Nutrient and Energy Recovery from anaerobic digester (AD) centrate using an algal system

Thinesh Selvaratnam
*Department of Civil &
Environmental Engineering,
Lamar University,*
Beaumont, TX 77705, USA.
tselvaratnam@lamar.edu

Hari Lal Kharel
*Department of Civil &
Environmental Engineering,
Lamar University,*
Beaumont, TX 77705, USA.
hkharel@lamar.edu

Melissa Tan
*Department of Civil &
Environmental Engineering,
Lamar University,*
Beaumont, TX 77705, USA.
mtan2@lamar.edu

Abstract— **Anaerobic digestion (AD) systems yield methane from wet sludge/biomass leaving carbon-depleted wastewater known as centrate in municipal wastewater treatment plants. Centrate is an ideal feedstock for nutrient/energy recovery because it is highly concentrated and is thus low volume. High nutrient levels are found in centrate in the form of ammonium and phosphate ions. This current study focused on the potential of using centrate to recover nutrients and energy through an algal-based system. A red-algal (*Galdieria sulphuraria*) strain was used for this application for the treatment of centrate from municipal AD systems.**

Keywords—bioremediation, microalgae, Galdieria sulphuraria, nutrient recovery.

I. INTRODUCTION

Anaerobic digestion systems yield methane from wet biomass leaving carbon-depleted wastewater known as centrate and residual solids. High nutrient levels are found in centrate in the form of ammonium (600-1200 ppm as ammoniacal N) and phosphate ions at levels up to 200 ppm. Centrate is an ideal feedstock for nutrient recovery because it is highly concentrated and is thus low volume. AD centrate cannot be discharged directly, so it is typically mixed with incoming primary wastewater in municipal WWTPs, representing a parasitic load on the plant. Agricultural AD system centrate is spread on fields with varying application rates that can lead to ground and surface water contamination. The AD centrate accounts for 0.5 to 1% of wastewater treatment (WWTP) influent flow but accumulates approximately 15 to 25% of the nutrient load to the WWTP [1].

The use of acidophilic algae in the genus *Galdieria* allows direct growth on undiluted centrate. Previous work has validated its use in wastewater treatment in a small wastewater treatment plant pilot operation [2-5]. Neutral pH algal platforms for centrate processing require an approximate five-fold dilution to avoid the potent toxicity of ammonia. Most of the current research efforts are focused on neutral pH algal strains and thus require multi-fold dilution in growth reactors [6,7]. *Galdieria* treatment occurs at a pH value that is 5-7 orders of magnitude below the pKa for NH_4^+/NH_3 and thus does not require dilution. The benefit is an estimated five-fold reduction in capital expenditures for photobioreactors relative to other algae systems. The growth advantage of *Galdieria* strains over other neutral pH strains can be elevated further by using mixotrophic metabolism. *Galdieria sulphuraria* is a red algal strain that thrives at very low pH values (1-4) and temperatures up to 56°C. The ability to grow in extreme conditions [8] enables ***Galdieria sulphuraria*** to be used in a multitude of bioremediation applications [9-11].

Several past studies demonstrated *Galdieria sulphuraria's* ability to treat municipal wastewaters to the desired effluent concentrations [11,12]. This study focuses on developing algal-based systems to treat AD centrate using ***Galdieria sulphuraria.*** The proposed technology has the potential to overcome the shortcomings of existing and planned AD installations through enhanced energy and nutrient recovery with concomitant options for renewable biofuels (gas and liquid) and bioproducts derived from the biochemical processing of red algal biomass feedstock produced during centrate water processing.

The potential impact of the proposed study stems from sustainable improvements in the energy budget and economics of municipal and agriculture anaerobic digestion operations across the United States. To validate the scope of using this algal strain for the bioremediation of AD centrate, this study evaluates the proof of this concept in lab-scale algal bioreactors.

II. MATERIALS AND METHODS

A. Culturing of algal strain and Anaerobic digestor centrate collection

The microalgae, *Galdieria sulphuraria* 5587.1, obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon), were used in this study. *Galdieria sulphuraria* was grown in an incubator (Percival, IA, USA) at 42°C with 24 h of continuous illumination (4000 lux) in Cyanidium media (CM) [11]. The cultures received from the culture collection were initially streaked onto agar plates, and then single colonies were picked to start axenic cultures from culture plates to CM, then scaled up the volume to 1 L Erlenmeyer flasks. The CO_2 concentration inside the incubator was maintained at 2 to 3%.

