Preliminary Evaluation of Anaerobic Production of Aminolevulinic Acid by Methanogens and Acetogens

Yance Syarif and Katherine A. Taconi, University of Alabama in Huntsville, 130 Engineering Building, Huntsville, AL 35899

Aminolevulinic acid (ALA) is an intermediate chemical generated and utilized for the biosynthesis of other compounds such as vitamin B12 and chlorophyll in animals, plants, algae, and bacteria. The unique properties of ALA allow it to be used as an effective, non-toxic, and biodegradable herbicide and insecticide as well as for the diagnosis and treatment of several types of cancer [1,4,5,6]. Anaerobic production of ALA using methanogens and acetogens is an attractive option for ALA production, as the substrates favored by these organisms are the primary components of many common industrial, municipal, and agricultural wastewater streams commonly treated using anaerobic digestion. Unfortunately, pure cultures of methanogens and acetogens have not been shown to produce sufficient amounts of ALA when compared to processes using other microorganisms.

The goal of this research was to evaluate ALA production by a mixed culture of acetogens and methanogens based on the hypothesis that together these two types of anaerobic microorganisms could produce more ALA than either could generate alone. Preliminary work focused on two important factors for facilitating ALA production: the type of organism used and the presence of an inhibitor that prevents further degradation of the ALA. Experiments utilized different combinations of substrates, including acetic acid, glucose and methanol, to promote and quantify ALA production by methanogens and acetogens, both individually and in a mixed culture. Methanogenic ALA production was promoted by providing acetic acid as the sole organic carbon source. Methanol and glucose were provided as co-substrates to facilitate both methanogenic and acetogenic ALA production. Experiments also evaluated the effect of adding levulinic acid, an inhibitor known to facilitate accumulation of ALA. Results were evaluated to determine the combination of inhibitor and substrate composition that shows the most promise for generating higher yields of ALA and to determine whether this process merits further investigation and optimization as a feasible alternative for ALA production.

All experiments were performed in custom-made 500 mL batch reactors containing 250 mL substrate and nutrient solution along with 20 mL of inoculum. Reactor performance was monitored by gas production, as measured by a pressure gauge fitted to the reactor. Inoculum was obtained from an anaerobic digester at a local wastewater treatment plant. Heat treatment (boiling for 20 minutes) was applied to the inoculum to enrich the culture with acetogens. Inoculum consisting of half heat-treated sludge and half un-heat-treated sludge was used in experiments containing a mixture of 2 g/L methanol and 10 g/L glucose. Experiments containing 1 g/L acetic acid as the substrate were inoculated with sludge that was not pasteurized. Preliminary results showed promising ALA production in experiments containing acetic acid. As expected, the inhibitor increased the amount of ALA produced. With the addition of 200 mM levulinic acid, the amount of ALA produced in acetic acid media was approximately 175 μM as shown in Figure 1. These experiments also produced approximately 8 mol of methane per mol acetic acid, as shown in Figure 2. The control containing only 1 g/L acetic acid but no levulinic acid produced only 1 μM ALA and yielded 1 mol of methane per mol acetic acid as shown in Figures 1 and 2. The higher yield of methane

produced in experiments containing the inhibitor is likely due to the fact that a fraction of levulinic acid was converted into methane. The chemical oxygen demand (COD) of the acetic acid media containing levulinic acid was 32,900 mg/L, where the COD of the acetic acid control media was only 1100 mg/L. The final COD of experiments containing levulinic acid was less than 30,000 mg/L, which indicates that some of the inhibitor was degraded into products, as shown in Figure 3. Similar data was expected in the methanol and glucose media, where the substrate used was 2 g/L methanol and 10 g/L glucose. The control experiment for using the glucose and methanol media is still in progress; however, to date, it has produced 62 μM ALA. Higher yields are expected with experiments in the presence of levulinic acid; however, these experiments are also still in progress, and data is not yet available.

To the best of our knowledge, the production of significant amount of ALA from methanogens using acetic acid as the organic substrate has not been reported in literature. Jaenchen, Gilles, and Thauer reported production of 40 μM ALA by methanogens using H_2 and CO_2 in the presence of 100 mM levulinic acid [2]. Lin, Nishio, and Nagai reported 67.3 μM ALA produced by methanogens from methanol in the presence of 50 mM levulinic acid [3]. These results show that acetic acid is a feasible substrate for higher ALA production by methanogens in the presence of levulinic acid, as it produced almost three times greater concentrations of ALA (175 μM) than reported for other substrates. A mixed culture of methanogens and acetogens to degrade a methanol/glucose substrate also exhibited promising ALA and CH_4 production in control experiment containing no inhibitor, and higher concentrations are expected from experiments containing methanol, glucose, and levulinic acid. Work is continuing to evaluate ALA production. Future work will also include maximizing the ALA production at minimum concentration of inhibitors and implementing other inhibitors available such as dioxoheptanoic acid (DOH) and oxoglutaric acid.

