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Assessment of Anammox Bacteria in the Enrichment Culture on Sand and Granular Activated Carbon

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ABSTRACT

Anammox bacteria are specific group of autotrophic bacteria that are capable of oxidizing ammonium with nitrite to produce nitrogen gas. This group of bacteria has low biomass yield and therefore, attached growth and granular sludge systems are often exposed to these bacteria for the removal of biological nitrogen from wastewater. This study investigated the deposition of anammox bacteria in the enrichment culture to the surfaces of silica sand and granular activated carbon (GAC). The interactions between the bacterial cells and these media could be described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. The DLVO theory predicted that the deposition of anammox cells onto the surfaces of both media was possible under the current experimental conditions. The comparison of net interaction energies between anammox cells and both media surfaces indicated that the deposition onto the surface of the GAC was more favorable. The predictions by the DLVO theory were in agreement with the physical observations of samples taken from the actual batch experiments using a scanning electron microscope.

Keywords: adhesion, Derjaguin-Landau-Verwey-Overbeek theory, interaction energy, surface potential, zeta potential

INTRODUCTION

Anaerobic ammonium oxidation or anammox is a process that is carried out by a specific group of autotrophic bacteria by oxidizing ammonium with nitrite to nitrogen gas. In general, it is quite difficult to enrich these bacteria in the laboratory because the biomass yield is low as the cells divide every 2 to 3 weeks (Noophan *et al*., 2008). Therefore, it is common to apply the anammox to wastewater treatment as attached growth systems and granular sludge systems (Thuan *et al*., 2004; Fernàndez *et al*., 2008). Adhesion of microorganisms, including bacteria, to various interfaces has been qualitatively explained by the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability (Hermansson, 1999). The theory is based on the interaction of two fundamental interfacial forces, that is, the electrical double-layer and the Lifishtz-van der Waals forces. Although later an extended DLVO theory (XDLVO) was developed and applied in relation to the classical DLVO to account for additional non-DLVO interactions (Ohki and Ohsima, 1999; Grasso *et al*., 2002; Bayoudh *et al*., 2009), under many circumstances,

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the classical DLVO is still adequate (Truesdail *et al*., 1998; Sharp and Dickinson, 2005). One important parameter for the calculation of interfacial forces is the surface potential. In the current study, the value of the surface potential of anammox bacteria was calculated based on the measurement of the zeta potential. The aims of the study were to investigate the deposition of an anammox culture on the surfaces of silica sand and granular activated carbon (GAC) in synthetic culture media and to compare the observed results with the predictions from the DLVO theory.

MATERIALS AND METHODS

Media preparation and surface characteristics

Ottawa sand (Fisher Scientific) of 20–30 mesh was repeatedly soaked in deionized water for 30 min three times. The sand was dried in the oven at 105 ^{o}C for 2 hr and allowed to cool to room temperature in the desiccator. According to Truesdail *et al*. (1998), the surface potential of Ottawa sand at pH 7.0 was -90 mV.

The granular activated carbon (GAC) (Carbokarn Co. Ltd., Thailand) was made from coconut shell (20–30 mesh). According to the manufacturer, this activated carbon has an iodine number less than 600 and an average macropore diameter of 1.64 ± 0.01 nm. The measurement of the zeta potential of the GAC was made with a Zetasizer Nano ZS (Malvern Instruments, United Kingdom) following the method described by Chiang *et al.* (2002). The activated carbon (about 100 mg) was ground into fine powder and mixed with distilled water, after which, the mixture was kept undisturbed for 45 min to allow heavy particles to settle. The colloidal-sized particles were collected. NaCl solution was added to the mixture as an electrolyte. The final concentration of electrolytes in the mixture was 1×10^{-2} M. Later, the pH adjustments were carried out using the same ionic strength solutions of 0.1M HCl and 0.1M NaOH.

