Ecotoxicology and Environmental Safety 157 (2018) 249–254

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Optimization of lactic acid fermentation for pathogen inactivation in fecal sludge

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ARTICLE INFO

Keywords:
Fecal sludge
Fecal coliform
Lactic acid fermentation
Lactobacillus
Fermented cassava flour
Fermented rice flour

ABSTRACT

The efficiency of lactic acid fermentation (LAF) as a pretreatment for human feces was investigated in laboratory-scale experiments that lasted for 3 weeks. The sanitization effect of LAF on fecal sludge (FS) was conducted in triplicate. This study used three materials, namely, lactobacillus of lactic acid bacteria, fermented cassava flour, and fermented rice flour, which were known to enhance the production of lactic acid. Each material was mixed in three different reactors at equal ratio with raw FS (i.e., 1:1 v/w, w/w, and w/w). The pH decline rate, lactic acid production rate, and fecal coliform suppression degree were monitored over the period of the treatment process as parameters to evaluate the efficiency of various LAF for pathogen inactivation in FS. Results showed that only fermented rice flour was able to completely inactivate the indicator organism (fecal *coliform*) at the end of fermentation. Final plate counts of 8.6×10^8 CFU/100 mL, 2.4×10^8 CFU/100 mL, and zero (0) were achieved from lactobacillus, fermented cassava flour, and fermented rice flour treatment processes, respectively. The final pH from the reactors that contained lactobacillus and FS, cassava flour and FS, and fermented rice flour and FS were 5.5, 8, and 3.9, respectively. This study revealed that not all LAF materials can effectively suppress pathogens in FS. The results serve as the foundation in developing an effective, cheap, and easy to use LAF on FS pretreatment for pathogen inactivation.

1. Introduction

Adequate facilities for the safe treatment or disposal of fecal sludge (FS) should be established globally. FS causes several harmful diseases if not properly managed (al-Ghazali and al-Azawi, 1990; Bracken and Ysunza, 2005; Jiang et al., 2002). Approximately 2.5 billion people globally do not have access to adequate sanitation facilities (UN, 2012), and more than 0.7 billion people globally lacked access to clean water because the water is polluted by people's own feces (UN, 2012). A study revealed that about 1800 children die every day due to diseases associated with inadequate hygiene, lack of sanitation, and contaminated water (UNICEF, 2013).

To address these issues, the millennium development goal (MDG) target aimed to reduce the 2.5 billion people without adequate sanitation in half by 2015. Unfortunately, this target was not feasible at the end of 2015. Considering that the world's population is expected to reach 9.6 billion in 2050, a suitable method for dealing with FS, especially on the processes that enable the sustainable and efficient recovery of resources, should be developed (UN, 2012; Yemaneh, 2015). In the past few years, dry and low water sanitation has gained worldwide attention with the concept that FS can be directly processed at the point of recovery or collected and transported to the point of treatment (Mackie Jensen et al., 2008; Verbyla et al., 2016; Yemaneh et al., 2012). Several products, such as fertilizers, biofuels, water, biogas and compost, can be recovered after treatment (Bracken and Ysunza, 2005; Jepsen et al., 1997; World Health Organization (WHO) (WHO) and (UNICEF), 2014).

Several methods have been introduced to successfully treat FS for hygienic end-product for re-use or disposal (Allievi et al., 1994; Anderson et al., 2015a; Factura et al., 2011; Feachem, 1983; Magri et al., 2015). Lactic acid fermentation (LAF) has been reported to successfully suppressed pathogens in FS and organic material preservation (Anderson et al., 2015b). LAF is a metabolic process in which lactic acid fermenting organisms (LAB) easily metabolize degradable carbohydrates to lactic acid. LAB have the capability to convert carbohydrates to lactic acid, and the genera *Leuconostic*, *Lactobacillus*, and *Streptococcus* are used for food preservation by fermentation industries and sanitation agencies (Vandenbergh, 1993). The reduction of pH during lactic acid fermentation and the production of antimicrobial compounds are effective natural and cheap processes to effectively

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https://doi.org/10.1016/j.ecoenv.2018.03.075
Received 5 December 2017; Received in revised form 24 March 2018; Accepted 27 March 2018

eliminate non-desirable microorganisms and pathogens when applied for hygienization (Noike et al., 2002). Lactic acid reduces the bulk pH of the surrounding medium that influences the activity of membrane-bound enzymes and exo-enzymes (Anderson et al., 2015b). In addition, the capability of lactic acid to suppress pathogens is partially attributed to its capability to penetrate the cytoplasmic membrane of micro-organisms in the associated form, which results in the decline on intracellular pH of pathogens. Zhu et al. (2006) reported that the survival of pathogens pH was reduced at pH less than 3.5 although the suppression of bacteria requires a pH of less than 2.5. Therefore, the key antimicrobial property of lactic acid is the capability to suppress the intracellular of bacteria (Anderson et al., 2015b). Scheinemann et al. (2015) reported that bacteria pathogens, such as *E. coli, salmonella, and Staphylococcus aureus* were eliminated from cow manure after few days of fermentation. Anderson et al. (2015b) also reported that fecal *coliform and E.* coli were reduced below detection limit within 1 week of FS treatment with LAB created through the mixture of fermented milk that contained *lactobacillus casei* and pasteurized whole milk.

