Bioresource Technology 206 (2016) 36–42

Contents lists available at ScienceDirect

Bioresource Technology

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

Potential application of *Alcaligenes faecalis* strain No. 4 in mitigating ammonia emissions from dairy wastewater

George M. Neerackal [a], Pius M. Ndegwa [a,*], Hung-Soo Joo [b], Xiang Wang [a], Craig S. Frear [a], Joseph H. Harrison [c], Marc W. Beutel [d]

[a] Department of Biological Systems Engineering, Washington State University, PO Box 646120, Pullman, WA 99164, USA
[b] School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 123 Cheomdan-Gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea
[c] Department of Animal Sciences, Washington State University, 2606 West Pioneer, Puyallup, WA 98371, USA
[d] School of Engineering, University of California Merced, 5200 North Lake Road, Merced, CA 95343, USA

HIGHLIGHTS

- A bioprocess for mitigating NH_3 emissions from dairy wastewater is presented.
- The bacterium *A. faecalis* strain No. 4 is viable for treating dairy wastewater.
- Flushing reactor headspace with oxygen enhances this bioprocess.
- Supplementing the wastewater with carbon also boosts this bioprocess.

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 9 January 2016
Accepted 10 January 2016
Available online 25 January 2016

Keywords:
Biological nitrogen removal
Batch aeration
Oxygenation
Nitrogen gas
Nitrification-denitrification

ABSTRACT

This research examined the potential mitigation of NH_3 emissions from dairy manure via an enhanced aerobic bio-treatment with bacterium *Alcaligenes faecalis* strain No. 4. The studies were conducted in aerated batch reactors using air and pure oxygen. Aeration with air and oxygen removed approximately 40% and 100% total ammoniacal nitrogen (TAN), respectively. Intermittent oxygenation (every 2 or 4 h) reduced oxygen consumption by 95%, while attaining nearly identical TAN removal to continuous aeration. The results revealed that adequate oxygen supply and supplementing dairy wastewater with carbon are essential for this bioprocess. Based on the nitrogen mass balance, only 4% of TAN was released as NH_3 gas, while the majority was retained in either the microbial biomass (58%) or converted to nitrogen gas (36%). The mass balance results reveal high potential for environmentally friendly bio-treatment of dairy wastewater using *A. faecalis* strain No. 4 with respect to NH_3 emissions.

1. Introduction

Biological treatments offer an attractive method for mitigating ammonia (NH_3) emissions from livestock wastewaters. Conventional nitrogen removal processes from wastewater traditionally consists of two stages: (i) aerobic autotrophic nitrification, and (ii) anaerobic heterotrophic denitrification. Nitrification transforms ammonium (NH_4^+) to oxidized nitrogen compounds ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$); and these compounds are further reduced to nitrogen (N_2) gas via denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (Sun et al., 2010; Junter et al., 1995; Focht and Chang, 1975). However, biological removal of ammonium in a conventional treatment system faces several problems including: (i) an extremely slow nitrification step, (ii) strong sensitivity to oxygen limitation, (iii) negative impacts via overloading of ammonium and organic matter, and (iv) requirement of two separate reactors for nitrification (an aerobic process) and denitrification (an anaerobic process) (Shoda and Ishikawa, 2014; Zhu et al., 2008; Joo et al., 2005). The low nitrification rates in this process result in the need for long hydraulic retention times or large reactor volumes to accomplish complete NH_4^+ removal. Consequently, conventional treatment demands multiple and larger reactors and high capital and operation costs (Zhu et al., 2008; Szögi et al., 2004).

Over the past two decades, several new bio-processes for ammonium removal from municipal and domestic wastewaters have been developed, including: simultaneous nitrification and denitrification, shortcut nitrification and denitrification, anaerobic ammonium oxidation (ANAMMOX), aerobic deammonification,

* Corresponding author. Tel.: +1 509 335 8167; fax: + 1 509 335 2722.
E-mail address: ndegwa@wsu.edu (P.M. Ndegwa).

http://dx.doi.org/10.1016/j.biortech.2016.01.063
0960-8524/

complete autotrophic nitrogen removal over nitrite (CANON), oxygen limited nitrification and denitrification (OLAND), advanced treatments using combination of theses process (anaerobic/oxic/anoxic process, step-feed multistage anaerobic/oxic process, and membrane bioreactors), and cell-immobilization systems. These technologies possess promising features for NH_4^+ removal from dairy wastewaters. However, these processes also have some potential problems or limitations similar to conventional nitrogen removal processes, such as reduced nitrification rates, longer retention times, large reactor volumes, and high operational costs which similarly limit their applications (Sun et al., 2010; Zhu et al., 2008; Junter et al., 1995).

In conventional aerobic treatment of high strength ammonia wastewaters, aeration represents the major operating cost (Ahmed et al., 2004; Water Pollution Control Federation, 1988). The use of pure oxygen gas to maintain aerobic systems has recently generated interest in wastewater treatment systems as an alternative to address the drawbacks of using air to oxygenate these systems (Palmer et al., 2009; Beutel and Horne, 1999; Brindle et al., 1998). Furthermore, use of pure oxygen to oxygenate aerobic systems has indicated potential of reducing the cost of aerating these systems (Speece, 1996). In general, systems that use pure oxygen gas rather than ambient air (21% O_2 by volume) demonstrate better oxygen transfer efficiency, are simple and compact, allow for easier gas storage and handling, and have lower operating costs. While numerous studies have evaluated aerobic treatment system using air-based aeration methods for removing ammonia from livestock wastewaters, no studies in the literature have focused on the use of oxygenation using pure oxygen gas to drive such systems. Therefore, one component of this study was to evaluate the use pure oxygen gas against ambient air, for driving such systems.