Samples of anammox bacterial cell suspension were taken from the stock culture used by the previous studies of Noophan *et al*. (2008; 2009). The measurement of the zeta potential of the anammox bacterial cells was also made with the Zetasizer Nano ZS. The measurement was conducted at an ionic strength of 1×10^{-2} M NaCl solution as a function of pH. The bacterial sample was washed three times in the electrolyte solution, centrifuged and resuspended at a final cell concentration of approximately 1×10^{6} to 1×10^{7} cell.mL^{-1}. The pH of the bacterial cell solution was adjusted from 5 to 8 by adding the same ionic strength solutions of 0.1M HCl and 0.1M NaOH.

The zeta potential measurements of the mixture were carried out after the pH adjustments. The surface potential at the surface of the particles was calculated from the solution to the Poisson-Boltzmann equation proposed by Wang *et al*. (2002) as shown in Equation 1:

$$\psi(x) = \frac{kT}{z_i e} sinh^{-1}\left[\frac{z_i e}{kT}\psi_0 \exp(-\kappa x)\right], \quad (1)$$

where $\psi(x)$ = the potential at any distance x within the double layer from the surface (V), x = the distance from the surface (m), κ = the inverse Debye length (m^{-1}), e = the electronic charge (C), z_i = the valences of ions, k = Boltzmann's constant (J.K^{-1}) and T = the absolute temperature (K).

If $\psi(x)$ was substituted by the zeta potential and x was substituted by the Debye length or κ^{-1}, Equation (1) becomes Equation 2:

$$\xi = \frac{kT}{z_i e} sinh^{-1}\left[\frac{z_i e}{kT}\psi_0 \exp(-\kappa\kappa^{-1})\right] \quad (2)$$

or Equation 3:

$$\psi_0 = \frac{kT}{(z_i e)\exp(-1)} sinh\left[\xi \frac{z_i e}{kT}\right] \quad (3)$$

where ξ = the zeta potential (V) and ψ_0 = the surface potential.

Calculation of interaction energies

The interaction energies between the anammox cells and the surfaces of different core media were calculated according to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability (Israelachvili, 1992). The interaction energy, W, was calculated based on the sum of electrostatic double layer repulsion, V, and van der Waals attraction forces, A_{vdW} (Equation 4):

$$W = V + A_{vdW} \quad (4)$$

Since the anammox cells were much smaller than the size of the core media, the van der Waals' attraction forces were assumed to be an interaction between a sphere (anammox cells) and a flat surface (of either the sand or GAC) as shown by Equation 5 (Elimelech *et al*., 1995):

$$A_{vdW} = \frac{-Ar}{6}\left[\frac{r}{D} + \frac{r}{D+2r} + ln\frac{D}{D+2r}\right], \quad (5)$$

where A = the Hamaker constant for a colloid interacting across water (J), r = the radius of the anammox cell (m) and D = the separation distance (m).

For anammox cell-core media surface interactions, the double layer repulsion, V, was calculated based on the expression presented by Gregory (1975), assuming constant charge and linear-superposition as shown in Equations 6 and 7:

$$V = \left(\sum_i N_A C_i\right)\frac{128\pi kT}{\kappa^2}\gamma_1\gamma_2\frac{rR}{(r+R+D)}\cdot exp(-\kappa D), \quad (6)$$

$$\gamma_j = \tanh\left(\frac{y_i}{4}\right), j = \text{either 1 or 2}, \quad (7)$$

where N_A = Avogadro's number (mol^{-1}), C_i = the concentration of ion species i ($mol.m^{-3}$), R = the average radius of the core media and y_j = the reduced potential at any distance from the surface of 1 and 2.

In this case, for the anammox cells and core media, the value of y_j was calculated by Equation 8:

$$y_j = \frac{\psi_0}{kT}\left(\sum_i z_i e\right), \quad (8)$$

where ψ_0 = the colloidal surface potential (V) and e = the electronic charge (C).