The following media recipe was used to prepare CM: $(NH_4)_2SO_4$, 1.32 g L^{-1}; KH_2PO_4, 0.27 g L^{-1}; NaCl, 0.12 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.25 g L^{-1}; $CaCl_2 \cdot 2H_2O$, 0.07 g L^{-1}; Nitch's trace element solution, 0.5 mL; $FeCl_3$ (solution = 0.29 g L^{-1}), 1.0 mL. The pH of the media was adjusted to 2.5 using 10 N H_2SO_4. The AD centrate used for this research was collected from a municipal wastewater treatment plant in Mesa (Mesa, Arizona) and stored in a standard refrigerator at 4 °C.

B. Bioremediation experiment

This study aimed to collect proof-of-concept type data and evaluate the feasibility for the bioremediation of AD centrate using *Galdieria sulphuraria*. This experiment was conducted using 1 L bubble column reactors (700mL of working volume) with carbon dioxide-enriched air supply (Fig. 1). Four different media compositions were used in this study: 1. Raw AD centrate; Raw AD centrate supplemented with CM components; 3. CM. The inoculum algae for this bioremediation study were grown in 1 L Erlenmeyer flasks, as described in section II A. At the start of the experiment, the inoculum was centrifuged at 2000 ×g for 10 min at 25°C (accuSpin 400 centrifuge, Fisher Scientific, USA), and the algae pellets were re-suspended in the four media compositions mentioned above.

Each media composition was prepared in triplicates in 1 L algal photobioreactors with a working volume of 700 mL. These reactors were housed in a temperature-controlled growth room and the room temperature was maintained at 40°C. For nutrient analysis, 5 mL samples were drawn from each bioreactor on days 1, 3, 5,

Fig. 1. Algal photo bioreactors used in the study

7,9,11,13,15,18, and 25. The samples were centrifuged for 10 min at 2000 ×g followed by the supernatants being transferred and stored in a refrigerator at 4°C for further analyses.

C. Sample analysis

Hanna pH meter (HI 5522, Hanna, Rhode Island, USA) was used for pH measurements. Ammoniacal nitrogen and phosphate were determined using HACH DR 3900 (HACH, Colorado, USA) spectrophotometer. Algal biomass density was quantified by measuring the optical density at the 750 nm wavelength using a spectrophotometer (HACH, Colorado, USA). The biomass density was evaluated in terms of 'ash-free dry weight (AFDW) (g L^{-1}), which was correlated to OD at 750 nm by the following equation:

