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*Original Article*

# Equilibrium and kinetic studies on the adsorption of humic acid by activated sludge and *Bacillus subtilis*

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## Abstract

This research is concerned with the adsorption of humic acid by activated sludge obtained from an alcoholic beverage plant and *Bacillus subtilis* (ATCC 6633). The effects of experimental factors such as initial pH, agitation speed, and types of adsorbent have been studied to compare adsorption capacity. The characterisation of activated sludge and *Bacillus subtilis* adsorption were examined by investigating the adsorption isotherm and kinetics. The results showed that the type of adsorbent had a significant relationship with adsorption capacity. In case of *Bacillus subtilis*, a maximum value of adsorption capacity of 11.44 mg humic acid /g biomass was observed based on the Langmuir adsorption isotherm and K value of 3.17x $10^{-2}$ ((mg/g)(mg/l)$^n$) from the Freundlich adsorption isotherm. The kinetic studies indicated that a pseudo second-order equation fitted the data remarkably well resulting in a rate constant ($k_2$) of about 0.019 g·mg$^{-1}$min$^{-1}$ for the adsorption onto *Bacillus subtilis*.

**Keywords:** adsorption isotherm, kinetic, humic acid, activated sludge, *Bacillus subtilis*

## 1. Introduction

Abrupt increases in manufacturing industries have caused excessive disposal of wastewater above the sanitary standards. The consequences lie not only upon the aquatic environment, but also on the health of general population. As the impacts have become more detrimental to the environment, waste treatments have gained more awareness. The global concern, however, needs also to be placed on the application of treatments that are in line with the different characteristics of the wastes. Appropriate methods to remove fluid pollutants include filtration, chemical precipitation, coagulation, solvent extraction, electrolysis, ion exchange, membrane process and adsorption. According to Martiinez *et al.* (2006), the most common and effective techniques are ion exchange and adsorption.

Among the environmental components that can be harmful to human health, the so-called humic substances (HS) representing complex compounds generated by plant's, animal's and microbial cell's decay are important. HS can be isolated from most soils, waters including ground and surface, and sediments. Based on their solubility, HS are categorized into three compositions including humins, humic acids (HAs) and fulvic acids (FAs). HAs consist high molecular-weight organic substances that are soluble in high-pH value water, whereas FAs consist of moderate molecular-weight organic substances soluble in water of any pH value (Trump *et al.*, 2006).

The adverse effects of HS occur as they have the ability to interact with organic and inorganic substances. Humic substances can interfere with the chlorination process in the drinking water manufacture, which leads to the generation of trihalomethanes (THMs). The US Environmental Protection Agency has classified THMs as a human carcinogen compound and suggested that maximum contamination level of THMs should be maintained below 80 µg/l in drink-

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ing water (Kim *et al.*, 2002). To prevent adverse effects on human health, natural organic compounds should be eliminated from water before supplying it for the chlorination process in the drinking water manufacture. Membrane separation and adsorption on activated carbon were the main processes available for removing natural organic matters from water (Moussavi *et al.*, 2011). Activated carbon adsorption is recommended by EPA as a treatment technique suitable for the elimination of natural organic matters from water to attain the established regulations. However, it has been shown that activated carbon was less efficient in practice for high molecular organic compounds such as natural organic matters (Moussavi *et al.*, 2011). In addition the application of activated carbon was not always technically advantageous due to the high regeneration cost involved and further disposal of the waste activated carbon was required (Sun and Lee, 2012).

Biotechnological approaches used for treating such pollutants are unsophisticated, relatively inexpensive, and environmentally friendly. For instance, microbiological processes have vital implication to determine metal mobility and potential in bioremediation of metal pollution. Some prospective uses of microorganisms in mining and similar fields are the bioleaching of heavy metals, biooxidation of precious metal from ores, desulfurization of coal and oil, and biosorption of metal ions.

Biosorption is the uptake of pollutant such as heavy metal from aqueous solution by biological materials known as biosorbents. A low cost sorbent is defined as one which is abundant in nature, or is a by-product or waste material. Humic substance is not easily decomposed by microorganisms as reported in the studies on the degradation of HA and FA in soil using fungi (Rezacova *et al.*, 2006) and cultivation (Bongiovanni and Lobartini, 2006). Various waste biomaterials such as activated clay, chitosan and composite beads (Chang and Juang 2004), chitosan-$H_2SO_4$ beads (Ngah et al., 2011) and activated sludge (Esparza-Soto and Westerhoff, 2003; Moura *et al.*, 2007) have been studied for the removal of humic acid. In addition to biomaterials, microorganisms have also been used as biosorbents; the adsorption of humic acid by *Bacillus subtilis* was investigated by Moura *et al.* (2007) and Fein *et al.* (1999). The activated sludge from wastewater treatment plants contains bacteria and protozoa. The cell walls of bacteria consist mainly of polysaccharides, lipids and proteins while outer membranes of protozoa consist of lipids and proteins, all of which are capable of adsorbing substances. The cell wall of *B. subtilis* is composed of peptidoglycans, salt, teichoic and teichuronic acids that it' exhibits as carboxyl, phosphoryl groups at the surface. In case of adsorption of humic acid onto *B. subtilis* where the humic acid and bacterial surface are lower charged, the interaction is mainly hydrophobic (Moura *et al.*, 2007).