References

[1]. Fukuda, H., A. Casas, and A. Batlle. "Aminolevulinic Acid: From Its Unique Biological Function to Its Star Role in Photodynamic Therapy." *International Journal of Biochemistry and Cell Biology*, vol. 37, pp. 272-276, 2005.

[2]. Jaenchen, R., H. Gilles, R. Thauer. "Inhibition of Factor F_{430} Synthesis by Levulinic Acid in Methanobacterium thermoautotrophicum." *FEMS Microbiology Letters*, vol. 12, pp. 167-170, 1981.

[3]. Lin, D., N. Nishio, and S. Nagai. "Production of 5-Aminolevulinic Acid by Methanogens." *Journal of Fermentation and Bioengineering*, vol. 68, No. 2, pp. 88-91, 1989.

[4]. Nishikawa, S. and Y. Murooka. "5-Aminolevulinic Acid: Production by Fermentation and Agricultural and Biomedical Applications." *Biotechnology and Genetic Engineering Reviews*, vol. 18, pp. 149-171, 2001.

[5]. Sasaki, K., M. Watanabe, T. Tanaka. "Biosynthesis, Biotechnological Production and Applications of 5-Aminolevulinic Acid." *Applied Microbiology and Biotechnology*, vol. 58, pp. 23-29, 2002.

[6]. Sasikala, C., C. Ramana, and P. Rao. "5-Aminolevulinic Acid: A Potential Herbicide and Insecticide from Microorganisms." *Biotechnology Progress*, vol. 10, pp. 451-459, 1994.

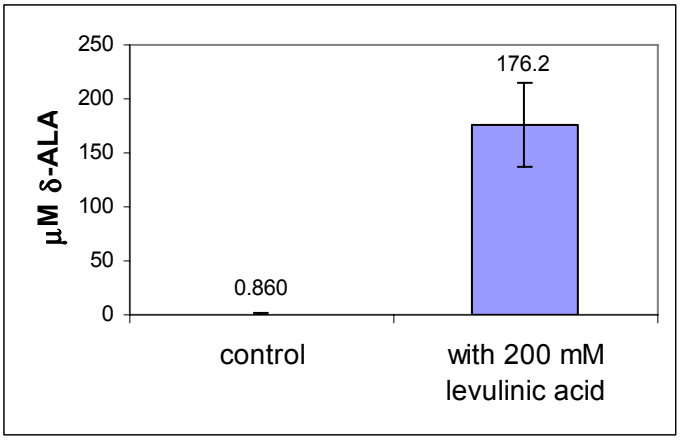

Figure 1: The effect of inhibitor on δ-ALA production by methanogens in acetic acid media.

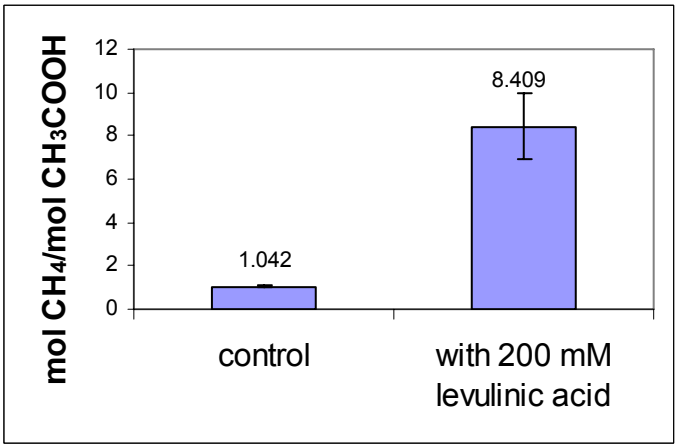

Figure 2: The effect of levulinic acid to CH_4 yield in acetic acid media

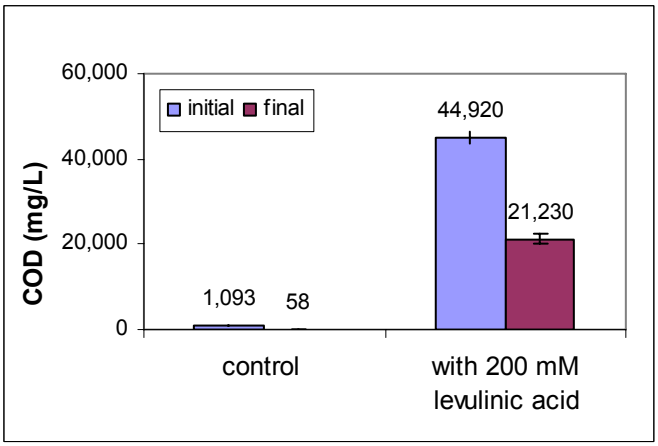

Figure 3: The Chemical oxygen Demand (COD) of the acetic acid media, with and without the inhibitor.