$$AFDW = 0.4775 * (OD@750nm) - 0.0163$$

$$n = 12, \ r^2 = 0.997$$

All the experiments and analytical measurements were carried out in triplicates. The averaged data were presented with error bars equal to one standard deviation. Microsoft Excel software (Version 16.0, Redmond, WA, USA) was used for the standard deviation calculations.
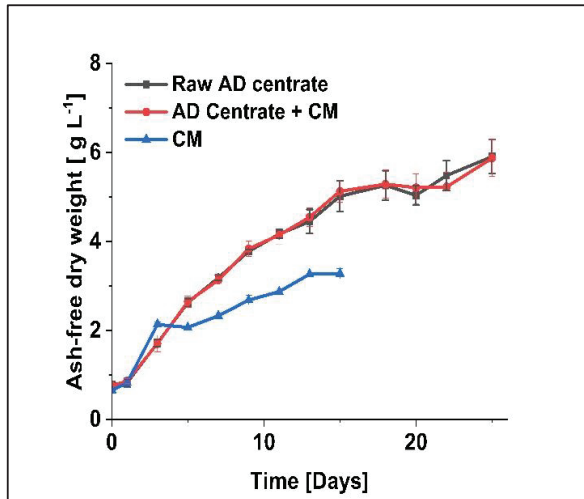
Fig. 2. Growth profiles of *Galdieria sulphuraria* in different media compositions. Data points represents average SD of n=3 biological replicates.
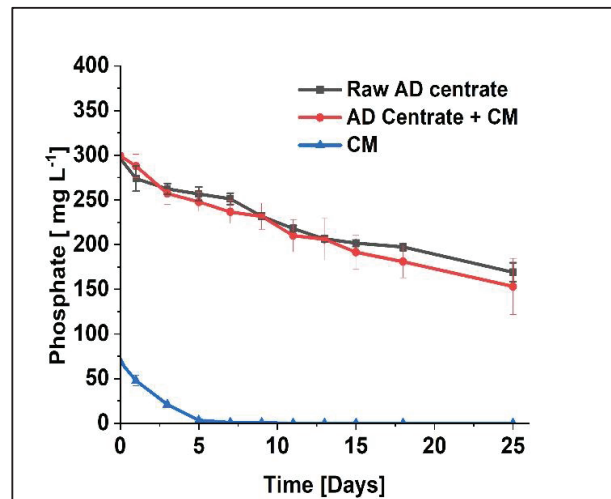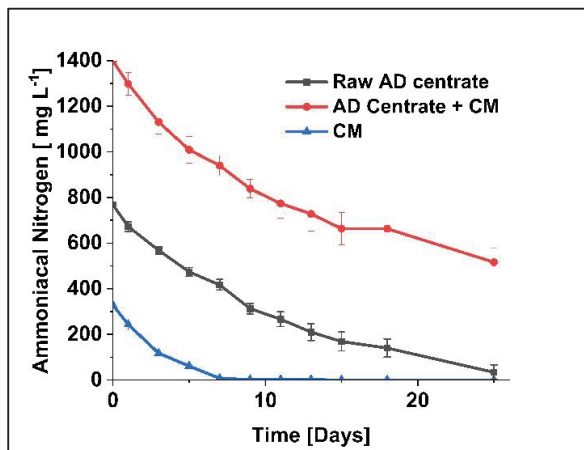

Fig. 3. Ammoniacal nitrogen removal by *Galdieria sulphuraria* in different media compositions. Data points represents average SD of n=3 biological replicates.

III. RESULTS AND DISCUSSION

The growth profiles of *G. sulphuraria*, cultured in different media compositions, are shown in Fig. 2 for 25 days. This figure shows that *G. sulphuraria* can grow in raw AD centrate comparable to AD centrate supplemented with CM media components and with standard growth media (CM). A similar growth pattern was observed on both media compositions with raw AD centrate and achieved a final biomass density of ≈ 6.0 g L^{-1}. The observed growth rates were 0.205 g L^{-1}d^{-1}, 0.204 g L^{-1}d^{-1}, and 0.174 g L^{-1}d^{-1} respectively for raw AD centrate, AD centrate with CM, and CM. Overall, the growth data

Fig. 4. Phosphate removal by *Galdieria sulphuraria* in different media compositions. Data points represents average SD of n=3 biological replicates.

demonstrated that *Galdieria sulphuraria* could be grown in raw AD without any potential growth inhibitions compared to the standard growth media and AD centrate supplemented with CM media compositions.

The removal of ammoniacal nitrogen and phosphate by *Galdieria sulphuraria* in the different media compositions is shown in Fig. 3 and Fig. 4. The removal patterns observed for both nutrients followed the similar trend observed for the growth profiles. A 95% ammoniacal nitrogen removal with a removal rate of 26.35 mg L^{-1}d^{-1} was observed with raw AD centrate. This result and the biomass growth rate observed in Fig. 1 provide necessary proof-of-concept type baseline data for an algal-based bioremediation system. The results again demonstrated the potential of using this specific algal strain for the bioremediation of AD centrate.

IV. CONCLUSIONS

Overall, this study evaluated the potential of using *Galdieria sulphuraria* for the bioremediation of anaerobic digestor centrate from the municipal wastewater treatment plants. Results obtained from the study showed the potential feasibility of the proposed system. Experimental data demonstrated that Galdieria sulphuraria could be grown in raw centrate and achieve up to 95% ammoniacal nitrogen removal.

REFERENCES

[1] Gustavsson, DJI; Syd, V. Biological Sludge Liquor Treatment at municipal wastewater treatment plants - A Review. Vatten 2010, 66, 179-192.

