Journal of Environmental Management 198 (2017) 63–69

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Lactic acid fermentation of human urine to improve its fertilizing value and reduce odour emissions

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ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form
12 April 2017
Accepted 19 April 2017
Available online 25 April 2017

Keywords:
Human urine
Lactic acid fermentation
Urine hydrolysis
Nutrient recycling
Odour emissions control
Ecological sanitation

ABSTRACT

During storage of urine, urea is biologically decomposed to ammonia, which can be lost through volatilization and in turn causes significant unpleasant smell. In response, lactic acid fermentation of urine is a cost-effective technique to decrease nitrogen volatilization and reduce odour emissions. Fresh urine (pH = 5.2–5.3 and NH_4^+-N = 1.2–1.3 g L^{-1}) was lacto-fermented for 36 days in closed glass jars with a lactic acid bacterial inoculum from sauerkraut juice and compared to untreated, stored urine. In the lacto-fermented urine, the pH was reduced to 3.8–4.7 and the ammonium content by 22–30%, while the pH of the untreated urine rose to 6.1 and its ammonium content increased by 32% due to urea hydrolysis. The concentration of lactic acid bacteria in lacto-fermented urine was 7.3 CFU ml^{-1}, suggesting that urine is a suitable growth medium for lactic acid bacteria. The odour of the stored urine was subjectively perceived by four people to be twice as strong as that of lacto-fermented samples. Lacto-fermented urine induced increased radish germination compared to stored urine (74–86% versus 2–31%). Adding a lactic acid bacterial inoculum to one week old urine in the storage tanks in a urine-diverting dry toilet reduced the pH from 8.9 to 7.7 after one month, while the ammonium content increased by 35%, probably due to the high initial pH of the urine. Given that the hydrolyzed stale urine has a high buffering capacity, the lactic acid bacterial inoculum should be added to the urine storage tank of a UDDT before urine starts to accumulate there to increase the efficiency of the lactic acid fermentation.

1. Introduction

Over the last decade, concern has grown in the world for efficient application of artificial nitrogenous fertilizers in order to reduce adverse environmental impacts including eutrophication, greenhouse gas effects and acid rain (Gastal and Lemaire, 2002). Alternative natural fertilizers capable of replacing or complementing mineral fertilizers need to be considered. Source-separated human urine is an excellent fertilizer that could be applied more widely owing to its nutrient content and universal availability. Fertilization of okra, cabbage, tomatoes and cucumber with urine generated similar or even higher yields compared to chemical fertilizers (Akpan-Idiok et al., 2012; Heinonen-Tanski et al., 2007; Pradhan et al., 2007, 2009). In fresh human urine, most of the nitrogen (75–90%) is present as urea [$CO(NH_2)_2$], with smaller amounts of uric acid, amino acids and other substances. When urine leaves the body (Kirchmann and Pettersson, 1994), only 7% is in the form of ammonia. In addition to nitrogen, urine contains phosphorous ($H_2PO_4^-$ and HPO_4^{2-}) and potassium (K^+) in ionic forms, calcium (Ca^{2+}), sulphate (SO_4^{2-}) and soluble organic matter (Maurer et al., 2006), which have a fertilizing effect as well.

Urine-diverting dry toilets (UDDT) and waterless urinals are ideal systems for harvesting urine for use as fertilizer. However, misuse of UDDT systems may lead to cross-contamination of urine with faeces and thus expose it to pathogens. To reduce the pathogen content to a safe level, it is recommended that urine be stored for 1–6 months (Jaatinen et al., 2016; WHO, 2006). During storage,

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http://dx.doi.org/10.1016/j.jenvman.2017.04.059
0301-4797/

under the influence of bacterial activity and particularly the enzyme urease, urea is degraded into ammonia and carbon dioxide, which raises the pH these being the crucial factors for reinforcing pathogen inactivation (Nordin, 2010). At pH 8.9–9.0, 95% of the nitrogen in the stored urine is constituted of ammoniacal nitrogen (Kirchmann and Pettersson, 1994). As a result of becoming more alkaline and increasing the bicarbonate and ammonia concentration, the buffering capacity of urine increases as well (Udert et al., 2006).

Once nitrogen is in form of ammonia, it can evaporate into the air (Udert et al., 2006). Aside from impacting the recovery efficiency of nitrogen, this triggers undesirable odour emissions which are intensified by other malodorous components, e.g. volatile fatty acids which are released by bacteria (Zhang et al., 2013). Odour emissions are a nuisance for toilet users, as well as for the neighbours of the agricultural fields where urine is applied.