The central hypothesis of this study was that using a unique heterotrophic microorganism *Alcaligenes faecalis* strain No. 4 and aeration using pure oxygen gas would enhance the bio-treatment process to mitigate NH_3 emissions from dairy wastewaters. Furthermore, it anticipated that this bio-treatment process would overcome the conventional nitrification–denitrification drawbacks outlined previously in this paper. The bacterium *A. faecalis* strain No. 4 has the unique ability to consume carbon (C) and remove ammonium from wastewater mainly via nitrogen (N_2) gas and microbial assimilation in a single aerobic process (Joo et al., 2007, 2006, 2005). This is an attractive approach to biological ammonium removal from dairy wastewater because the conventional pathway of its removal via N_2 can be shortened via single aerobic process ($NH_4^+ \rightarrow NH_2OH \rightarrow N_2$). Although a few studies have been conducted with *A. faecalis* under various ammonium (low and high strength) concentrations (Shoda and Ishikawa, 2014; Zhao et al., 2012; Joo et al., 2007, 2006, 2005), to date, no studies have used *A. faecalis* strains for treating high strength ammonium dairy-cattle manure wastewater.

The goal of this study was to search for a cost-effective bioprocess for mitigation of NH_3 emissions from dairy wastewater. To achieve this goal, the following two specific objectives were formulated: (i) examine potential mitigation of NH_3 emissions from high-strength-TAN dairy-cattle wastewater using *A. faecalis* strain No. 4, and (ii) assess the enhancement of this bioprocess using pure oxygen gas, instead of air, to maintain aerobic conditions in the system.

2. Methods

2.1. Preparation of bacterial culture

The bacterium *A. faecalis* No. 4 was cultivated in a basal medium prepared by dissolving the following in 1 L lab grade water: 14 g K_2HPO_4, 6 g KH_2PO_4, 51 g trisodium citrate dihydrate, 6 g of $(NH_4)_2SO_4$, 0.2 g $MgSO_4{\cdot}7H_2O$ and 2 ml trace mineral solution. The trace mineral contents included (per liter): 57.1 g EDTA·2Na, 3.9 g $ZnSO_4{\cdot}7H_2O$, 7 g $CaCl_2{\cdot}2H_2O$, 5.1 g $MnCl_2{\cdot}4H_2O$, 5.0 g $FeSO_4{\cdot}7H_2O$, 1.1 g $(NH_4)_6\ Mo_7O_{24}{\cdot}4H_2O$, 1.6 g $CuSO_4{\cdot}5H_2O$, and 1.6 g of $CoCl_2{\cdot}6H_2O$ (pH = 6.0). The media was autoclaved for 20 min at 120 °C. A 1.2 mL of stock solution of strain No. 4 was inoculated into a 150 mL of the basal medium in a 500 mL and cultivated at 37 °C at the shaker agitation rate of 120 rpm for 48 h prior to startup of experiments (Joo et al., 2005).

2.2. Dairy wastewater collection and preparation

Manure samples for this study were collected from a commercial dairy farm lagoon located in central Washington, in the Pacific Northwest of the U.S.A. Samples of liquid dairy manures were transported to the lab in 20-L sealable plastic buckets and frozen prior to use for this study. The manure samples were thawed at lab temperatures prior to startup of experiments. The dairy wastewater was screened through a 1.40 mm sieve to remove debris and larger solid particles. This screening was essential to avoid pipet-tip clogging during wastewater sampling and to ensure integrity of samples.

2.3. Instrumentation

The experimental set-up and instrumentation used in this study is shown in Fig. 1. This system is similar to those used in similar bio-treatments of wastewaters (Shoda and Ishikawa, 2014; Zhao et al., 2010; Joo et al., 2006). Briefly, the bioreactor system consisted of a sealed Erlenmeyer flask (reactor), agitation plus temperature control system (hot plate with stirrer, catalogue No. 03407-10, Cole-Parmer Instrument Company, IL) or a shaking water bath (model 50, Thermo Scientific, OH), variable area flowmeters (catalogue No. 32460-42, 5% full-scale accuracy, Cole-Parmer Instrument Company, IL) to regulate aeration in the bioreactor system, graduated cylindrical acid traps (250 mL, catalogue No. 03-007-34, Fisherbrand, Mexico), and either an air compressor (model LA-5706, PUMA Industries Inc., TN) or oxygen tank to aerate the reactor.

2.4. Aerated batch culture experiments

Two sets of aerated batch culture experiments were conducted. The first set (phase I) of experiments to test the efficacy of *A. faecalis* strain No. 4 in dairy wastewaters were conducted in a batch mode in which air or oxygen were introduced directly into the wastewater in 1 L reactors. A sample of 50 mL of the preculture (10% inoculum) was introduced into each reactor containing 500 mL of dairy wastewater. The reactors were tightly sealed, stirred

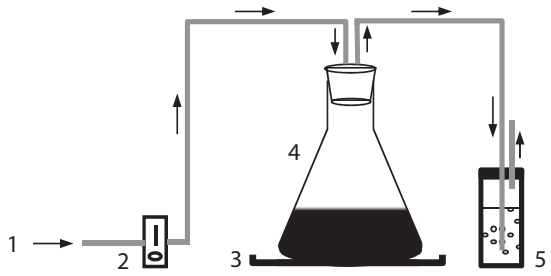

Fig. 1. The bioreactor system used to conduct batch experiments: (1) air compressor pump or oxygen-gas tank; (2) flow meter; (3) stirring hot plate for aerated experiments or shaking water bath for flushing experiments; (4) reactor; and (5) acid trap. The arrows indicate the flow direction of either air or oxygen.