

Kinetic Modelling of Free Energy for Simultaneous Nitrification and Aerobic Denitrification under High Cyanide Environments

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Abstract—The Gibbs free energy is significant to understand simultaneous nitrification and aerobic denitrification (SNaD), it is also imperative to study Gibbs free energy to determine whether or not the reaction is plausible. This study aims to investigate the effect of cyanide (CN⁻) on SNaD when cyanide degrading mix consortia is used. The relationship between the rate of nitrification and Gibbs free energy was also investigated. The results attained show that 37.55 mg/L of CN⁻ does not have an effect on SNaD. The consortia degraded CN⁻ and achieved SNaD, with degradation efficiency of 92.9%, 97.7% and the degradation rate of 0.0234 and 0.139 mg/L/hr for nitrification and CN⁻ respectively. Moreover, all the free Gibbs energy was found to be negative, with the lowest Gibbs free energy being -756.4 and -1830.9 Kcal/mol for nitritation and nitratation in the first 48 hr of the reaction respectively. Additionally, a linear relationship between the rate of NH₄-N and NO₂⁻ degradation with their respective Gibbs free energy was observed. Linear model was also used to predict the relationship between NH₄-N, NO₂⁻ degradation and Gibbs free energy. The results obtained showed a good correlation between the models and the experimental data with correlation efficiency being 0.94 and 0.93 for nitritation, and nitratation respectively. From the results found it can be deduced that SNaD is plausible under high cyanide conditions when cyanide degrading or tolerant microorganisms are employed. This can be a sustainable solution to SNaD inhibition by CN⁻ compounds during wastewater treatment.

Keywords—Cyanide, Denitrification, nitrification and Gibbs free energy.

I. INTRODUCTION

Cyanide (CN⁻) is a toxic compound known to inhibit most biological process that is being performed in wastewater treatment plants (WWTP). Nitrification and denitrification are among the process that are heavily affected by CN⁻ compounds [1], [2]. Remedial procedures such as the use of activated carbon have been recognized to eradicate inhibition effect of toxic compounds on nitrification and denitrification; however, the use of activated carbon is not feasible in CN⁻ containing

wastewater due to its low absorption capability; furthermore, the use of activated carbon may also result in additional costs [2]. Reference [3] proposed that cyanide degrading bacteria must be employed to eliminate the inhibition effect of CN⁻ toward nitrification and denitrification. Reference [4] has proven the feasibility of using cyanide degrading bacteria as a remedial option for inhibition of nitrification and denitrification by CN⁻. However, the application of cyanide degrading microorganisms is not yet established in a large scale WWTP due to fear of irretrievable process failure, thus more information that will ease the process control is still required. This study investigates the thermodynamics to understand the simultaneous nitrification and aerobic denitrification (SNaD) by cyanide degrading consortium in the presence of high CN⁻ conditions. The Gibbs free energy of nitritation and nitratation were investigated since nitritation is known to be a rate-limiting step. A linear relationship is known to exist between the rate of biochemical reaction and their respective free energies [5]. The Gibbs free energy was selected for this study since it is known to be a driving force of a reaction. The reaction is judged based on the principles of whether the reaction is spontaneous ($\Delta G < 0$), at equilibrium ($\Delta G = 0$) and impossible if ($\Delta G > 0$) [6].

II. MATERIALS AND METHODS

A. Isolation and Inoculum Development

The consortia were isolated from CN⁻ containing waste at the Bioresource Engineering research group (BioERG), Cape Peninsula University of Technology (CPUT). They were cultured into a complex media at sterile conditions for 5 days; thereafter, it was transferred into 100 ml of basal media containing: 7.9 g Na₂HPO₄, 1.5 g KH₂PO₄, 0.5 g MgSO₄·7H₂O and 1 mL trace elemental solution. The trace elemental solution was composed of: 1.1 g (NH₄)₆Mo₇O₂·4H₂O, 50 g EDTA, 2.2 g ZnSO₄·7H₂O, 5.5 g CaCl₂, 5.0 g FeSO₄·7H₂O, 5.06 g MnCl₂·4H₂O, 1.61 g CoCl₂·6H₂O, 1.57 g CuSO₄·5H₂O (per liter). The consortia were incubated at 36.5°C for 48 hrs and was used as an inoculum for the reactor experiment.