The objectives of this project were (i) to determine the equilibrium time of HA adsorption onto activated sludge as a function of agitation speed, (ii) to study the effect of adjusting the pH in aqueous solutions on the adsorption and (iii) to investigate the potential of activated sludge, biomass (*Bacillus subtilis*) and selected waste biomaterials for absorbing humic substance from aqueous solution. Later, the data of HA adsorption onto activated sludge and *Bacillus subtilis* was analyzed using Langmuir and Freundlich adsorption models. In addition, the data were examined for the adsorption kinetics including pseudo first and second-order equations.

## 2. Materials and Methods

### 2.1 Biosorbent preparation

Activated sludge was obtained from a factory producing alcoholic beverage in Thailand, and then was dried outside the building under good air circulation for a few days. Dried activated sludge was ground and kept in a desiccator before testing. The concentration of activated sludge was 25 g dry basis per liter of humic acid solution.

*Bacillus subtilis* (ATCC 6633) were cultured at 10% inoculum in LB medium (tryptone 1%, yeast extract 0.5%, and NaCl 1%) at 37°C for 24 h in an orbital shaker at 100 rpm. Cells were removed from the nutrient broth (100 ml) by membrane filtration (Millipore 0.45 mm), and then dried at about 60°C, finally weighed and kept in a desiccator. The viable cell concentration of *Bacillus subtilis* was determined using a microscope counting chamber (hemocytometer) to be $1.8 \times 10^8$ cell/ml, corresponding to the average cell yield of about 0.24 g of cell dry weight per liter broth.

Waste materials such as bagasse, husk corn, coconut coir, and un-ripened banana peel gel were also used to study the absorption capacity. Dried bagasse, corn husk, and coconut coir were cut, ground in a grinder, then dried again in the oven at 45-50°C, and finally screened into the size range of less than 0.3 mm. Un-ripened banana peel was cut into small pieces (about 0.5 cm size), and mixed with sulfuric acid (at the ratio of 60 ml solvent to 100 g material) and stirred at about 100°C for 24 hours under reflux control for gel cross-linking. The slurries after neutralization with $NaHCO_3$ were filtered, washed with distilled water and dried in the oven at 100°C. Dried gel was ground in a grinder, and also screened into size range of less than 0.3 mm. Dry powders of waste materials were kept in a desiccator. The concentration of each waste material was 20 g dry basis per liter of humic acid solution.

### 2.2 Humic acid solution

The chemical used for this study was analytical grade humic acid purchased locally from Sigma-Aldrich, Switzerland. The humic acid solution was prepared by dissolving the respective amounts in distilled water to the required concentration with pH adjustment. The exact concentration was analyzed by an UV/ Visible spectrophotometer (Perkin Elmer Lambda 20). A calibration curve was determined using standardized humic acid solutions. The solutions were diluted with distilled water and later the same day the humic acid concentrations were determined by the UV/ Visible spectrophotometer. The wavelength selected was 272 nm for humic acid analysis (Traina *et al.*, 1989; Haderlein *et al.*, 2001; Young *et al.*, 2004).

### 2.3 Procedures

In a 250 ml Erlenmeyer flask, biosorbent and 50 ml of humic acid solution (with pH adjustment) were added. The flasks were placed in an shaker incubate at 30°C, 100 rpm and samples were taken out after intervals of 5, 10, 20, 40, 60, 120 minutes for the study of equilibrium contact time. The humic acid solution was passed through a filter paper (Whatman No. 1) to separate solid and liquid phases and the filtrate was used for analyzing the humic acid concentration. All experiments were conducted in duplicate. In order to study the effects of initial pH on adsorption capacity, the initial pH levels of humic acid solution were adjusted in 2 to 11 range with 0.5 M HCl or NaOH. The flasks were taken out at optimal equilibrium contact time.

