrogen containing urine using deammonification process.

3.1.1 Struvite precipitation

Synthetic human urine containing 11 solutes, in concentrations equivalent to the daily average urine of normal healthy men was prepared according to conventional urological methods (Griffith et al., 1976) for use in the experiments. Struvite precipitation batch tests were conducted under three different conditions: 1) synthetic human urine supplemented with external urease (pH=9), 2) synthetic human urine without the addition of urease (pH=7), 3) synthetic human urine without the addition of urease (pH=9). Furthermore, under each condition, three different molar ratios (1:1, 1:1.5 and 1:2) of P: Mg were tested by adding $MgCl_2*6H_2O$ externally. The pH was adjusted using 10(N) NaOH solution and the tests were performed at the room temperature (20° C) with a reaction time of 3-hr.

3.1.2 Deammonification:

The deammonification includes two stages (nitritation, followed by anammox) and single stage deammonification processes. The reactors were fed with diluted urine after P recovery through struvite precipitation with 1:1.5 of P: Mg treatment, with extra bicarbonate.

1) Two stages: A 4 L nitritation sequencing batch reactor was seeded with biomass from a nitrification SBR as mentioned elsewhere (Meher et al., 2013). The nitritation reactor was operated on hydraulic retention time (HRT) of 2 day. Each cycle started with the addition of 1 L of 5%-15% synthetic urine after struvite precipitation, followed by 12 h of aerobic reaction and, concluded with 30 min of settling followed by decanting of 1 L of supernatant. The reactor temperature was maintained at ~30 °C using a heating pad. The effluent from nitritation reactor reactor was taken to the anammox reactor through an intermediate collection vessel. The anammox reactor started to operate as Meher at al., 2013 mentioned.

2) Single stage: A batch reactor is being operated 90hr to develop the operational strategy for one stage deammonification reactor in which case, the ammonia oxidizers were kept suspended but the anammox bacteria was immobilized on biocarrier. Dissolved oxygen (DO) was controlled around 0.5mg/L using DO sensor.

3.2 Effect of E2 on reactors performance

50mL serum bottle tests were performed using 1) biomass from nitritation reactor with diluted urine after struvite precipitation, under aeration condition; 3) anammox granules obtained from anammox bioreactor with diluted nitritation reactor, under anaerobic condition; 4) biomass from deammonification reactor with diluted urine after struvite precipitation, under low DO condition. In each group, controlled (without E2 spiked) and tested (with E2) samples were included.

3.3 Analytical method

Samples were collected, filtered (0.45μm) and analyzed. Chemical oxygen demand (COD), ammonia (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), and dissolved phosphorus (PO_4^{3-}P), were quantified using HACH methods 8000, 10031 (Salicylate method), 1002 (Chromotropic acid method), and 8153 (Ferrous sulfate method), and 8048 (Ascorbic acid method), respectively.

4. RESULTS AND DISCUSSION

4.1 nutrients recovery and removal from urine

4.1.1 Struvite precipitation

Table 1 shows the results of struvite crystallization. Since NH_4-N concentrations are much higher than PO_4^{3-}P after the hydrolysis, the molar ratio of Mg:P was the most significant factor for experiments conducted with urease added and the pH was maintained at 9 (condition 1). The PO_4^{3-}P removal efficiency is 95.06%, 98.98% and 99.79% for the Mg: P (molar ratio) as 1:1, 1:5 and 2:1, respectively. Although, these removal percentages are in close proximity, the general trend was that as the molar ratio of Mg:P increased, the percentage P removal also increased. The effluent PO4-P decreased to 1.31mg/L when the Mg:P increased to 2:1. When the struvite precipitation test without urease, which means no hydrolysis happen and the pH is neutral, only 40-50% P were removed (as table 1, condition 2 results shows).