Different methods have been proposed to reduce ammonia volatilization and inhibit urea decomposition in urine. This can be successfully achieved by adding strong acetic or sulphuric acids (2.9 g L^{-1}) to keep the pH below 4 (Hellström et al., 1999), but this is not widely applied due to the cost of the acids and health risks involved in their handling (Maurer et al., 2006). There is also limited knowledge about the impacts these acids may have on soils and crops. An alternative method for stabilizing nitrogen is biological nitrification with the use of ammonia and nitrite-oxidizing bacteria, however sustaining bacterial activity in high strength ammonia solutions as urine is challenging and requires skilled staff (Udert and Wächter, 2012). Thus, there is a need to develop a cost-effective method to acidify urine that safeguards its fertilizer value and land applicability, minimizes energy input, and does not require sophisticated technical skills.

Lactic acid fermentation (LAF) of urine is a simple and economical technique that can be carried out with different homemade fermented products that contain lactic acid bacteria (LAB), e.g. sauerkraut juice (Beganovic et al., 2014), and a source of carbohydrates. Moreover, there are no major health or environmental risks during urine treatment and there is no need for large investments or highly skilled staff. This study, therefore, focused on the efficiency of LAF for treating urine. The change in pH, chemical oxygen demand (COD) and ammonium concentration, buffering capacity and odour as well as the potential biological effects on plant germination of LAF treated urine was compared to that of (untreated) stored urine.

This research expands the knowledge regarding the sensitive practicalities of resource-oriented sanitation, which is currently limited. Maurer et al. (2006) suggest that focusing on the prevention of urea hydrolysis as the ultimate goal of urine stabilization, as it prevents nitrogen loss via ammonia volatilization and organic matter degradation (the main causes of odour), together with the precipitation of phosphorous compounds (the main cause of pipe clogging). This research benefits urine management of UDDTs since LAF of urine can reduce ammonia loss and unpleasant odours, while improving the fertilizing value of urine. The LAF process can also contribute to the potential of urine for fertigation (incorporation of fertilizer into irrigation water), by decreasing the risks of blocking the drip irrigation sets due to phosphorus precipitation.

2. Material and methods

2.1. Experimental set-up

Storage and LAF of fresh urine was performed under laboratory conditions in two trials, each with three replicates, over two periods of 36 days each time (in this period the pH is lowered to 3.8–4.7, thus hampering the urea hydrolyzation). The experiment was carried out from December 2015 to January 2016 and from April to May 2016. The urine was kept at room temperature (20 ± °C), and shielded from direct sunlight exposure. Urine samples were collected from 2 donors (a female 44-year old and a 7-year boy) for a period of three days and stored in 1 L glass jars tightly closed with a plastic lid. At the end of the collection period, all urine of both donors was thoroughly mixed, chemical analysis was performed and then the urine was separated into two parts. The first part was mixed with a LAB solution (1:1) and LAF proceeded in the glass containers for a period of 36 days. The second part was stored in parallel in tightly closed glass containers, without any additions for the same period of time.

The LAB solution was obtained by fermenting chopped cabbage over a period of one month, after which sauerkraut juice was extracted, collected, then mixed with sugar beet molasses and water at a proportion of 1:1:9 and kept in a closed plastic jar until the pH was reduced to below 5.

After the treatments, chemical analysis was performed in both the LAF and stored urine samples. In addition, the LAB solution as well as the LAF urine was analysed for their *E. coli* and LAB concentration. No analysis of *E. coli* was performed in the untreated, simply stored urine.

The efficiency of LAF was also evaluated at field conditions in a functioning household UDDT in the vicinity of Chisinau (Moldova), used by a family of two adults (45-year-old male and 44-year-old female) and one seven-year-old boy. In this test, the 300 L plastic urine storage tank and the urine pipes were thoroughly washed and rinsed with vinegar prior to the experiments. Then, urine was collected in the tank for a period of one week (for pH and ammonia analysis) after which the LAB inoculum and molasses were added to the tank at the same ratio as in the laboratory experiments, which reduced the pH below 4.5. Each time the toilet was used, the urinal and urine compartment were sprayed with the inoculated LAB solution.

2.2. Odour evaluation

The intensity of the odour of LAF and stored urine was evaluated by four people (2 men and 2 women) independent from each other. Perception of the strength of the perceived odour was evaluated according to a rank scale from 0 (no odour) to 6 (extremely strong odour) as described in Table 1 (Misselbrook et al., 1993).

2.3. Germination tests

To evaluate if the urine samples stimulate germination, they were firstly diluted 1:10 with distilled water and then 3 ml was added to Petri dishes on Whatman filter pads together with twenty seeds of radish *Raphanus sativus*. As control, 3 ml of distilled water was used. The urine dilution rate (1:10) was obtained after a few germination tests and was required to adjust for the low pH of the LAF urine. After 72 h, germination was terminated by adding 3 ml of 50% alcohol to each of the Petri dishes (Tiqua et al., 1996). The

Table 1
Rank scale for different perceived urine odour strengths.

Perceived odour strength	Rank scale
No odour	0
Very faint odour	1
Faint odour	2
Distinct odour	3
Strong odour	4
Very strong odour	5
Extremely strong odour	6