Proceeding of the 1st International Conference on Tropical Agriculture
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Characteristics of *Alcaligenes* sp. LS2T Heterotrophic and Aerobic Ammonium Removal for Potential Livestock’s Wastewater Treatment

Aldyon Restu Azkarahman, Yuny Erwanto, Widodo Hadisaputro, Lies Mira Yusiati, and Nanung Agus Fitriyanto

Abstract *Alcaligenes* spp. was found to have the ability to grow and remove ammonium in the medium through the nitrification-denitrification process. This ability was also known as an ammonium treatment in organic wastewater including livestock’s wastewater. In order to further understand its potential to remove ammonium in livestock’s wastewater, microbial growth, ammonium, nitrite, and nitrate production of *Alcaligenes* sp. LS2T were investigated in phosphate medium at different carbon/nitrogen ratios (C/N ratios) along with constant airflow. Ammonium sulfate was used as sole nitrogen source with acetate and citrate as an organic carbon source in a separate medium. Results showed that *Alcaligenes* sp. LS2T could utilize ammonium as sole nitrogen source which associated with acetate and citrate as carbon source at different C/N ratios, resulting in ammonium removal and production of nitrite and nitrate at various concentrations in the medium. The highest ammonium removal was found in the acetate C/N 28 medium where 94.44% of initial ammonium was removed.

Keywords *Alcaligenes* sp. LS2T • Ammonium removal • Heterotrophic nitrification

1 Introduction

Development in livestock’s industry was followed by the intensive farming system, causing accumulation of ammonium in livestock’s waste area. Excess of ammonium was known to cause several environmental problems, one of it is eutrophication. Therefore, ammonium removal in wastewater treatment became essential to
overcome this problem; thus, the sustainable farming system could be achieved. Conventional ammonium removal in wastewater treatment plants consist of nitrification by autotrophic bacteria under aerobic conditions [1] and difficult to apply because autotrophic bacteria are vulnerable to the high concentration of ammonium and organic matters with C/N ratio of 0 to 2 [2]. Thus, wastewater pretreatment must be done to reduce the C/N ratio [3] or diluting the wastewater [4, 5]. Other researches have been done to observe potential of various heterotrophic microbes which could live in the higher C/N ratio medium and are able to remove ammonium on livestock’s wastes like on dairy wastewater [6], piggery wastewater [7], and poultry manure [8]. *Alcaligenes* spp. was known as heterotrophic bacteria that could be used for removing ammonium in wastewater [9] through simultaneous heterotrophic nitrification and aerobic denitrification [1, 10, 11]. *Alcaligenes* sp. LS2T is one bacterium that could remove ammonium in the medium [12], allegedly through heterotrophic nitrification and aerobic denitrification process. In this research, we further observe the potential of *Alcaligenes* sp. LS2T for livestock’s wastewater treatment. Investigation on its characteristics to remove ammonium at different C/N ratios in the phosphate medium was done, and nitrite and nitrate production which was also the result of the nitrification-denitrification process was also determined.

2 Methods

2.1 Microorganisms and Culture Conditions

*Alcaligenes* sp. LS2T was collected from the soil around layer farm in Yogyakarta, Indonesia, and then cultured and stocked in agar medium in the Laboratory of Animal Skins, By-products, and Animal Wastes Technology, Faculty of Animal Science, Universitas Gadjah Mada. The phosphate medium was prepared by dissolving the following in 1 L of distilled water: \((\text{NH}_4)_2\text{SO}_4 0.472 \text{ g}, \text{MgSO}_4.7\text{H}_2\text{O} 0.05 \text{ g}, \text{K}_2\text{HPO}_4 0.2 \text{ g}, \text{NaCl} 0.12 \text{ g}, \text{MnSO}_4.\text{H}_2\text{O} 0.01 \text{ g}, \text{and FeSO}_4 0.01 \text{ g} \). Sodium acetate and trisodium citrate were used as organic carbon source separately in the medium, abbreviated as acetate medium and citrate medium, respectively. The growth of *Alcaligenes* sp. LS2T in these experiments was performed in shaken Erlenmeyer flasks containing 500 mL medium and 1% preculture of *Alcaligenes* sp. LS2T on a rotary shaker at 120 rpm with constant airflow.

