Title
1,4-Dioxane Biodegradation Using Bioaugmented Granular Activated Carbon

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Publication Date
2016

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

1,4-Dioxane Biodegradation Using Bioaugmented Granular Activated Carbon

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Civil Engineering

by

Michelle Allison Myers

2016

ABSTRACT OF THE THESIS

1,4-Dioxane Biodegradation Using

Bioaugmented Granular Activated Carbon

by

Michelle Allison Myers

Master of Science in Civil Engineering

University of California, Los Angeles, 2016

Professor Shaily Mahendra, Chair

1,4-Dioxane is a probable human carcinogen and a contaminant that has been emerging in surface water and groundwater resources in the US and internationally. Many traditional water treatment technologies, such as air stripping and UV photolysis have not been effective for 1,4-dioxane, while others, such as advanced chemical oxidation, are costly and energy intensive. Biodegradation of 1,4-dioxane is a low cost, energy-efficient, and environmentally friendly *in situ* method. *Pseudonocardia dioxanivorans* CB1190 (CB1190), an aerobic bacterial strain, uses 1,4-dioxane as its sole carbon and energy source. *Mycobacterium austroafricanum* JOB5 (JOB5), another aerobic bacterium, co-metabolizes 1,4-dioxane, while using propane, as its sole carbon and energy source. Both CB1190 and JOB5 have been primarily studied in the laboratory planktonic cultures, while most environmental microbes grow in biofilms on surfaces. Thus, it is

important to characterize the removal of 1,4-dioxane by bacteria in attached growth mode. Other hydrophilic water contaminants, such as methyl tert-butyl ether, tert-butyl alcohol, and 4-chlorophenol, have been previously reported to degrade in bioaugmented sorbent reactors.

This study investigated 1,4-dioxane biodegradation by CB1190 and JOB5 cultures growing attached to inorganic and carbonaceous sorbents. In abiotic controls, the sorption capacity and kinetics of selected commercial sorbents were evaluated. Abiotic 1,4-dioxane batch reactors were set up with Norit 1240, Ambersorb 560, Bayoxide E33, US1076, and US1078 to establish isotherms and model the isotherm kinetics. 1,4-Dioxane rapidly adsorbed to all tested sorbents, except Bayoxide E33, displaying Freundlich behavior. Desorption of 1,4-dioxane occurred to various extents into water or ionic buffer at the same temperature. Norit 1240 and Ambersorb 560, sorbents, which demonstrated high affinity for 1,4-dioxane and possessed physical characteristics compatible with a flow-through column reactor, were used in CB1190 and JOB5 bioaugmented sorbent batch reactors. Bioaugmented sorbents removed significantly more 1,4-dioxane than abiotic sorbents. Abiotic sorbents reduced aqueous concentrations by 85-89% of initial concentration, whereas JOB5 and CB1190 bioreactors reduced aqueous concentrations to below the 95% and 98% of initial concentration, respectively. Bacterial growth and attachment was visualized using fluorescence microscopy and was confirmed by amplification of taxonomic genes by quantitative polymerase chain reaction (qPCR) and an ATP assay. Filtered samples of industrial wastewater and contaminated groundwater were tested in the bioreactors to ensure that these bioreactors could be used for nutrient-poor environmental waters. Both CB1190 and JOB5 demonstrated 1,4-dioxane removal greater than that of the

abiotic sorbent controls. This study suggested bioaugmented sorbents could be an effective and novel technology for 1,4-dioxane removal in heavily contaminated water resources.

The thesis of Michelle Allison Myers is approved.

Jennifer Ayla Jay

Michael K. Stenstrom

Shaily Mahendra, Committee Chair

University of California, Los Angeles

2016

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List of Acronyms

Chlorinated Volatile Organic Compounds	CVOCs
1,1-Dichloroethene	1,1-DCE
1,1,1-Trichloroethane	TCA
Trichloroethylene	TCE
Gas Chromatography-Flame Ionizing Detector	GC-FID
Gas Chromatography-Mass Spectrometer	GC-MS
Ammonium Mineral Salts	AMS
Nitrate Mineral Salts	NMS
Granular Activated Carbon	GAC
Biological Granular Activated Carbon	BioGAC
Quantitative Polymerase Chain Reaction	qPCR
Adenosine Triphosphate	ATP
Phosphate Buffered Saline	PBS
Extracellular Polymeric Substance	EPS
United States Environmental Protection Agency	US-EPA
Lethal Dose for 50% for Population	LD_{50}
Unregulated Contaminant Monitoring Rule 3	UCMR3

Acknowledgements

I have many people to thank for their help and support throughout my Master's at UCLA. I would first like to thank my advisor, Dr. Shaily Mahendra for her support and guidance throughout this research project. I came into this lab as a geologist, and Shaily has helped transform me into a microbiologist and an environmental engineer (and geologist).