B. Reactor Experimental Runs

The basal media as indicated above was used for the reactor experiment. 1 L reactor was used for this experiment. It was inoculated with 10% of the 48 hrs culture and the

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microorganisms were grown for 5 days, the contaminants were added on day 6. The pH and temperature were maintained at 6.5 and 36.5°C throughout the experiment respectively. A concentration, i.e. 285 and 37.55 mg/L of NH₄-N and CN⁻ were then added to the reactors, respectively. After the addition of the toxicant, the reactors were tightly closed and covered with foil to avoid ammonium stripping and volatilization of CN⁻. Samples were collected every 24 hrs to analyze residual CN⁻, NH₄-N, NO₂⁻ and NO₃⁻ using test kits obtained from Merck SA and Merck ANOVA Spectroquant. All the analysis was performed in duplicates.

C. Bioenergetic/Thermodynamic Models

The free energy (ΔG) equation for nitrification can be expressed as:

$$NH_4 - N + 1.05O_2 \rightarrow NO_2^- + H^+ \Delta G = 65 + RT \ln \left\{ \frac{[NO_2^-](10^{-pH})^2}{[NH_4 - N][O_2]^{1.5}} \right\} \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \Delta G = 18.5 + RT \ln \left\{ \frac{[NO_3^-]}{[NO_2^-][O_2]^{0.5}} \right\} \quad (2)$$

Where ΔG is a free energy change in Kcal/mol, NH₄-N is molar concentration of ammonium ions, NO₂⁻ is molar concentration of NO₂⁻ and O₂ is a molar concentration of oxygen gas, R is the universal gas constant and T is temperature. However, when the experiment is operated at steady state with constant Temperature, pH and abundance of O₂ Eq. (1) and (2) becomes:

$$\Delta G = 65 + RT \ln \left(\frac{[NO_2^-]}{[NH_4 - N]} \right) \quad (3)$$

$$\Delta G = 18.5 + RT \ln \left(\frac{[NO_3^-]}{[NO_2^-]} \right) \quad (4)$$

The model used to estimate the rates in respect to their corresponding Free energies is as explained by [7]. The model assumes that when nitrifying bacteria remain constant then the rate of microbial growth could be described as a function of ΔG , therefore:

$$\mu = F \Delta G \quad (5)$$

Where μ is a growth rate, F is a linear function. However $\mu = (1/X)(dx/dt)$ and $Y = dx/dt$, ds/dt the equation can be rearranged as follow:

$$\frac{ds}{dt} = \frac{X}{Y} F \Delta G \quad (6)$$

Where X is biomass concentration and Y is biomass yield. At steady state and when the bacterial yield is constant the equation can be rearranging as:

$$\frac{ds}{dt} = F \Delta G \quad (7)$$

The value of ΔG was determined as using with equation 3 and 4, thereafter, Eq. 7 was for linear regression using polymath 10.0.

III. RESULTS AND DISCUSSION

An experiment was conducted to study the effect of high CN- concentrations on the ability of cyanide degrading mix consortia to achieve SNaD. The experiment was conducted in a batch reactor. The results obtained reveal that 37.55 mg/L of CN- did not affect the SNaD. The consortia rapidly degraded CN- from 37.55 to 12 mg/L in the first 48 hrs of the experiment. This could be a defensive mechanism for the consortia to detoxify the CN- concentration into less toxic concentrations. The consortia achieved nitrification, denitrification and cyanide degradation simultaneously.

The degradation efficiency over 288 hrs was found to be 92.9%, 97.7% with the degradation rate of 0.0234 and 0.139 mg/L/hr for nitrification and CN⁻ degradation respectively. The removal efficiency found in this study is greater than that of a study conducted by [2]. Furthermore, [3] found similar results when using cyanide degrading bacteria for nitrification subsequent denitrification. However, the conversion of NH₄-N to NO₂⁻ did not make a stoichiometric logic as the concentration of NH₄-N used did not correspond with the NO₂⁻ produced. This phenomenon could be an indication of the residual NO₂⁻ being used for microbial proliferation or may be caused by high nitrite reductase which rapidly converts NO₂⁻ into NO₃⁻. Jin *et al.* [8] also found similar results in a study conducted using *Pseudomonas* sp. ADN-42 for Heterotrophic SNaD under saline conditions. The existence of denitrification was demonstrated by the decrease in the NO₃⁻ -see Fig. 1. Nevertheless, the production of the nitrogenous gasses was not examined during this study.