[2] Henkanatte-Gedera, S.M.; Selvaratnam, T.; Caskan, N.; Nirmalakhandan, N.; Van Voorhies, W.; Lammers, P.J. Algal-based, single-step treatment of urban wastewaters. Bioresour. Technol. 2015, 189, 273-278, doi:10.1016/j.biortech.2015.03.120.

[3] Selvaratnam, T.; Pegallapati, A.K.; Reddy, H.; Kanapathipillai, N.; Nirmalakhandan, N.; Deng, S.; Lammers, P.J. Algal biofuels from urban wastewaters: Maximizing biomass yield using nutrients recycled from hydrothermal processing of biomass. Bioresour. Technol. 2015, 182, 232-238, doi:http://dx.doi.org/10.1016/j.biortech.2015.01.134.

[4] Selvaratnam, T.; Pegallapati, A.; Montelya, F.; Rodriguez, G.; Nirmalakhandan, N.; Lammers, P.J.; van Voorhies, W. Feasibility of algal systems for sustainable wastewater treatment. Renew Energ 2015, 82, 71-76, doi:http://dx.doi.org/10.1016/j.renene.2014.07.061.

[5] Selvaratnam, T.; Pegallapati, A.K.; Montelya, F.; Rodriguez, G.; Nirmalakhandan, N.; Van Voorhies, W.; Lammers, P.J. Evaluation of a thermo-tolerant acidophilic alga, Galdieria sulphuraria, for nutrient removal from urban wastewaters. Bioresource Technology 2014, 156, 395-399, doi:http://dx.doi.org/10.1016/j.biortech.2014.01.075.

[6] Arias, D.M.; Uggetti, E.; García-Galán, M.J.; García, J. Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment. Science of The Total Environment 2017, 587-588, 157-167, doi:https://doi.org/10.1016/j.scitotenv.2017.02.097.

[7] Ren, H.; Tuo, J.; Addy, M.M.; Zhang, R.; Lu, Q.; Anderson, E.; Chen, P.; Ruan, R. Cultivation of Chlorella vulgaris in a pilot-scale photobioreactor using real centrate wastewater with waste glycerol for improving microalgae biomass production and wastewater nutrients removal. Bioresource Technology 2017, 245, 1130-1138, doi:https://doi.org/10.1016/j.biortech.2017.09.040.

[8] Toplin, JA; Norris, T.B.; Lehr, C.R.; McDermott, T.R.; Castenholz, R.W. Biogeographic and phylogenetic diversity of thermoacidophilic Cyanidiales in Yellowstone National Park, Japan, and New Zealand. Applied and Environmental Microbiology 2008, 74, 2822-2833, doi:10.1128/aem.02741-07.

[9] Nirmalakhandan, N.; Selvaratnam, T.; Henkanatte-Gedera, S.; Tchinda, D.; Abeysiriwardana-Arachchige, I.; Delanka-Pedige, H.; Munasinghe-Arachchige, S.; Zhang, Y.; Holguin, F.; Lammers, P. Algal wastewater treatment: Photoautotrophic vs. mixotrophic processes. Algal Research 2019, 41, 101569.

[10] Henkanatte-Gedera, S.M.; Selvaratnam, T.; Karbakhshravari, M.; Myint, M.; Nirmalakhandan, N.; Van Voorhies, W.; Lammers, P.J. Removal of dissolved organic carbon and nutrients from urban wastewaters by Galdieria sulphuraria: Laboratory to field scale demonstration. Algal Research 2017, 24, Part B, 450-456, doi:https://doi.org/10.1016/j.algal.2016.08.001.

[11] Selvaratnam, T.; Henkanatte-Gedera, S.M.; Muppaneni, T.; Nirmalakhandan, N.; Deng, S.; Lammers, P.J. Maximizing recovery of energy and nutrients from urban wastewaters. Energy 2016, 104, 16-23, doi:http://dx.doi.org/10.1016/j.energy.2016.03.102.

[12] Selvaratnam, T.; Pegallapati, A.K.; Montelya, F.; Rodriguez, G.; Nirmalakhandan, N.; Van Voorhies, W.; Lammers, P.J. Evaluation of a thermo-tolerant acidophilic alga, Galdieria sulphuraria, for nutrient removal from urban wastewaters. Bioresour. Technol. 2014, 156, 395-399, doi:https://doi.org/10.1016/j.biortech.2014.01.075.