### 2.4 Adsorption capacity

Adsorption capacity of the biosorbent was defined on the basis of difference between the concentration of humic acid in the liquid solution before and after adsorption by the following equations:

$$Ad = \left[\frac{C_i - C_{eq}}{C_i}\right] \times 100 \tag{1}$$

$$q_{eq} = (C_i - C_{eq})(\frac{V}{w}) \tag{2}$$

where: Ad = adsorption percentage (%)

$q_{eq}$ = humic acid adsorbed on biosorbent (mg/g dry basis)

$C_i$ = humic acid concentration in liquid solution before adsorption (mg per liter)

$C_{eq}$ = humic acid concentration in liquid solution after adsorption (mg per liter)

V = solution volume (liter)

w = amount of dry biosorbent used (gram)

### 2.5 Adsorption isotherm

The Langmuir and Freundlich equations are widely used to interpret the adsorption data. At the equilibrium adsorption time, the data can be characterized by the following equations:

Langmuir equation: $$\frac{1}{q_{eq}} = \frac{1}{q_m b C_{eq}} + \frac{1}{q_m} \tag{3}$$

where: $q_m$ is the maximum adsorption capacity per unit weight of biosorbent and b is the adsorption equilibrium constant.

The equilibrium data for the bacterial adsorption is used to plot as 1/ $q_{eq}$ vs 1/$C_{eq}$ and the two constants $q_m$ and b are then calculated from the slope (1/$q_m$b) and intercept (1/$q_m$) of the fitted line, respectively.

Freundlich equation: $$q_{eq} = KC_{eq}^{1/n} \tag{4}$$

where: K and n are empirical constants indicative of the adsorption capacity and adsorption intensity.

The parameters K and n were obtained by fitting the adsorption data to the linearized equation of the graph between log $q_{eq}$ and log $C_{eq}$.

## 3. Results and Discussion

### 3.1 Equilibrium contact time

Adsorption equilibrium is defined as the equilibrium distribution of a given component between an adsorbate and adsorbent. The humic acid concentration in the liquid-phase decreased rapidly with time apparently due to the adsorption of humic acid on the activated sludge. Adsorption of humic acid on activated sludge and the cells of *Bacillus subtilis* reached equilibrium levels for all samples as shown in Figure 1. The equilibrium adsorption levels were attained after about

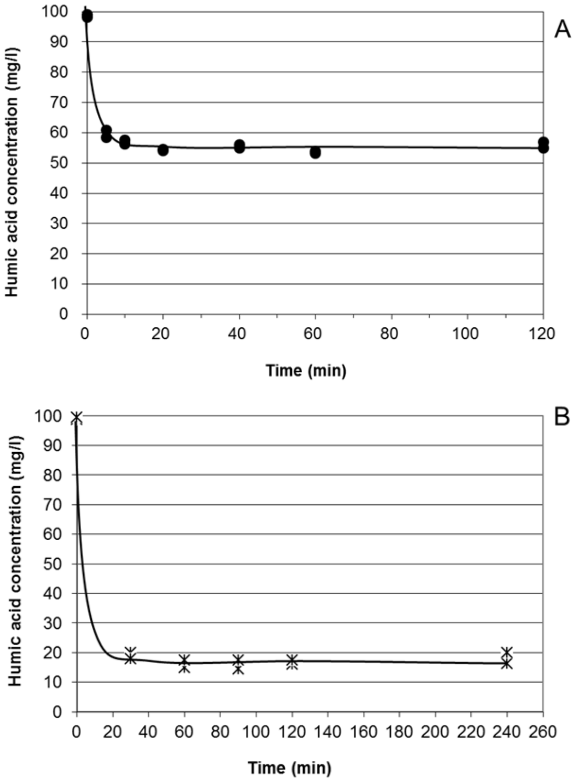

Figure 1. Adsorption of humic acid in solution as a function of time by activated sludge (A) in 100 mg/l $[HA]_0$ at initial pH of 10.0 and the cells of *Bacillus subtilis* (B) in 100 mg/l $[HA]_0$ at initial pH of 2.0. Adsorption conditions: 30°C, 50 ml humic acid solution and 1.25 g dry basis of activated sludge or 0.02 g dry basis *Bacillus subtilis*.

20 and 60 minutes of exposure for activated sludge and the cells of *Bacillus subtilis*, respectively. The equilibrium adsorption trends of copper (II) were in good agreement with the results of Moura *et al.* (2007). An equilibrium time of more than 30 min was sufficient for the adsorption of humic acid onto activated sludge at a concentration of 25 g/L and pH 6.2. and *Bacillus subtilis* at a concentration of 10.5 g/L and pH 6.02. In addition to adsorption retention times, Esparza-Soto and Westerhoff (2003) concluded that the biosorption of activated sludge and not the biodegradation took place at retention times shorter than 3 hours.