I would also like to thank my professors, Dr. Jennifer Jay, Dr. Michael Stenstrom, and Dr. Issam Najm, for their interesting and engaging courses. These courses provided me the background I needed in order to conduct the research within this thesis. Issam was particularly helpful in his insight and advice with regards to my bioreactors. I will be forever grateful that I was able to serve as a teaching assistant for and participate in Jenny's environmental student outreach course.

Thanks to all Mahendra Lab members, past and present; I appreciate your support and friendship over the past 2 years. Dr. Phil Gedalanga introduced me to 1,4-dioxane, taught me laboratory techniques, and has been enormously supportive. Dr. Peerapong "Ice" Pornwongthong introduced me to 1,4-dioxane adsorption and the idea of using bioaugmented sorbents as a treatment technique for 1,4-dioxane. Ice helped me in my first months, as I was doing preliminary sorption experiments, as well as conducted some of the initial sorbent studies. I would also like to thank Shu Zhang, Nick Johnson, Nancy Merino, Meng Wang, and Dr. Linda Tseng, for their encouragement and feedback on my research, as well as occasional culture maintenance and sampling. I would also like to thank my fabulous undergraduate research

assistants, Erick Zerecero Marin and Jamie Liu, for their hard work in the lab. I am grateful for their willingness to stay extra hours to finish experiment set-up and come in weekends for sampling as well as their positive attitudes.

My parents deserve special thanks for all of the sleep-deprived, late-night calls they have taken from me in the past 2 years. You have been overwhelmingly supportive and encouraging, and appreciate your constant confidence in me. Thank you to my friends Katya and Sam for being a reliable source of support. Lastly, I would like to thank Jason Utas for his constant optimism and support throughout the past four years. He has helped me to gain perspective when I needed it most.

This research project was conducted with the support of Strategic Environmental Research and Development Program award ER-2307, Dow Chemical Company contract 244633, DuPont Corporate Remediation Group contract MA-03653-13 and was carried out in a renovated collaboratory supported by the National Science Foundation award number 0963183 under the American Recovery and Reinvestment Act of 2009 (ARRA).

1. Introduction

1.1 1,4-Dioxane Relevance and Occurrence in Water

First identified in 1863, 1,4-dioxane was first used as a solvent in the late 1920s to early 1930s for a variety of commercial and raw products, such as cellulose acetate, plastic

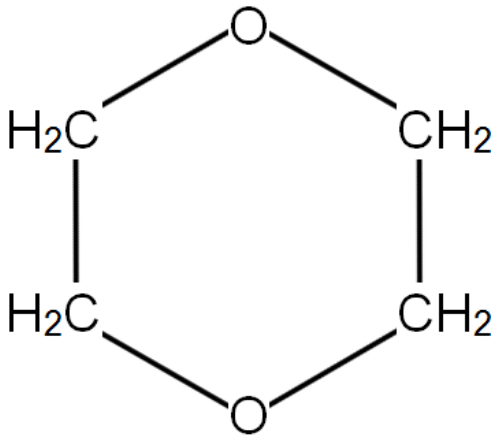


Figure 1 1,4-Dioxane Chemical Structure.

manufacturing, wool scouring, dye production, printing, degreasing, varnishes, paints, cosmetics, glues, preservatives, fumigants, and deodorants[1]. In 1945, a patent was filed for the use of 1,4-dioxane as a solvent stabilizer[2]. 1,4-Dioxane has been used as a solvent stabilizer for such solvents as methyl chloroform (TCA). Additionally, 1,4-dioxane can be found in a variety of personal care products, where ethylene oxide dimerization can also occur when ethylene oxide is added to alcohol, a practice that is common in order to make the ethylene oxide more soluble[3]. The most common sources of 1,4-dioxane environmental exposure are: its historical use as a solvent stabilizer, especially in conjunction with the chlorinated solvent trichloroethane (TCA);

its production during the synthesis of surfactants found in detergents and personal care products; and its direct use as a solvent. Some introductions of 1,4-dioxane into the groundwater have historically been through its use in aerospace applications. 1,4-Dioxane was an additive in deicing and antifreeze fluids before 2000, and was used to stabilize TCA for the degreasing of aircrafts and machinery[1]. 1,4-Dioxane groundwater contamination, which is primarily due to its use as a solvent stabilizer, and surface water contamination, which is primarily due to its presence in detergents and personal care products, represent significant human exposure routes and have the potential to be a public safety concern, especially where a reliance on groundwater or surface water for drinking water exists.

Table 1 Physiochemical Properties of 1,4-Dioxane.