The value of κ was then calculated from Equation 9:

$$\kappa = \left[\sum_i \frac{2N_A C_i e^2 z_i^2}{\varepsilon\varepsilon_0 kT}\right]^{1/2}, \quad (9)$$

The value of A was calculated from Equation 10 according to the Lifshitz theory:

$$A = \frac{3}{4}kT\left(\frac{\varepsilon_1-\varepsilon}{\varepsilon_1+\varepsilon}\right)\left(\frac{\varepsilon_2-\varepsilon}{\varepsilon_2+\varepsilon}\right) + \frac{3h\nu_e}{8\sqrt{2}}\cdot \frac{(n_1^2-n^2)(n_2^2-n^2)}{(n_1^2+n^2)^{1/2}(n_{12}^2+n^2)^{1/2}\left[(n_1^2+n^2)^{1/2}+(n_{12}^2+n^2)^{1/2}\right]}, \quad (10)$$

where h = Planck's constant (J.s), ν_e = the absorption frequency (s^{-1}), ε = the dielectric constant of water, ε_1 = the dielectric constant of the anammox cell wall, ε_2 = the dielectric constant of either the sand or the GAC, n = the refractive index of water, n_1 = the refractive index of the anammox cell and n_2 = the refractive index of either the sand or the GAC.

Anammox cultures

The study used the same anammox enrichment culture from previous studies (Noophan *et al*., 2008; 2009; Saricheewin *et al*., 2010). This particular enrichment culture has a distinct red color that is common to anammox cultures. Based on the data of molecular analyses by Saricheewin *et al*. (2010), this culture contained at least 80% anammox bacteria. The enriched anammox culture was grown in two identical Erlenmeyer Flasks (500 mL) for the sequencing batch experiments. The working volume of each flask was 400 mL. To each flask, 100 mL of anammox stock culture and 300 mL of synthetic wastewater were added. The anammox stock culture and synthetic wastewater were prepared according to Noophan *et al*. (2009).

The measured pH of the synthetic wastewater was 7.0 and the calculated ionic strength was 0.014M.

The two different reactors were added with either 2 g of silica sand or 2 g of the GAC, then sealed and placed on a shaker at 50 rpm. The reactors were manually operated in cycles of 24 hr distributed in four periods: mixed fill (10 min), mix react (23.5 hr), settle (15 min) and draw (5 min). The core media were sampled after 20 min into the mix react period of the experiments. The attachment of anammox cells onto different media surfaces was observed using a scanning electron microscope (SEM; JSM 5600, LV JEOL, Japan).

RESULTS AND DISCUSSION

Using Equations 1–3, the values of the surface potential were calculated based on the zeta potential values at different pH levels. The zeta potential values and surface potentials of the GAC and anammox bacterial cells at different pH levels are shown in Table 1. The zeta potential values of the GAC were in the same range as previously reported by Chiang *et al.* (2002). For the anammox bacterial cells, at the same electrolyte concentration and pH level, the value of the surface potential found in this study (-77.2 mV) was higher than the value for the phospholipid membrane (-85 mV) reported by Ohki and Ohshima (1999) and lower than the values of the surface potential of various strains of bacterial cell walls (-60 to -40 mV) reported by van der Wal *et al*. (1997).

Under the experimental conditions used in this study, the DLVO interaction energy was calculated between the anammox bacterial cells and the core media (either the GAC or sand). Literature values for several parameters were used for the surface potential of the sand, the dielectric constants and the refractive indices. The value of the dielectric constant of the GAC was estimated from the value of complex permittivities reported by Atwater and Wheeler (2003). The reported value of the refractive index of elemental carbon was used for the value of the refractive index of the GAC (Schkolnik *et al*., 2007). Although the anammox cells possessed proteinaceous cell wall-like archaea (Bothe *et al*., 2007), the values of the dielectric constant and refractive index for the bacterial cell wall were used in the calculations. Even though the actual values of the dielectric constants and refractive indices may differ from the estimated values used in the study, the error associated with the values of these constants was expected to be very small since the calculated Hammaker constants were relatively insensitive to these parameters. The parameters for the interaction energy calculation are provided in Table 2.

Figure 1 shows the DLVO interaction energy for both cases. The calculations indicated that the net interaction energy, W, between anammox cells and the two surfaces were negative at all separation distances, which indicated attraction. However, between the two surfaces, the anammox cells had a higher attraction to the GAC than to the sand.