Moreover, the equilibrium depended on the interactions of many of the same parameters affecting the adsorption capacity, among which shake flask speed played an important role. Figure 2 shows the biosorption of humic acid on activated sludge at initial pH of 2.0 with different agitation speeds. Apparently, equilibrium adsorption levels were attained after about 20 minutes of exposure for all agitation speeds investigated in this study. During the equilibrium adsorption at 250 rpm, the adsorption capacity gave the lowest adsorption of humic acid of 2.1 mg/g, which was much lower than the adsorption capacity at 100 rpm. The higher shear forces at 250 rpm may have resulted in adsorbate detachment or prevention of attachment in the first place. In addition the adsorption capacity of 2.4 mg/g determined at 100 rpm was highest in comparison to the values obtained at 50, 150 and 200 rpm. Taking into account these results, a contact time of 60 minutes and agitation speed of 100 rpm were selected for further experiments.

### 3.2 Effect of pH on adsorption capacity

Humic acid is a subclass of humic substances (humins, HAs, and FAs) of macromolecular characteristics, containing both hydrophobic and hydrophilic moieties as well as chemical functional groups such as carboxylic, phenolic, carbonyl and hydroxyl groups connected with the aliphatic or aromatic carbons in the molecules (Zhang and Bai, 2003). Biosorption of humic acid was greatly affected by pH, resulting in a decrease in adsorption capacity with increasing pH (Figure 3). A maximum adsorption of 2.25 mg adsorbed humic acid per gram of activated sludge was observed at initial pH value of 2. When the adsorption data from mean average experiments at initial pH 2 was compared to experiment at initial pH 3, a difference of a less than 0.86 mg/g adsorption capacity was observed. This was followed by a decrease in adsorption capacity to end of the set of pH experiments. Also the minimum adsorption capacity was found to be only 0.39 mg/g at initial pH 11, resulting in a sharp decrease in adsorption capacity by more than 5-fold from 2.25 mg/g at initial pH 2. In addition, the humic acid solution showed an increase in initial pH of 2 to equilibrium pH of 5 after 60 minutes. It is believed that humic acid usually contains a wide variety of functional groups (-COOH, -OH, $-NH_2$, etc.), which are in uncharged state at lower pH, and hence tend to adsorb more (Wang *et al.*, 2009). The decreased amount of $H^+$ ions by the end of experiment may be consistent with uncharged state of those functional groups of humic acid. This was assumed that the system became less hydrophilic and thus humic acid, would bind hydrophobic compounds more effectively.

Comparison of this work with that of Moura *et al.* (2007), Wang *et al.* (2009) and Weng *et al.* (2007) shows a good agreement. These authors also reported an increase in the initial pH of humic acid solution with decreasing adsorption capacity. The maximum percentage of HA adsorption on bentonite (Doulia *et al.*, 2009) and carboxymethylchitosan (Zhao *et al.*, 2008) were achieved at pH 3 and 3.5, respectively. The adsorption of humic acid onto *B. subtilis* and activated sludge increased with decreasing pH above the 3.8-10.0 pH range, corresponding to a maximum adsorption of about 5 mg adsorbed per gram of *B. subtilis* biomass and about 2.4 mg adsorbed per gram of activated sludge at pH value of 4.0 [8]. In addition, humic acid adsorption onto bacterial surfaces was highest at pH 2.5 (Fein *et al.*, 1999).

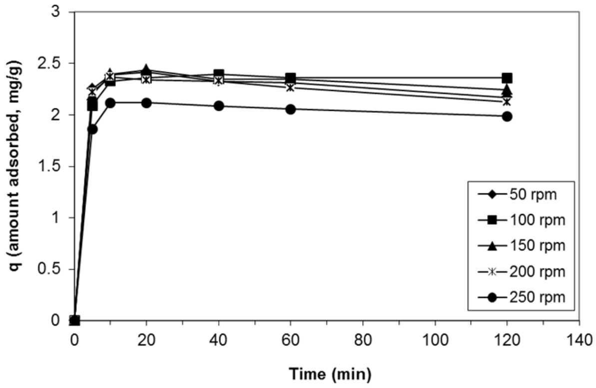

Figure 2. Biosorption of humic acid on activated sludge as a function of time for different agitation speeds. Adsorption conditions: 100 mg/l $[HA]_0$ at initial pH of 2.0, 25 g dry basis of activated sludge per liter of humic acid solution (1.25 g activated sludge per 50 ml humic acid solution) and 30°C.

