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The recycle of water and nitrogen from urine in bioregenerative life support system

Shengda Deng [a,b,c], Beizhen Xie [a,b,c,*], Hong Liu [a,b,c,*]

[a] School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China
[b] Institution of Environmental Biology and Life Support Technology, Beihang University, Beijing 100191, China
[c] International Joint Research Center of Aerospace Biotechnology & Medical Engineering, Beihang University, Beijing 100191, China

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ABSTRACT

The recycle of the wastewater is one of the main factors for realizing a higher closure degree of bioregenerative life support system (BLSS), among which the treatment and recovery of the crew's urine are the most difficult and critical issues. Researchers have paid a lot of attention on the desalination of urine in the previous studies, however, if the nitrogen could be recycled simultaneously while desalting the urine, the substance circulation and the closure of BLSS could be improved more significantly. In this study, two-step method was conducted to treat the urine and recycle the water and nitrogen. The urine was hydrolyzed firstly, and then the water vapor and ammonia gas were cooled and collected by using reduced pressure distillation in alkaline condition. High temperature acidification method (HTAM) and immobilized urease catalysis method (IUCM) were investigated in the hydrolysis pretreatment of urine. The treatment conditions of both methods were optimized and the hydrolysis efficiencies were compared. The results showed that the optimum treatment temperature and acidity for HTAM were 99 °C and $[H^+]$ =2 mol/L when the reaction time was 7 h, and the maximum nitrogen recycle efficiency was 39.7%. While, the optimum treatment conditions for IUCM were 60 °C, pH=7.0 and 40 min, and the maximum nitrogen recycle efficiency could reach 52.2%. Therefore, compared with HTAM, IUCM has higher hydrolysis efficiency with milder reaction temperature and pH and shorter reaction time which means it could adapt to the heavy urine treatment workload in BLSS. This investigation has provided a promising method to recycle the urine in BLSS, and all the results will contribute to the further BLSS experiments conducted in the stage II of the "Lunar Palace 1".

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1. Introduction

For long-duration deep space exploration missions, oxygen, water, food and other supplies needed by humans can no longer entirely rely on the ground [1,2]. Thus, bioregenerative life support system (BLSS) should be established to satisfy the supply demands. The BLSS is featured by the in-situ regeneration of water, oxygen and food through biotechnologies and engineering control technologies basing on the principle of ecological system [3], in which the water regeneration is an important part for achieving high closure.

Urine is the most complex wastewater with a great deal of treatment difficulties in BLSS. The daily water demand for each crew is 1.86 kg/d, while the amount of urine excretion is 1.5 kg/d per crew with 95% of water. Thus if the water in crew's urine can be recycled, the pressure of the water supplies in deep space exploration will be greatly eased, and the material circulation will be highly promoted [4]. Furthermore, urine is rich in nitrogen, phosphorus, potassium and other nutrients which are required for plant growth. However, the large amount of NaCl dissolved in urine will lead to the salinization of the substrate which is negative for the plant growth. Therefore, how to effectively recover the N, P, K and other nutrients while desalting the urine is an important issue in BLSS [5].

Currently in the field of space life support system, many researches have been focusing on the water recycling technologies of the urine, which can be mainly divided into physical/chemical and biological methods. The physical/chemical methods, including distillation [8,9] and membrane separation methods [6,10] etc., are well developed for recycling most of the water in the urine by separating and discharging the organic pollutants and mineral salts [7,8]. Normally, urine was biologically treated as plants' fertilizer [11], or to remove urea and recycle nitrogen by bioreactors

Abbreviations: BLSS, bioregenerative life support system; HTAM, high temperature acidification method; IUCM, immobilized urease catalysis method; TN, total nitrogen; UHE, urine hydrolysis efficiency; T, temperature; RT, reaction time

* Corresponding authors at: School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China.
E-mail addresses: xiebeizhen@buaa.edu.cn (B. Xie), LH64@buaa.edu.cn (H. Liu).

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[12,13]. Compared to physical/chemical methods, biological methods have comparatively low energy consumption, relatively simpler devices, and bigger possibility to recycle a large number of elements. However, longer processing time may also be required. In Micro-Ecological Life Support System Alternative (MELiSSA) [14], thermophilic anaerobic bacteria, photoautotrophic/heterotrophic bacteria and nitrifying bacteria cells were used to convert the nitrogen in urine into nitrate [15,17], which was finally supplied as a nitrogen source for photoautotrophy algae and higher plants [16]. Our research team have previously studied the cultivation of *Spirulina platensis* for urine treatment, and the results showed that the consumption of Cl^- could reach to 75% while using the N and P in urine as a nutrient source for spirulina [18]. These studies provided references for the design of urine desalination and nitrogen recycling technology in BLSS.