Table 1 Zeta potential values and surface potentials of granular activated carbon (GAC) and anammox bacterial cells at different pH levels.

GAC			Anammox bacterial cell		
pH	Zeta (mV)	Surface potential (mV)	pH	Zeta (mV)	Surface potential (mV)
5	3.80	10.37	5	-20.48	-61.76
6	-4.10	-11.19	6	-21.71	-66.29
7	-17.30	-50.66	7	-24.51	-77.20
8	-24.10	-75.55	8	-17.57	-51.57

After the media had been sampled, the deposition of anammox cells to the surfaces of both media could be observed by the naked eye as the appearance of a slightly reddish, biofilm color on the media surface. Figure 2 shows the SEM photographs of the core media surfaces with deposition of anammox cells onto both media in different quantities. It was obvious that more anammox cells were deposited onto the surface of the GAC than onto the surface of the sand. Although it was not possible to conclude that the depositions were all anammox bacteria, it could be assumed that the depositions were mostly anammox bacteria due to the reddish color appearing on the media surfaces and the fact that this was an enrichment culture that contained mostly anammox bacteria (Saricheewin *et al*., 2010). Therefore, the results from physical observations were in agreement with the prediction by the DLVO theory. The data and calculations demonstrated here could apply to the selection of proper materials for core media to promote cell

Table 2 Values of parameters used in the calculation of interaction energy.

Parameter	Symbol	Value	Remarks
Sum of concentration of positive ions in the culture media (mol.m^{-3})	ΣC_i	23.0	This study
Sum of concentration of negative ions in the culture media (mol.m^{-3})	ΣC_i	23.0	This study
Radius of anammox cells (m)	r	4.8×10^{-7}	This study
Radius of sand (m)	R	2.5×10^{-4}	This study
Radius of GAC (m)	R	5×10^{-4}	This study
Dielectric constants			
Water	ε	78	
Bacteria cell wall	ε_1	60	van der Wal *et al*. (1997)
Wet silica sand	ε_2	19	Hubbard *et al*. (1997)
GAC	ε_2	1.0	Atwater and Wheeler (2003)
Surface potentials (V)	ψ_0		
Anammox bacteria cell		-0.08	This study
Wet silica sand		-0.09	Truesdail *et al*. (1998)
GAC		-0.05	This study
Water absorption frequency (s^{-1})	v_e	3×10^{15}	Israelachvili (1992)
Avogadro's number (mol^{-1})	N_A	6.02×10^{23}	
Electronic charge (C)	e	-1.60×10^{-19}	
Boltzmann's constant (J-K^{-1})	k	1.38×10^{-23}	
Absolute temperature (K)	T	298	This study
Planck's constant (J-s^{-1})	h	6.63×10^{-34}	
Refractive indices			
Water	n	1.33	
Bacteria cell wall	n_1	1.45	Heavens (1990)
Wet silica sand	n_2	1.448	Truesdail *et al*. (1998)
GAC	n_2	1.91	Schkolnik *et al*. (2007)

GAC = granular activated carbon.

deposition in the attached growth systems as well as the selection of materials for different parts of the system to prevent fouling.

CONCLUSIONS

The deposition of anammox cells onto the surfaces of silica sand and the GAC was observed in the culture vessels. The net interaction energy between the anammox cells and the surfaces of the sand and the GAC under the experimental conditions was negative at all separation distances, which indicated attraction. However, the DLVO predicted that the deposition of anammox cells onto the surface of the GAC was more favorable than the deposition of anammox cells onto the surface of the sand. The SEM images taken after the experiments showed that more anammox cells