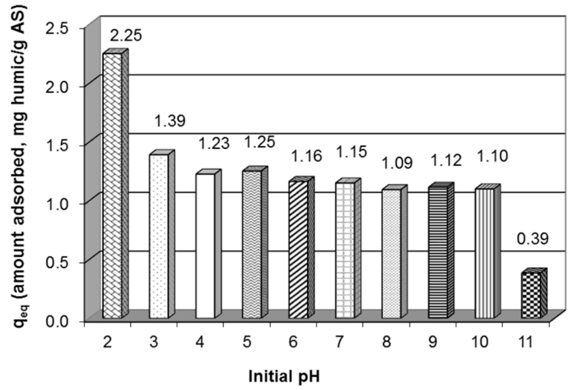

Figure 3. Biosorption of humic acid on activated sludge for different initial pHs. Adsorption conditions: 75 mg/l $[HA]_0$, 25 g dry basis of activated sludge per liter of humic acid solution, 60 minutes of adsorption time and 100 rpm incubate shaker at 30°C.

A reduced level of ineffective adsorption was observed at higher pH value above 7. As pH is lowered the overall surface becomes uncharged and the greater degree of adsorption observed suggesting hydrophobic interactions [8, 9]. This is in agreement with Esparza-Soto and Westerhoff (2003) that higher biosorption at low pH may be attributed to hydrophobic interactions between humic substances and activated sludge biomass extracellular polymers (EPS). Approximately 75% of the EPS organic carbon has been characterized as amino acids with 25% of these amino acids identified as acidic amino acids. The remaining fraction of EPS amino acids has been described as hydrophobic amino acids, which gives a hydrophobic character to the bacteria surface (Esparza-Soto and Westerhoff, 2003).

### 3.3 Type of biosorbent

Figure 4 shows the comparison of adsorption capacities of humic acid by different adsorbents. The results showed some differences in adsorption capacities of humic acid, probably due to different structure of materials. Sugarcane bagasse, corn husk and coconut coir indicated lower adsorption efficiency in agreement with the work reported by Kurakake *et al.* (2001). The untreated sugarcane bagasse (finer than 36 mesh) and untreated corn husk (finer than 80 mesh) had no capacities for the adsorption of enzyme CMCase and xylanase (Kurakake *et al.*, 2001). Sugarcane bagasse, husk corn and coconut coir consisted of high proportion of well-defined polymeric structures: cellulose, hemicellulose and lignin (Kurakake *et al.*, 2001; Wu *et al.*, 2006; Nilza *et al.*, 2008). These components may have resulted in adsorbate detachment or prevention of adsorbate attachment in the first place.

Un-ripened banana peel is also generally composed of cellulose, hemicellulose and lignin containing polyphenolic groups (Wachirasiri *et al.*, 2009). The tannin content of un-ripened banana (*Musa seientum linn*) in this study was found to be 0.7 milligram tannin per gram material. Un-ripened banana peel gel was formed by tannin polymerization mainly by the -OH, C=O, C=C and C-H stretching group of tannin (Rubcumintara *et al.*, 2010). The adsorption by un-ripened banana peel gel (5.52 mg humic acid /g adsorbent) was significantly higher than by sugarcane bagasse, corn husk, coconut coir and activated sludge.

The adsorption of humic acid on activated sludge in present work was slightly greater than that observed by Moura *et al.* (2007). This difference may be due to a complex microbial community of microorganisms in the activated sludge. The activated sludge from the wastewater treatment plant of an alcoholic beverage industry might have contained mainly yeast *Saccharomyces cerevisiae*. The cell walls of *Saccharomyces cerevisiae* consist mainly of D-glucan as the major polysaccharides, along with an insoluble pellet highly enriched with D-glucan (Kwiatkowski *et al.*, 2009). The cell wall proteins may affect hydrophobicity of the cells, which is important for adhering to polystyrene and other abiotic surface (Klis *et al.*, 2006). The non-reducing ends of the 1,3 -β-glucan may function as sites for covalent attachment of other polysaccharides (Klis *et al.*, 2006). This could have resulted in a better capability for adsorption.

*Bacillus subtilis* is a rod-shaped organism which is known for its structure and the function of the cell wall. The chemistry of the cell wall peptidoglycan has been well established (Hayhurst *et al.*, 2008). The great difference in surface structure resulted in a greatest biosorption capacity corresponding to 17.8 mg/g adsorbent for experimental conditions of 75 mg/l initial humic acid solution, initial pH of 2.0, 60 minutes of adsorption time and 100 rpm incubate shaker at 30°C.

