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Microbial ureolysis in the seawater-catalysed urine phosphorus recovery system: Kinetic study and reactor verification

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ABSTRACT

Our previous study has confirmed the feasibility of using seawater as an economical precipitant for urine phosphorus (P) precipitation. However, we still understand very little about the ureolysis in the Seawater-based Urine Phosphorus Recovery (SUPR) system despite its being a crucial step for urine P recovery. In this study, batch experiments were conducted to investigate the kinetics of microbial ureolysis in the seawater-urine system. Indigenous bacteria from urine and seawater exhibited relatively low ureolytic activity, but they adapted quickly to the urine-seawater mixture during batch cultivation. During cultivation, both the abundance and specific ureolysis rate of the indigenous bacteria were greatly enhanced as confirmed by a biomass-dependent Michaelis–Menten model. The period for fully ureolysis was decreased from 180 h to 2.5 h after four cycles of cultivation. Based on the successful cultivation, a lab-scale SUPR reactor was set up to verify the fast ureolysis and efficient P recovery in the SUPR system. Nearly complete urine P removal was achieved in the reactor in 6 h without adding any chemicals. Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis revealed that the predominant groups of bacteria in the SUPR reactor likely originated from seawater rather than urine. Moreover, batch tests confirmed the high ureolysis rates and high phosphorus removal efficiency induced by cultivated bacteria in the SUPR reactor under seawater-to-urine mixing ratios ranging from 1:1 to 9:1. This study has proved that the enrichment of indigenous bacteria in the SUPR system can lead to sufficient ureolytic activity for phosphate precipitation, thus providing an efficient and economical method for urine P recovery.

1. Introduction

Phosphorus (P) recovery from source-separated urine has been widely accepted as a promising method not only to combat P depletion (Cordell et al., 2009; Mihelcic et al., 2011), but also to downsize the downstream wastewater treatment works and reduce severe eutrophication of water matrixes (Wilsenach and van Loosdrecht, 2003; Wilsenach and van Loosdrecht, 2004). P recovery is generally achieved by magnesium- and calcium-based precipitation of P, via dosing with magnesium and calcium salts (Rittmann et al., 2011). Seawater was recently proved to be an efficient precipitant for urine P recovery because of its high magnesium and calcium content (approximately 1200 mg/L and 470 mg/L in this study respectively) (Liu et al., 2013; Dai et al., 2014), which is particularly suitable for Hong Kong where seawater toilet flushing has been practiced since 1958 (Leung et al., 2012). In this new process, ureolysis ($(NH_2)_2CO + 2H_2O \rightarrow NH_3 + NH_4^+ + HCO_3^-$) is a prerequisite for the urine P recovery, as it provides abundant ammonium and a sufficiently high pH environment for phosphate precipitation (Musvoto et al., 2000; Udert et al., 2003a, 2003b; Tilley et al., 2008; Dai et al., 2014). In a previous study we have preliminarily shown that an extent of ureolysis of more than 40% can induce 98% urine P recovery in our Seawater-catalysed Urine Phosphorus Recovery (SUPR) system (Dai et al., 2014). The ureolysis rate should be much lower than the phosphate precipitation rate so that ureolysis would influence phosphate precipitation and consequently affect the P recovery from urine (Gethke et al., 2006; Tilley et al., 2008; Peng, 2012). Before the SUPR system can be applied in the real world, however, a thorough understanding of the ureolysis rate and its influencing factors is needed.

Ureolysis is generally catalysed by a group of enzymes called ureases, which are produced in numerous organisms including bacteria, plants, algae, fungi and invertebrates. Bacterially induced

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ureolysis can be 10^{13} times faster than uncatalysed ureolysis (Mobley et al., 1995; Krajewska, 2009). The presence of a high concentration of urea stimulates ureolytic bacterial growth in urine, both inside the human body (e.g. an infected urinary tract) and outside (e.g. a urine-collecting system) (Krajewska, 2009). For source-separated urine with indigenous ureolytic bacteria only, it takes more than a month to achieve complete ureolysis during storage, which is too slow for practical purposes (Fittschen and Hahn, 1998; Kabdaşli et al., 2006; Gethke et al., 2006). When contaminated by faeces, the time can be reduced to less than 20 days (Hotta and Funamizu, 2008a, 2008b). In the urine collecting pipes of NoMix system, complete ureolysis can be achieved within a few days because of a high bacterial abundance, but ureolysis-induced precipitation causes phosphorus loss and blockage problems (Udert et al., 2003a, 2003b). Ureolytic bacteria are also widely present in seawater, but their ureolytic activity varies significantly among bacteria isolated from different environments (Kelly and Stroh, 1989; Baffone et al., 2001; Obiri-Danso et al., 2001; Bansal, 2011). Therefore, urine and/or seawater indigenous bacteria could provide an *in-situ* ureolytic population to facilitate phosphate precipitation in the SUPR system, thus eliminating the need for a separate storage tank for preureolysis (Maurer et al., 2006). However, their ureolytic activity in this new, complicated system remains unknown. This knowledge is needed in the optimization and application of the system. Besides, enrichment might be necessary to achieve a high ureolysis rate to facilitate acceptable hydraulic retention time (HTR) in SUPR if the ureolytic potential of indigenous bacteria is too low (Fujita et al., 2008).