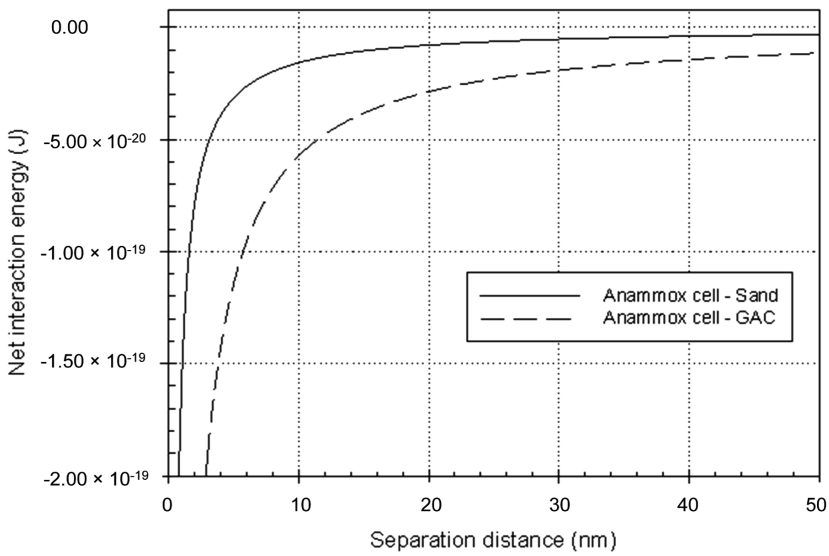

Figure 1 Net interaction energy between anammox cell and sand, and between anammox cell and granular activated carbon (GAC).

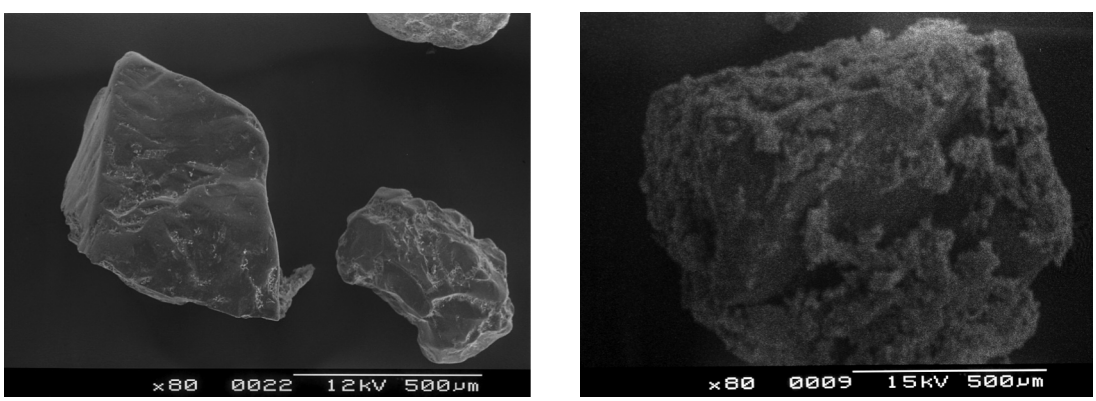

Figure 2 Scanning electron microscope images for (a) anammox cells deposited on sand and (b) anammox cells deposited on granular activated carbon (GAC).

were deposited onto the surface of the GAC than onto the surface of sand. The results from the physical observations were in agreement with the prediction by the DLVO theory.

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LITERATURE CITED

Atwater, J.E. and R.R. Wheeler. 2003. Complex permittivities and dielectric relaxation of granular activated carbons at microwave frequencies between 0.2 and 26 GHz. **Carbon** 41: 1801–1807.

Bayoudh, S., A. Othmane, L. Mora and H. Ben Ouada. 2009. Assessing bacterial adhesion using DLVO and XDLVO theories and the jet impingement technique. **Colloid. Surface. B.** 73: 1–9.

Bothe, H., S. Ferguson and W.E. Newton. 2007. **Biology of the Nitrogen Cycle.** Elsevier Science. 452 pp.

Chiang, H.L., C.P. Huang and P.C. Chiang. 2002. The surface characteristics of activated carbon as affected by ozone and alkaline treatment. **Chemosphere** 47: 257–265.

Elimelech, M., J. Gregory, X. Jia and R.A. Williams. 1995. **Particle Deposition and Aggregation.** Butterworth-Heinemann. Boston. 448 pp.