The low P removal was not because of unavailability of ammonium but possibly due to kinetic limitations at low pH. Past research has shown that higher pH is preferred for struvite precipitation. The results (condition 3) with higher pH but no urease added were similar to the condition 1 when urease was added. The PO43-P removal efficiency in this case was 94.54%, 99.21% and 99.74% for the Mg: P (molar ratio) as 1:1, 1.5:1 and 2:1, respectively. It was surprising to see a significant P removal with no urease added at the higher pH. The addition of urease enhances ammonification of urine to generate ammonium. Struvite contains equal molar ratio of Mg, phosphate and ammonium. Based on our ammonium measurements, it is concluded that the amount of ammonium initially present even without adding urease was sufficient to support struvite precipitation.

Table 1 Result of struvite precipitation

Condition	Urease	NaOH	pH	Mg:P (molar ratio)	% P removal
1	added		9	1:1	95%
				1.5:1	99%
				2:1	100%
2			7	1:1	39%
				1.5:1	45%
				2:1	48%
3		added	9	1:1	95%
				1.5:1	99%
				2:1	100%

4.1.2 Deammonification

1) Two stage

Nitritation reactor proved to convert partial total ammonia concentrations in the influent from 300mg/L to 1000mg/L (Figure 1) to nitrite. The nitrite to ammonium ratio was 0.76± 0.17 on average, was lower than other studies (Udert et al., 2003; Feng et al., 2008; Udert et al., 2012). The reason could be the DO concentration in the reactor was not high enough compared with others, more air pumps will be

added. Nitrate in the effluent was only 5% of the influent total ammonia, which is closed with previous researches (Udert et al., 2003; Udert et al., 2012). The anammox reactor was in the start-up phase with the feed from nitritation reactor effluent.

1) Single stage

Table 2 shows results from a batch study of the single stage deammonification reactor. The results show that the single stage deammonification reactor can remove 66 $mgNd^{-1}$. In order to evaluate the nitrogen removal in this system, several relationships were considered: (i) the molar ratio of NH_4-N / NO_2-N, in nitritation is 1:1, (ii) the stoichiometric consumption (molar ratio) of NH_4-N / NO_2-N in anammox process is 1:1.32, and produces 0.26 moles of NO_3-N, subsequently that can be utilized in denitrification, (iii) 1 mg/L of NO_3-N is used for consuming 1.74 mg/L COD in denitrification. Based on these published ratios, we concluded that around 92.8% of the TN removal was due to deammonification and the rest was due to heterotrophic dinitrification of nitrate to nitrogen gas.

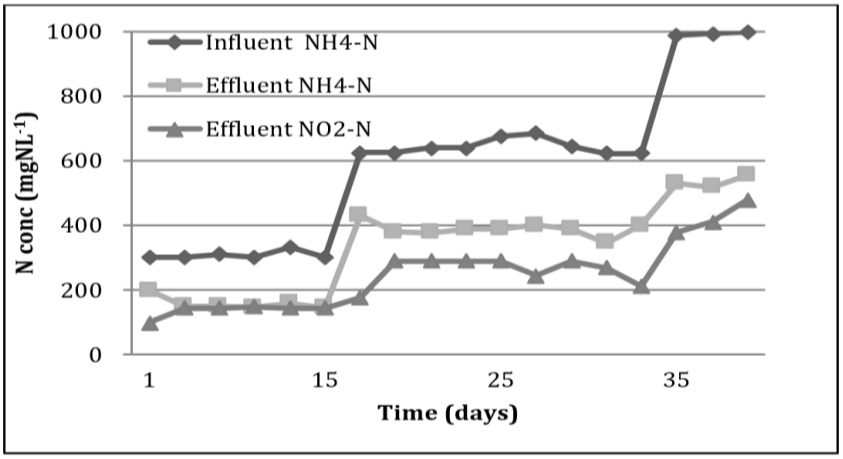

Figure 1 Performance of nitritation reactor

Table 2 Results from a batch study for single stage deammonification

	COD	NH_4-N	NO_2-N	NO_3-N	TIN	%N removal
Time (hr)	mg/L					%
0	78	250	0	0	250	-
18	76	201	7	0	208	19%
36	76	161	30	10	201	26%
54	70	103	52	20	175	37%
72	64	40	0	24	64	73%
90	60	0	0	20	20	83%