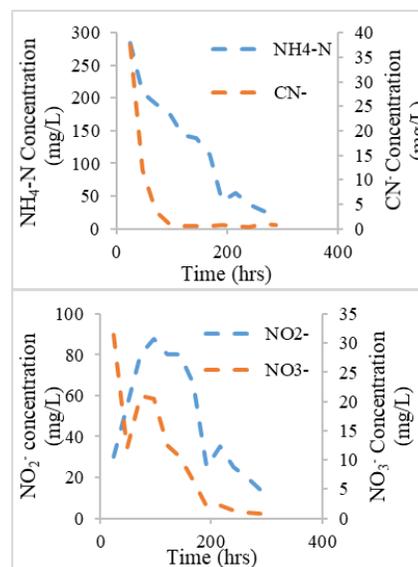


Fig. 1. graphs representing simultaneous nitrification, denitrification, and cyanide degradation. A: nitrification and Cyanide degradation. B: NO₂⁻ and NO₃⁻ accumulation and degradation.

TABLE I: MODEL VARIABLES FOR NITRITATION AND NITRATATION

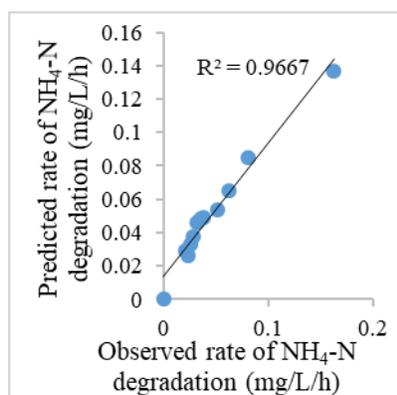
Model	F	a	R ²	variance	R ² adj
$\frac{d[\text{NH}_4\text{-N}]}{dt} = F\Delta G$	-0.0001	-	0.93	0.0001	0.93
$\frac{d[\text{NO}_2^-]}{dt} = F\Delta G + a$	0.001	1.85	0.93	0.0140	0.93

Gibbs free energy (ΔG) was plot as a function of $[\text{NO}_2^-]/[\text{NH}_4\text{-N}]$ and $[\text{NO}_3^-]/[\text{NO}_2^-]$, all the valued of Gibbs free energy were found to be negative for both nitrification and nitrification. This is an indication of thermodynamic feasibility and the spontaneity of nitrification and nitrification under the CN-conditions [9,10]. At the beginning of the experiment, the Gibbs free energy was low with the lowest free energy of -756.4 and -1830.9 Kcal/mol for nitrification and nitrification respectively. This shows that the reaction was more rapid at the beginning of the experiment [11], as the concentration of the toxicant becomes exhausted the free Gibbs energy increases. It has been documented that when a rate of reaction has a strongly negative ΔG values, it implies that they are kinetically controlled, meaning they are feasible in wide concentration ranges, this is more likely to occur in oxidation reaction with stronger electron acceptor like oxygen and nitrate [12].

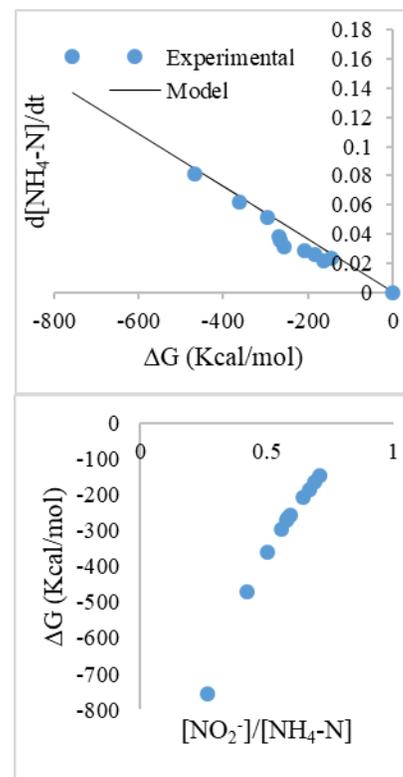
This could also be linked with microorganisms reaching the stationary phase, thus the decrease in energy. Literature claims that there's a linear relationship exist between biochemical reaction with their corresponding ΔG [5]. The relationship between the rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation and their corresponding ΔG was studied and it was apparent that there is a linear correlation between the rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation and their corresponding ΔG . Lüttge *et al.* [13], also reported a linear relationship between the rate of albite dissolution and their corresponding ΔG . Furthermore, the Linear models were used to predict the relationship between the rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation with their corresponding ΔG . the model fitted well into the experiment with correlation coefficient of 0.94 and 0.93 for nitrification and nitrification respectively. Additionally, the observed rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation was plotted against the predicted rate of degradation for both $\text{NH}_4\text{-N}$ and NO_2^- , the result showed that the observed rate and the predicted rate had a good linear