Table 1 shows the comparison of adsorption efficiencies on different adsorbents. The results showed some differences in equilibrium concentration of adsorbed humic acid per unit weight of adsorbent. Fly ash and zeolite tended to yield greater adsorption efficiency. These different results are probably due to different pH conditions corresponding to various initial humic acid solutions. In the present work, *Bacillus subtilis* (ATCC 6633) showed a significant difference in equilibrium concentration of adsorbed humic acid per unit weight of adsorbent compared to the work reported by Moura *et al.* (2007) when using *Bacillus subtilis* (CECT 4522).

### 3.4 Adsorption isotherms

The equilibrium adsorption isotherms data for humic acid by activated sludge and *Bacillus subtilis* according to Langmuir equation and Freundlich equation are characterized in Figures 5A and 5B, respectively. The concentration of adsorbed metal per unit weight of biosorbent (q) and the

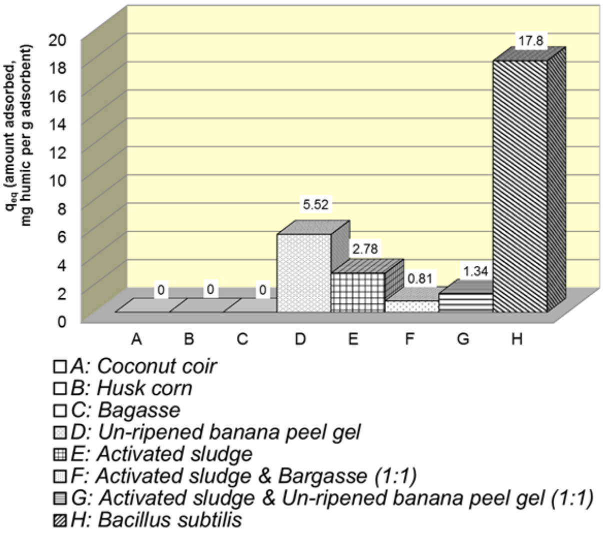

Figure 4. Adsorption of humic acid on different adsorbents. Adsorption conditions: 75 mg/l $[HA]_0$ at initial pH of 2.0, 60 minutes of adsorption time and 100 rpm incubate shaker at 30°C.

Table 1. Comparison of adsorption efficiencies on different adsorbents.

| Type of adsorbent | Studied conditions | q (mg humic acid/ g biosorbent) | Ref. |
|---|---|---|---|
| Chitosan-coated granules | 15 mg/l $[HA]_0$ at initial pH of 6.5 | 0.07 | Zhang and Bai (2003) |
| Activated clay | 100 mg/l $[HA]_0$ at initial pH of 8.0 | 12 | Chang and Juang (2004) |
| Fly ash | 50 mg/l $[HA]_0$ at initial pH of 3.0 | 40 | Wang and Zhu (2007) |
| Zeolite | 50 mg/l $[HA]_0$ at initial pH of 5.0 | 42 | Wang *et al.* (2008) |
| Bentonite | 50 mg/l $[HA]_0$ | 1.75 | Doulia *et al.* (2009) |
| Activated sludge | 100 mg/l $[HA]_0$ at initial pH of 4.4 | 2.4 | Moura *et al.* (2007) |
| *Bacillus subtilis* (CECT 4522) | 100 mg/l $[HA]_0$ at initial pH of 4.1 | 4.9 | Moura *et al.* (2007) |
| Natural zeolite | 20 mg/l $[HA]_0$ at initial pH of 7.0 | 8.7 | Moussavi *et al.* (2011) |
| polyaniline/attapugite (ATP–PANI) | 60 mg/l $[HA]_0$ at initial pH of 5.0 | 61 | Wang *et al.* (2011) |
| Activated sludge | 75 mg/l $[HA]_0$ at initial pH of 2.0 | 2.78 | This work |
| *Bacillus subtilis*(ATCC 6633) | 75 mg/l $[HA]_0$ at initial pH of 2.0 | 17.8 | This work |