In order to solve the theoretical and practical problems in BLSS, our research team developed a integrative experimental system "Permanent Astrobase Life-support Artificial Closed Ecosystem"–"Lunar Palace 1" (stage I) in October 2013, and successfully carried out a 105-day closed bioregenerative life support experiment with multi-crew involved from January to May in 2014 [3]. During the 105-day experiment, the crew's urine in Lunar Palace 1 was treated by reduced pressure distillation method. The results indicated that all the water in urine has been recovered, but the nitrogen recycle efficiency was merely 20.5%. Thus, the process of the nitrogen recycle from urine still needs to be further optimized.

In this study, two-step method was conducted to treat the urine and recycle the water and nitrogen simultaneously. The urine was pre-hydrolyzed, and then the water vapor and ammonia gas were collected by using reduced pressure distillation in alkaline condition. High temperature acidification method (HTAM) and immobilized urease catalysis method (IUCM) were studied during the urine hydrolysis pretreatment. The processing factors of both methods were optimized and their influences on the nitrogen recycle efficiency were compared. The results might be applied to the further BLSS experiments in "Lunar Palace 1" (stage II).

2. Materials and methods

2.1. Urine samples

Real human urine collected from the crew of "Lunar Palace 1" was used in this research. Before each experiment, the ionic composition and the urea concentration of urine were tested as well as total nitrogen (TN) (Table 1), and the results indicated that the crew's urine contains large amounts of urea and inorganic salts. Since the water in urine would be recycled to irrigate the higher plants in BLSS, it is necessary to desalt the urine in case it would inhibit the higher plants growth, and recycle the nitrogen as much as possible at the same time. The main organic composition of real human urine is shown in Table 2 [19,20].

Table 1
Ionic composition, urea and TN concentrations of real human urine.

Real human urine ions (mg/L)	
Na^+	8740.3 ± 80.6
NH_4^+	304.2 ± 4.1
K^+	1628.75 ± 52.77
Mg^{2+}	146.03 ± 9.94
Ca^{2+}	109.19 ± 8.24
Cl^-	9534.51 ± 10.59
NO_3^-	111.55 ± 0.12
SO_4^{2-}	1542.94 ± 1.91
PO_4^{3-}	1186.44 ± 2.60
Urea	25744.7 ± 1534.7
TN (g/L)	12.02 ± 0.72

Table 2
Organic composition of real human urine.

Organic impurities (g/L)	
Creatine	1.3–1.5
Hippuric acid	0.10–1.6
Uric acid	0.20–0.80
Phenol	0.10–0.30
Fatty acid	0.03–0.06

2.2. Optimization of HTAM

In HTAM, the urea in urine was firstly hydrolyzed to ammonia in high temperature and acidic conditions, and then distilled in reduced pressure to recover the ammonia and water as much as possible. Temperature and pH were two significant parameters in HTAM. Therefore, single factor experiments were designed to optimize them and the experimental procedures were as follows: 1) Four samples of urine, each 150 mL, were added to 500 mL round-bottom flasks and placed in the water bath of rotary evaporator (RE-2000A, Shanghai Yarong, China), and the bath temperatures were respectively adjusted to 70 °C, 80 °C, 90 °C and 98 °C. The pH of all samples was maintained at 2.0 by hydrochloric acid (1 mol/L) and the reaction time was set to 7 h. When the hydrolysis process finished, the urine was adjusted to alkaline condition by adding sodium hydroxide solution (1 mol/L) and then distilled. The TN of urine before distillation and the NH_4^+–N concentration of the condensate water of each sample were detected, and the nitrogen recycle efficiency and the constant K of urine hydrolysis rate were calculated and compared accordingly to obtain the optimal temperature. The volume of urine and condensate water were also measured to calculate the water recycle efficiency; 2) Another four samples of urine were prepared and hydrolyzed under the pH of 1.5, 2.0, 2.5 and 3.0, respectively, and the temperature was maintained at 90 °C. After hydrolysis treatment for 7 h and distillation, the same operation as above was repeated to confirm the optimal pH.

2.3. Optimization of IUCM

2.3.1. Urease immobilization process

The urease powder (TOYOBO CO, Japan) was dissolved in 6% gelatin solution at 35 °C and the urease concentration was controlled to 1.0 g/L, followed by 30 min condensing at 4 °C. Thereafter, the condensed urease–gelatin mixture was cut into 1 cm × 1 cm masses, and cross-linked with 5 g/L glutaraldehyde solution for 90 min. The prepared immobilized urease was restored in 1% urea solution at 4 °C.

2.3.2. Optimization of immobilized urease pretreatment conditions

Single factor experiments were also conducted to optimize the three significant parameters, which were temperature, pH and reaction time, in IUCM, and the experimental procedures were as follows: (1) Six samples of urine, each 150 mL, were added to 500 mL round bottom flasks, each with 50 g of immobilized urease. Then the samples were hydrolyzed in the water bath of rotary evaporator under the temperature of 30 °C, 40 °C, 50 °C, 60 °C, 70 °C and 80 °C, respectively. The pH of each sample was maintained at 7.0 by adding phosphate buffered solution (0.1 mol/L Na_2HPO_4 + 0.1 mol/L NaH_2PO_4) and the reaction time was set to 60 min. After the hydrolysis process, the immobilized urease was

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