Model: Change = F*G

Parity plot



Simulation of the model



ΔG as a function of $[\text{product}]/[\text{reactant}]$

Fig. 2. Parity plots of predicted rate nitrification values versus experimental values. simulations of the linear model data into a rate of $\text{NH}_4\text{-N}$ degradation versus Gibbs free energy data and ΔG as a function of $[\text{product}]/[\text{reactant}]$.

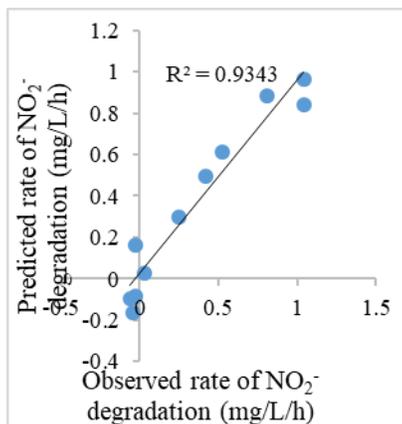
correlation with the correlation coefficient of 0.97 and 0.93 for nitrification and nitrification respectively- Fig 2 and 3.

IV. CONCLUSION

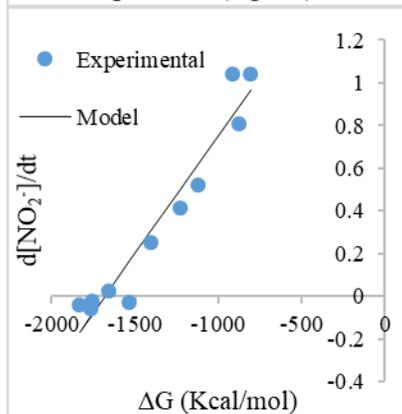
The isolated consortia presented an interesting characteristic, it performed nitrification, denitrification and degraded CN^- simultaneously. The Gibbs free energy was also studied to confirm the feasibility of the nitrification denitrification under high CN^- conditions. Negative ΔG were obtained for both nitrification and nitrification, this indicated that the reactions are feasible, spontaneous and rapid. Furthermore, the relationship between the rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation and ΔG was investigated, the results showed that there is a linear correlation between rate of $\text{NH}_4\text{-N}$ and NO_2^- degradation and their respective ΔG . The equilibrium constant was also investigated (results not shown) since the nature of equilibrium constant can also articulate whether the reaction is spontaneous or not, an increase in equilibrium constant with the decrease in ΔG was observed, this is also a proof that SNaD reaction occurs spontaneously and does not require additional energy.

Model: Change = a + F*G

Parity plot



Simulation of the model



ΔG as a function of [product]/[reactant]

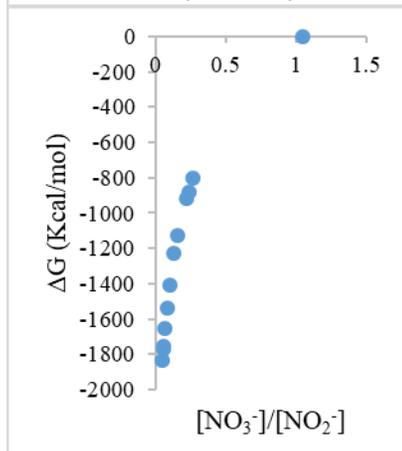


Fig. 3. Parity plots of predicted rate nitration values versus experimental values. simulations of the linear model data into a rate of NH₄-N degradation versus Gibbs free energy data and ΔG as a function of [product]/[reactant].

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