Fernàndez, I., J.R. Vazquez-Padín, A. Mosquera-Corral, J.L. Campos and R. Méndez. Biofilm and granular systems to improve Anammox biomass retention. **Biochem. Eng. J.** 42: 308–313.

Grasso, D., K. Subramaniam, M. Butkus, K. Strevett and J. Bergendahl. 2002. A review of non-DLVO interactions in environmental colloidal systems. **Rev. Environ. Sci. Biotech.** 1: 17 –38.

Gregory, J. 1975. Interaction of unequal double layers at constant charge. **J. Colloid Interface Sci.** 51: 44–51.

Heavens, O.S. 1990. Cell studies of total internal reflection fluorescence: Effect of lipid membranes. **J. Cell Sci.** 95: 175–176.

Hermansson, M. 1999. The DLVO theory in microbial adhesion. **Coll. Surfaces B.** 14: 105–119.

Hubbard, S.S, J.E. Peterson Jr., E.L. Majer, P.T. Zawislanski, K.H. Williams, J. Roberts and F. Wobber. 1997. Estimation of permeable pathways and water content using tomographic radar data. **The Leading Edge** 16: 1623–1628.

Israelachvili, J.N. 1992. **Intermolecular and Surface Forces.** 2nd ed. Academic Press. San Diego. California. 450 pp.

Noophan, P., C. Wantawin, S. Sripiboon, S., Sirivithayapakorn and M. Damrongsri. 2008. Nitrogen removal by autotrophic ammonium oxidizing bacteria enrichment under anaerobic conditions. **Songklanakarin J. Sci. Technol.** 30: 659–665.

Noophan, P., S. Sripiboon, M. Damrongsri and J. Munakata-Marr. 2009. Anaerobic ammonium oxidation by *Nitrosomonas* spp. and ANAMMOX bacteria in a sequencing batch reactor. **J. Environ. Manage.** 90: 967–972.

Ohki, S. and H. Ohshima. 1999. Interaction and aggregation of lipid vesicles (DLVO theory versus modified DLVO theory). **Colloid. Surface. B.** 14: 27–15.

Saricheewin, K., S. Sirivithayapakorn, P.L. Noophan, C. Wantawin, S. Techkarnjanaruk and J. Munakata-Marr. Nitrogen removal of anammox cultures under different enrichment

conditions. **J. Environ. Sci. Heal. A.** 45: 1832–1838.

Schkolnik, G., D. Chand, A. Hoffer, M.O. Andreae, C. Erlick, E. Swietlicki and Y. Rudich. 2007. Constraining the density and complex refractive index of elemental and organic carbon in biomass burning aerosol using optical and chemical measurements. **Atmos. Environ.** 41: 1107–1118.

Sharp, J.M. and R.B. Dickinson. 2005. Direct evaluation of DLVO theory for predicting long-range forces between a yeast cell and a surface. **Langmuir.** 21: 8198–8203.

Thuan, T.H., D.J. Jahng, J.Y. Jung, D.J. Kim, W.K. Kim, Y.J. Park, J.E. Kim and D.H. Ahn. 2004. Anammox bacteria enrichment in upflow anaerobic sludge blanket (UASB) reactor. **Biotechnol. Bioproc. E.** 9: 345–351.

Truesdail, S.E, J. Lukasik, S.R. Farrah, D.O. Shah and R.B. Dickinson. 1998. Analysis of bacterial deposition on metal (hydr)oxide-coated sand filter media. **J. Colloid Interface Sci.** 203: 369–378.

van der Wal, A., M. Minor, W. Norde, A.J.B. Zehnder and J. Lyklema. 1997. Electrokinetic potential of bacterial cells. **Langmuir.** 13: 165–171.

Wang, Z.W., G.Z. Li, D.R. Guan, Z.Z. Yi and A.J. Lou. 2002. The surface potential of a spherical colloid particle: functional theoretical approach. **J. Colloid Interface Sci.** 246: 302–308.