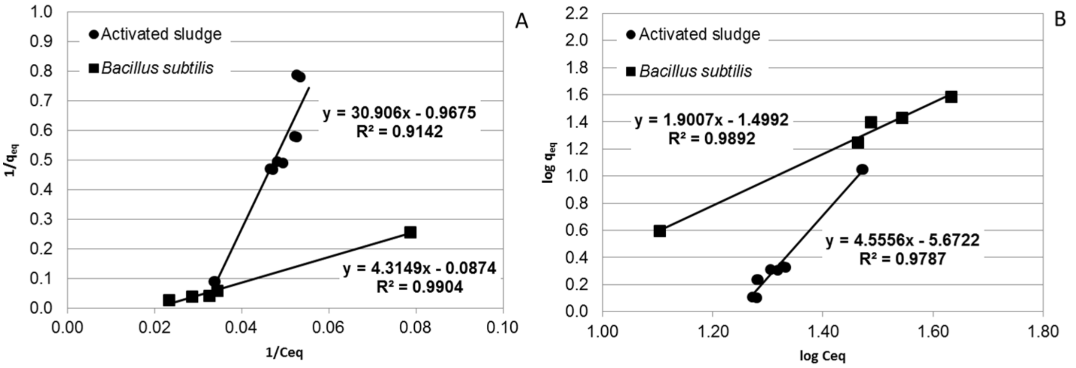

Figure 5. The fitted Langmuir (A) and Freundlich (B) isotherms for the adsorption of humic acid onto various adsorbents. Adsorption conditions: humic acid solution at initial pH of 2.0, and 100 rpm incubate shaker at 30°C.

concentration of humic acid in the liquid phase ($C_{eq}$) were determined for equilibrium time of 60 minutes. The equilibrium data were plotted as 1/q vs $1/C_{eq}$ and log q vs log $C_{eq}$ according to Equations 3 and 4, respectively. Subsequently, the parameters in Langmuir equation ($q_m$ and b) and Freundlich equation (K and n) were determined from the slope and intercept of the least-squares fits. Table 2 presents the results of regression analysis for determining the parameters of Langmuir and Freundlich equations. The coefficient of determination ($R^2$) ranged from 0.914 to 0.979 and 0.990 to 0.989 for adsorption onto activated sludge and *Bacillus subtilis*, respectively. These results indicated that the equilibrium adsorption data onto *Bacillus subtilis* conformed remarkable well to the Langmuir and Freundlich equations. However, the equilibrium adsorption data onto activated sludge showed better fitting with Freundlich equation.

### 3.5 Adsorption kinetics

In the past, various kinetic models have been examined to investigate the mechanism of adsorption. Two simplified kinetic models consisting of pseudo first-order and pseudo second-order equations [6, 16] were selected for analysis. The pseudo first-order equation was given by:

$$\frac{dq_t}{dt} = k_1(q_{eq} - q_t) \qquad (5)$$

where: $k_1$ = pseudo-first order rate constant ($min^{-1}$).
$q_{eq}$ = pseudo-equilibrium adsorption corresponding to the initial humic acid concentration $[HA]_0$.
$q_t$ = amount of adsorption at time t.

The integration of eq. (5) with the conditions $q_t = 0$, t = 0 and $q_t = q_t$, t = t, yielded:

$$\ln(q_{eq} - q_t) = \ln q_{eq} - k_1 t \qquad (6)$$

The values of $q_{eq}$, $k_1$ and the correlation coefficients were determined from the linear plots of $\ln(q_{eq}-q_t)$ versus t as shown in Figure 6A.

In addition, the pseudo-second-order kinetic model was also examined as follows:

$$\frac{dq_t}{dt} = k_2(q_{eq} - q_t)^2 \qquad (7)$$

where: $k_2$ = pseudo-second order rate constant ($g \cdot mg^{-1} min^{-1}$). After integration, the linear equation is given by:

Table 2. Regression parameters of Langmuir and Freundlich equations for biosorption of humic acid solution by activated sludge and *Bacillus subtilis* at initial pH of 2.0

| Adsorbents | Langmuir equation | | | Freundlich equation | | |
|---|---|---|---|---|---|---|
| | $q_m$(mg/g) | b (l/mg) | $R^2$ | K ((mg/g)(mg/l)$^n$) | n | $R^2$ |
| Activated sludge | 1.03 | 0.03 | 0.9142 | 2.13x10$^{-6}$ | 0.22 | 0.9787 |
| *Bacillus subtilis* | 11.44 | 0.02 | 0.9904 | 3.17x10$^{-2}$ | 0.53 | 0.9892 |

$$\frac{t}{q_t} = \frac{1}{k_2 q_{eq}^2} + \left(\frac{1}{q_{eq}}\right) t \qquad (8)$$

The $q_{eq}$ and $k_2$ parameters were obtained by fitting the adsorption data to the linearized equation of the graph between $t/q_t$ and t as shown in Figure 6B.