Although several studies have been conducted to investigate the sanitizing potential of lactic acid within the food industry (Anderson et al., 2015b; Mante et al., 2003), studies that focused on the sanitizing potential of lactic acid on pathogens present in FS are limited. In addition, no concrete studies have been conducted to compare the efficiency of LAF of FS. An experiment was conducted in this study to compare the LAF treatment efficiency of FS with LAF materials, namely, lactobacillus strain of LAB, fermented cassava flour, and fermented rice flour. The results served as the foundation for the research on producing effective, cheap and easy to use LAF for safe application or disposal of FS hygienization.

2. Materials and method

2.1. Origin of fecal sludge and experimental set-up

FS was collected from the septic tank of the University of Science and Technology, Beijing. The initial characteristics are shown in Table 1. FS was collected from the septic tank at the same day of fermentation with the prepared lactic acids to avoid the changes in microbial communities and pH variation. FS was transported to the laboratory in a 10 L bucket. In the laboratory, a suction pump was used to fill each of the four 5 L buckets with approximately 1 kg for control bucket, 1 kg for bucket marked with 1:1 of FS and lactobacillus, 1 kg for the bucket with 1:1 of FS and fermented cassava flour, and 1 kg for bucket marked with 1:1 of FS and fermented rice flour. Each of the buckets was designated as reactors 1–4. Prior to the commencement of the stabilization experiments, 50 g of raw FS was used for the analysis on physical and microbial properties. The indicator organism, fecal coliform, was used to assess the overall sanitation efficiency of various fermentations. Serial dilution of the fecal sample was conducted in deionized water for microbial test. Fecal coliform count was determined using a membrane method with coliform agar, followed by incubation at 48 °C for 24 h.

Table 1
Initial fecal sludge characteristics.

Parameters	Unit	Initial fecal sludge characteristics Values
Temperature	°C	21
pH		7.53
Total solid	%	14.41
Fecal coliform	CFU/100 mL	3.1×10^8

3. LAF

The current approach for lactic acid production uses food waste and agricultural products. The three materials used were distinguished into different materials. *Lactobacillus* strain and cassava were classified as starch materials, and rice was classified as cellulose products. All the three fermentations lasted for 6 d. The initial volume of lactobacillus used was 40 mL, cultured every 24 h, and was enlarged to the required volume of 1 L with the addition of deionized water for the period of 6 d at room temperature for the LAB to grow to a large quantity before mixing with FS for the treatment process. For the fermentation in cassava flour, dry cassava flour was soaked with deionized water and was allowed to ferment for a period of 6 d. The main stages involved in the lactic acid production from fermented rice flour included steaming, soaking, extruding, boiling, cooling, and fermenting. Soaking is important for the production of fermented rice flour because it increases the water content of rice and enhances natural fermentation. pH values of 4.2, 3.8, and 3.4 were achieved from *lactobacillus*, fermented cassava flour, and fermented rice flour on the 6th day of fermentation. Fig. 1 shows the LAF process of lactobacillus, fermented cassava flour, and fermented rice flour.

3.1. Analytical method

The total solid content of initial FS and after treatment were assessed using the 2540E standard method for wastewater examination. The LAF treatment process was monitored by measuring the concentration and accumulation of lactic acid bacteria and pH variation. The use of lactic acid for the inactivation of fecal coliform was conducted by evaluating the survival rates of pathogens through a total viable plate count method and pH changes during the treatment process. LAB was cultured using a pour plate technique on lactobacillus select agar and were incubated at 37 °C for 24 h. The total viable counts of bacteria colonies on solidified agar plates using chromogenic agar technique were used to measure the level of fecal coliform suppression. The fecal coliform count was determined using the membrane method with coliform agar, followed by incubation at 48 °C for 24 h. The treatment period was set as the time required for fecal coliforms to appear on the agar. The number of fecal coliforms (CFU/100 mL) was determined for each analyzed sample by using Chromocult® Coliform agar. The culture media was prepared using standardized protocols and reagents under sterile conditions. This process was conducted in an electric oven operated aerobically. The pH values during the treatment processes were determined by taking 10 g of sample from each reactor that was dissolved in 100 mL distilled water. The dissolved portions were stirred for 15 min. After settling, the liquid portion was measured through potentiometric measurement using standard pH electrode.

4. Results and discussion

4.1. Lactic acid production

To determine the lactic acid content and production efficiency of LAF from fermented materials and their fermentation efficiency when applied in LAF of FS, the community composition and dynamics of LAB associated with *lactobacillus*, cassava flour, and rice flour fermentation were investigated by using a culture-dependent approach. Single strain of the fermentation materials (i.e., *Lactobacillus fermentum*, *Lactobacillus amylovorus*, and *Lactobacillus Sp.*) was isolated. The results showed that *Lactobacillus Sp.,* which was a strain from fermented rice flour, produced high lactic acid content compared to cassava flour and *lactobacillus*. Furthermore, the lactic acid production efficiency of fermented rice flour was higher than that of cassava flour and *lactobacillus*, as shown in Table 2. This result revealed that fermented rice flour can be an effective substrate for LAF of FS for pathogen reduction and odor control due to its efficiency in producing high amounts of lactic acid