2.2 Shaking Culture Experiment

In order to elucidate the influence of carbon-to-nitrogen molar ratio (C/N) on the nitrifying capacity, the amount of N-ammonium was fixed at approximately 100 mg/L, while the amount of carbon was adjusted to the appropriate C/N ratio...
which was 7, 14, 21, and 28. The ammonium removal ability was investigated in the acetate medium and citrate medium separately. Samples were taken periodically to examine changes in growth, concentrations of ammonium, nitrite, and nitrate.

2.3 Analytical Methods

The growth of *Alcaligenes* sp. LS2T was monitored by measuring the optical density at 600 nm (OD 600 nm) of the culture medium using a spectrophotometer (Shimadzu, Japan) [11]. Culture samples were centrifuged at 10,000 rpm for ammonium, nitrite, and nitrate analysis. Ammonium concentration was analyzed by Nessler’s reagent photometry method [13]. Nitrite concentration was determined by N-(1-naphtalene)-diaminoethane photometry method [13]. Nitrate concentration was measured by using EPA [14]-modified colorimetric, brucine method. All tests were conducted in triplicate.

3 Results and Discussion

3.1 Growth Characteristics

The growth of *Alcaligenes* sp. LS2T at different C/N ratios in the acetate and citrate medium in shaking culture is shown in Fig. 1. Growth curves were established by measurement of the OD value at 600 nm.

In the acetate medium, the log phase of the bacterium was all similar started at 3 h. However, the growth peak in the C/N 7 medium was relatively lower than any other medium, and the stationary phase starts later than any other acetate medium, indicating that carbon source was deficient for cell synthesis. The shorter stationary phase was found in the C/N 14 medium which lasts for 9 h, while the longest stationary phase was found in the C/N 28 medium which lasts for 24 h. In the citrate medium under same conditions, the log phase of the bacteria was all similar started at 3 h. The growth peak in the citrate C/N 7 medium was also relatively lower, and the stationary phase was also started later than any other citrate medium. The growth phase trend in citrate medium was relatively the same than in the acetate medium, with the longest stationary phase found in the citrate C/N 21 and C/N 28 media which lasts for 21 h and the shortest was found in the citrate C/N 14 medium which lasts for only 9 h.
3.2 Heterotrophic Nitrification

In order to investigate the correlation between microbial growth, ammonium removal, and release of products during heterotrophic nitrification, *Alcaligenes* sp. LS2T was inoculated into acetate and citrate media under different C/N ratios. Cultures were sampled every 12 h for analysis. Figure 2 shows the changes by nitrification (ammonium, nitrite, and nitrate) during the 96 h growth period.

In the acetate medium, the highest ammonium removal after 96 h was found in C/N 28 medium, and about 94.44% of initial ammonium removed, followed by C/N 21, C/N 14, and C/N 7 which removed 93.49%, 93.12%, and 93.04% of initial ammonium, respectively. Furthermore, the highest nitrite production was found in C/N 28 medium (0.79 mg/L), followed by C/N 21 (0.68 mg/L), and C/N 7 (0.63 mg/L). The highest nitrate production was found in C/N 28 medium (35.53 mg/L), while in the C/N 7 medium was 22.59 mg/L, 23.31 in C/N 14, and 26.71 mg/L in the C/N 21 medium.

In the citrate medium after 96 h growth period, the highest ammonium removal was found in the C/N 21 medium, with 89.98% of initial ammonium removed,
followed by C/N 28 with 88.45%, C/N 14 with 86.36%, and C/N 7 with 83.49% of initial ammonium removed. The highest nitrite production was found in the C/N 7 and C/N 14 medium which both produced 0.27 mg/L, while in the C/N 21 and C/N 28 medium both produced 0.24 mg/L nitrite. The C/N 7 medium was found to produce the highest nitrate production, with 23.89 mg/L of nitrate produced after 96 h, followed by C/N 21, C/N 14, and C/N 28 medium which produced 22.45 mg/L, 22.08 mg/L, and 20.13 mg/L of nitrate, respectively. Heterotrophic nitrification process in citrate medium under different C/N ratios is shown in Fig. 3.