The pseudo first-order kinetic model and pseudo second-order kinetic model basically include all steps of adsorption such as external film diffusion (Chang *et al.*, 2005). The correlation coefficients for the fit of the linear regression of pseudo first-order equation to the data were only 0.23 and 0.69 for adsorption onto activated sludge and *Bacillus subtilis*, respectively, indicating that the kinetic distribution data did not conform to the pseudo first-order linear regression. If all the data points were fitted with the pseudo second-order linear regression (Figure 6B), the trend line achieved $R^2$ values of more than 0.99, indicating a very good data fit. From the regression analysis, the values of pseudo second-order rate constants ($k_2$) for the adsorption onto activated sludge and *Bacillus subtilis* were calculated to be about 2.66 and 0.019 g·mg$^{-1}$min$^{-1}$, respectively. The pseudo-equilibrium adsorption coefficient, $q_{eq}$ (corresponding to the initial humic acid concentration) were 2.37 and 17.54 mg/g for the adsorption onto activated sludge and *Bacillus subtilis*, respectively. A high correlation coefficient for the fit of the pseudo second-order linear regression to the data indicated that the adsorption data conform to the pseudo second-order linear regression.

The adsorption of humic acid onto activated clay, chitosan beads and composite beads was better represented by the fitted pseudo first-order model (Chang and Juang, 2004). On the other hand, Wang *et al.* (2009) stated that the pseudo second-order kinetics and the two site kinetics of humic acid adsorption onto unburned carbons gave better results than the pseudo first-order kinetics. The adsorption of humic acid on fly ash could be modeled by pseudo second-order kinetics rather than first-order kinetic (Wang *et al.*, 2008). It is difficult to interpret the results reported in the literature due to their conflicting nature. These conflicting results are probably the consequence of the nature of the adsorbents, i.e. a pseudo second-order model for humic acid adsorption onto activated sludge and *Bacillus subtilis* as observed in present study, and a pseudo first-order model for humic acid onto activated clay, chitosan beads and composite beads reported by Chang and Juang (2004).

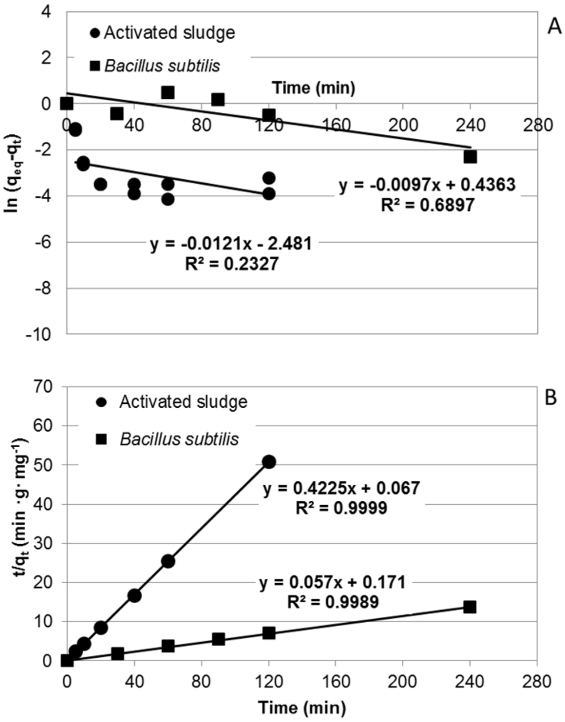

Figure 6. Plots of pseudo first-order kinetic model (A) and pseudo second-order kinetic model (B) for the adsorption of humic acid on different adsorbents. Adsorption conditions: 100 mg/l $[HA]_0$ at initial pH of 2.0, and 100 rpm incubate shaker at 30°C.

## 4. Conclusion

Adsorption of humic acid on activated sludge attained equilibrium after about 20 minutes of exposure for agitation speed ranging from 50 to 250 rpm. The optimum agitation speed and initial pH level for HA adsorption by activated sludge were found to be 100 rpm and 2.0, respectively. The overall adsorption efficiency increased with a decrease in the initial pH, giving a maximum adsorption capacity per unit dry weight of activated sludge of 2.25 at initial pH value of 2 and agitation speed of 100 rpm. *Bacillus subtilis* represented the most suitable adsorbent for HA adsorption amongst the adsorbents considered in this study. Results showed maximum adsorption capacity based on Langmuir isotherm to be 11.44 and 1.03 mg HA per g dry weight *Bacillus subtilis* and activated sludge, respectively. The adsorption data of HA adsorption on *Bacillus subtilis* and activated sludge conformed to the pseudo second-order linear regression. The pseudo second-order rate constant ($k_2$) for the adsorption onto *Bacillus subtilis* and activated sludge were calculated to be about 0.019 and 2.66 $g{\cdot}mg^{-1}min^{-1}$, respectively.

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