This study was designed to tackle these issues, and in particular to evaluate the influence of enrichment on the ureolytic activity of seawater and urine indigenous bacteria through batch cultivation and lab-scale Seawater-catalysed Urine Phosphorus Recovery (SUPR) reactor operation. It is expected that more than 40% of ureolysis and 90% of P removal in SUPR reactor can be achieved within 6 h of HRT. The corresponding microbial ureolytic activities and bacterial communities were investigated to reveal the role of microbial ureolysis in the seawater-urine system. Hopefully, a full-scale SUPR reactor with 90% of P recovery in 2–3 h can be realized in the near future based on the ureolysis mechanism revealed in this study.

2. Materials and methods

2.1. Urine and seawater collection

Urine was collected from a male toilet at The Hong Kong University of Science and Technology using a source separation urinal equipped with a cooling box. The collected urine was stored at 4 °C and was used within 24 h to inhibit ureolysis. Seawater was directly collected from a urinal flush nozzle in that toilet every day. The composition of fresh urine and seawater has been described in a previous study (Dai et al., 2014).

2.2. Batch study

In order to increase the ureolysis rate in seawater-urine mixture system, uroelytic activity of indigenous bacteria in seawater and urine without and with enrichment should be firstly investigated and compared to verify the feasibility of SUPR reactor. Therefore, experiment I and II were conducted.

2.2.1. Batch experiment I: ureolytic activity of indigenous bacteria in seawater and urine

Batch experiments were conducted to compare the ureolytic activity of two different bacterial communities—seawater autochthonous bacteria and urine autochthonous bacteria, hereafter referred to as seawater bacteria and urine bacteria. Toilet flush seawater and urine were collected and the bacterial abundance were determined to be $4.41 \pm 0.94 \times 10^5$ cells/mL and $3.93 \pm 2.76 \times 10^5$ cells/mL, respectively. Four different mixtures of seawater and fresh urine were sterilized by 0.22 μm filtration and put into an autoclaved beaker, with a total solution volume of 1 L and a seawater-to-urine ratio of 1:1, to assess the ureolytic activity of the bacteria. Control experiments with both sterilized urine and seawater were conducted in parallel. The initial bacterial abundance in each batch experiment, except the control experiment, was determined to be at the same order of magnitude (10^5 cells/mL), which allows the specific comparison. The ureolysis condition was closely monitored by measuring the total ammonium nitrogen ($NH_3 + NH_4^+$) during the experiments, and the ureolytic activity was reflected by the rate of ammonium nitrogen production (Zantua and Bremner, 1975; Kandeler and Gerber, 1988). Once the ammonium nitrogen concentration became stable, the ureolysis was assumed to be completed. As real urine was used in this study, the urine composition varied across batches. The ureolysis extent is defined by Eq. (1) for specific comparison.

$$\text{Ureolysis Extent} = \frac{\text{Ammonium Nitrogen (mgN/L)}}{\text{Total Nitrogen (mgN/L)}} \cdot 100\% \quad (1)$$

2.2.2. Batch experiment II: changes in overall ureolytic activity with bacterial population size during the cultivation process

Initially a 1 L seawater-urine mixture (1:1, if not specified) was used as the culture medium for the indigenous bacteria. Urea was hydrolysed by the ureolytic bacteria within the mixture. Once the ureolysis was completed, the solids and bacteria were harvested by centrifugation at 8500 g for 10 min and washed three times with a filter-sterilized seawater-urine mixture. All of the solids and bacteria were then resuspended to a 1 L filter-sterilized seawater-urine mixture for the next cycle of cultivation. These steps were repeated four times to achieve complete ureolysis within 4 h. During the experiments, the bacteria abundance and total ammonium nitrogen were closely monitored in each cycle. The ureolysis extent was calculated according to Eq. (1).

All the batch experiments (I and II) were repeated in triplicate and the results are given as the mean value and standard deviation unless otherwise specified. All experiments were conducted in autoclaved beakers with magnetic stirring homogenization in the dark at 23 ± 1 °C. Sampling and pH measurement were conducted regularly, and the sample amount was limited to a maximum total volume loss of 10%.

2.2.3. Modelling of microbial ureolysis

In order to assist the comparison of this study with other findings, modelling of microbial ureolysis was conducted based on the data obtained from experiment II. Kinetic rate parameters of microbial ureolysis were acquired by fitting the experimental results of the batch experiments II to a biomass-dependent Michaelis–Menten equation (Roden, 2008):

$$v = \frac{V_m \cdot [Urea]}{K_m + [Urea]} [B]/1000 \quad (2)$$

where v is the ureolysis rate (mmol/L/h); V_m is the maximum specific rate of ureolysis (mmol/cell/h); [Urea] is the concentration of urea (mmol/L); K_m is the half-saturation substrate concentration (mmol/L) at which $v = 0.5\ V_m \cdot [B]$; and [B] is the bacterial abundance (cell/mL).