The previous study done by [12] showed that Alcaligenes sp. LS2T has the ability to utilize ammonium as a nitrogen source for metabolism. Various studies showed that some Alcaligenes spp. has the ability to perform heterotrophic nitrification under aerobic condition [1, 10, 11]. In this study, we investigate the growth profiles of Alcaligenes sp. LS2T at different C/N ratios in the acetate and citrate medium. The results indicate that log phase by Alcaligenes sp. LS2T was found in 3 h in both acetate and citrate medium at all C/N ratios researched. In all C/N 7 medium, the stationary phase was found later than in any other C/N ratio medium. Furthermore, the growth was relatively lower; this could be caused by the lack of carbon source for metabolism, resulting in less ammonium removal in the medium.
The highest ammonium removal in acetate medium was found in C/N 28 and for the citrate medium was found in C/N 21; both were known to achieve earlier and longer stationary phase than in any other C/N in the respective medium. The correlation between earlier and longer stationary phase with higher ammonium removal is because the assimilation ratio is as high as heterotrophic nitrification ratio [15, 16], the assimilated nitrogen is associated with cell growth, and the nitrogen removal rate is affected by cell doubling time [17]. Therefore, microorganisms with earlier and longer stationary phase can take ammonium quickly into the cell, resulting in more effective ammonium removal [18]. At four different C/N ratios in both medium, nitrite and nitrate were formed by *Alcaligenes* sp. LS2T during heterotrophic ammonium removal process. The experimental results showed that the formation of nitrate is higher than nitrite. Nitrite and nitrate concentration in this research demonstrates a whole time nitrification process until 96 h; these results correspond to the present study that indicates heterotrophic nitrite/nitrate production is linked with growth and supports that heterotrophic nitrification can take place during the entire growing phase [19, 20]. Nitrate as dominant product in the process of heterotrophic nitrification by *Alcaligenes* sp. LS2T is similar with A. *faecalis* strain NR [10], *Achromobacter* sp.GAD3, and *Comamonas* sp. GAD4 [21], while other strains

![Graphs](https://example.com/graphs.png)
like *A. faecalis* C16 [11] and *Bacillus methylotrophicus* strain L7 [22] had nitrite as the dominant product over nitrate in the process of heterotrophic nitrification.

Carbon dependency has been shown in this research. A possible reason is that ammonium is oxidized by a sequence involved in some way with the metabolism of a certain or specific carbon source that could provide the acceptor molecules for the synthesis of intermediate organic nitrogen compounds [23]. Results of this research also showed that even if the C/N ratio is as high as 28, which is too high for autotrophic nitrifying bacteria, *Alcaligenes* sp. LS2T still exhibits nitrification ability. These results showed *Alcaligenes* sp. LS2T potential in livestock’s wastewater treatment like in piggery wastewater with C/N ratio around 5 to 20 [7] or even chicken manure with C/N ratio around 8 [24].

### 4 Conclusions

*Alcaligenes* sp. LS2T was capable of removing ammonium in the medium through nitrification process, utilizing ammonium in the medium for cell synthesis, and producing nitrite and nitrate with organic carbon sources. The highest ammonium removal was found in the acetate medium at C/N 28; this indicates that *Alcaligenes* sp. LS2T could perform heterotrophic nitrification under relatively high C/N ratio. Results of this research suggest deeper research on the ammonium removal ability of *Alcaligenes* sp. LS2T which is in the production of hydroxylamine and nitrogen gas and its application on various livestock’s wastewater, so that clearer nitrogen pathway in this process could be conducted.

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**References**

CERTIFICATE OF PARTICIPATION

This is to certify that
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