4.2 Effect of E2 on reactors performance

E2 concentration as high as 135ug/L (Wei et al., 2007; Hoffmanna et al., 2010) have been observed in the human urine, and 0.57pg/L in the river (Wei et al., 2007). In this study, we spiked 100mg/L E2 into the serum bottles to find out the effect of E2 on the deammonification processes. In the Table 3, 100mg/L E2 proved to be not an inhibitor during the deammonificaiton process. During the nitrification, the nitrification rate in then controlled bottle was 264 mg-N

oxidized/ (L×day), which was as similar as the one contained E2, 264 mg-N oxidized/ (L×day) (as Table 3 shown).

For the anammox, the initial serum bottle tests were performed using common dissolved ions that may found in the wastewater treatment plan. After the struvite presentation with urine, extra Mg could be left in the influent will continue to the anammox reactor, also high sulphate concentration is also contained in the urine. However, up to 500 mg-P/L, 1200mg-Mg/L, and 500mg-SO_4/L have been found did not affect the anammox processes (Dapena-Mora et al., 2007). According to Table 3, 100mg/L of E2 did not inhibit the anammox activity either. 37% Total inorganic nitrogen (TIN) were removed in the controlled bottle compare with the 36% in the tested (with E2) bottle, the 3% differences can be accounted for the method accuracy. The removal ratios of NO_2-N: NH_4-N (not shown in table) were consistently in the range of 1.1-1.3:1, which agrees with other observed removal ratios ranging from 1:1 to 1.7:1. Also the nitrate production was closed with Strous et al., 1999 study NO_3-N: NH_4-N ratio of 0.26:1.

In the single stage deammonification serum bottle test, the relative changes based on NH_4-N removal showed no difference between the serum bottles with and without E2. What's more, the nitrate production ratio (NO_3-N: NH_4-N) is 0.08-0.11:1, which is also closed to the theoretical stoichiometry of single stage deammonificaiton of 1:0.11.

Table 3 Nitrification, anammox and single stage deamonification activity in the serum bottles with the presence of E2

		A	B	C	D	E
Name	**E2 conc.**	**TIN rem.**	**NH_4-N rem.**	**NO_2-N rem.**	**Nitrification rate**	**relative changes**
	µg/L	**%**	**%**	**%**	**mg-N oxidized /(L×day)**	**%**
Nitritation[a]		**-**	**71%**	**-**	**264**	**100%**
	100	**-**	**71%**	**-**	**263**	**100%**
Anammox[b]		**37%**	**53%**	**39%**	**-**	**100%**
	100	**36%**	**50%**	**38%**	**-**	**97%**
Single stage[c]		**15%**	**19%**	**-**	**-**	**100%**
	100	**14%**	**19%**	**-**	**-**	**100%**

a:initial NH_4-N=370mg/L; $E = \frac{D(with\ E2)}{D\ (no\ E2)}$

b:initial TIN=107mg/L; $E = \frac{A(with\ E2)}{A\ (no\ E2)}$

c:initial NH_4-N=415mg/L; $E = \frac{B(with\ E2)}{B\ (no\ E2)}$

5. SUMMARIES AND FUTURE RESEARCH

This study investigated a continue process to recover phosphorous and remove nitrogen with urine. The recovery efficiency of PO_4^{3-}P using struvite precipitation process could reach above 95% with molar ratio of Mg:P of 1-2:1 simultaneously with urine hydrolysis. The

deammonification processes were developed to remove nitrogen from diluted synthetic urine after struvite precipitation in lab scale reactors. The serum bottles tests revealed that the reactors performances were not affected by up to 100ug/L E2. For the future research, the effect of other estrogens and illicit drugs on the reactor performance will be tested, also the degradation of estrogens and illicit drugs through the treatment process are also going to start.

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