Chemical and Physical Properties	1,4-Dioxane
Molecular Weight (g/mol)	88.11 [a]
Density (g/mL at 20^{o}C)	1.0329 [a]
Melting Point (^{o}C at 760 mm Hg)	11.8 [a]
Boiling Point (^{o}C at 760 mm Hg)	101.1 [a]
Octanol-Water Partition Coefficient log(K_{ow})	-0.27 [b]
Water Solubility (g/L)	miscible [c]
Henry's Law Coefficient K_H at 25^{o}C (atm-m^3/mol)	4.80x10^{-6} [d]
Vapor Pressure (mm Hg at 25^{o}C)	38.1 [e]

[a] (O'Niel et al, 2001)
[b] (Hansch et al, 1995)
[c] (Riddick et al, 1986)
[d] (Park et al, 1987)
[e] (Daubert et al, 1985)

A recent national survey of public drinking water supplies conducted by the United States Environmental Protection Agency (US-EPA) Unregulated Contaminant Monitoring Rule 3 (UCMR3) Occurrence Database found that of 7171 samples taken in the first year of the program, the maximum concentration found was 9.2 μg/L, with 11.9% of samples exceeding the 0.07 μg/L detection limit and 3.9% of samples exceeding the 0.35 μg/L reference concentration, which represents the 1×10^{-6} cancer risk for 1,4-dioxane for the average consumer[4-5].

1.2 1,4-Dioxane Toxicity

The predominant exposure pathway for 1,4-dioxane is ingestion of drinking water, as 1,4-dioxane does not bioaccumulate and does not significantly penetrate the skin via dermal contact[6-8]. 1,4-dioxane is also miscible in water, meaning it would not significantly partition into the gaseous phase in concentrations likely to be present in the environment[9]. However, personnel who work with pure or high concentrations of 1,4-dioxane may experience inhalation exposure occupationally. A volunteer inhalation study showed that humans exposed to the high 1,4-dioxane concentration of 50 mg/L for 6 hours receive this dose as over 99% 2-hydroxyethoxyacetic acid, a 1,4-dioxane metabolite[10]. Occupational, high concentration 1,4-dioxane vapor exposure has been known to cause a number of side effects, such as drowsiness, headache, nausea, irritation of mucous membranes, and damage to the liver and kidneys[11]. Fetal death has been documented in the cases of five textile factory workers as a result of acute 1,4-dioxane inhalation[11]. In these cases, the cause of death was found to be fetal kidney injury[11].

1,4-Dioxane is considered a probable human carcinogen (B2) by the International Agency for Research on Cancer. This classification is based mainly on laboratory animal studies, as there is insufficient human exposure data to definitively determine human carcinogenicity. Animal studies have shown increased incidence of liver and nasal cavity carcinomas in rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs[12-16]. Extrapolations on animal studies suggest a human ingestion 1×10^{6} lifetime cancer risk of 1.5×10^{-5} (mg/kg-day) [17].

1,4-Dioxane has known toxicity beyond carcinogenicity. Chronic, low-dose exposure to 1,4-dioxane has the ability to damage the kidneys, the liver, and the nervous system[18]. The lethal dose (LD_{50}) of 1,4-dioxane for tested laboratory animals was a body burden between 4 and 14 mg/L, and death has been documented in humans at a body burden on 470 mg/L[11, 18].

1.3 1,4-Dioxane Contamination and Co-occurrence

1,4-Dioxane is a synthetic organic chemical and probable human carcinogen that often in found as a groundwater co-contaminant of chlorinated volatile organic compounds (CVOCs), such as 1,1-dichloroethene (1,1-DCE), 1,1,1-trichloroethane (TCA), and trichloroethylene (TCE). Due to the low Henry's Law coefficient and miscibility in water (Table 1), 1,4-dioxane preferentially stays in the aqueous phase in a water-1,4-dioxane solution, as opposed to volatilizing into the gaseous phase. It is for these reasons that 1,4-dioxane that has been introduced to the groundwater via point sources can rapidly develop into groundwater contaminant plumes, as the 1,4-dioxane diffuses and flows with the groundwater[1, 5].

1.4 Existing Abiotic 1,4-Dioxane Treatment Technologies

Many abiotic technologies used in conventional water treatment are largely ineffective for remediation of 1,4-dioxane. 1,4-Dioxane is miscible in water, and air stripping has difficulty moving the 1,4-dioxane into the gaseous phase. The process would require much air and energy in order to be effective, and would have difficulty bringing the concentration to acceptable limits. A reactor designed to strip chlorinated solvents used on 1,4-dioxane contaminated waters saw a decrease from 610 μg/L to 430 μg/L, which is still well above the recommended concentration for drinking water[19]. Chemical oxidation, such as with permanganate and chlorine, were found to be largely ineffective[20]. Sodium hypochlorite degraded 1,4-dioxane to byproducts with much higher toxicities than 1,4-dioaxane [21]. This process also requires energy intensive changes in pH and temperature conditions, which would make this process prohibitively expensive[22]. Direct photolysis also has a low efficiency as a sole degradation technique, as 1,4-dioxane is a relatively poor UV-absorber[23].

Advanced oxidation can be an effective means for 1,4-dioxane degradation. These techniques typically rely on degradation due to hydroxyl radicals. Hydrogen peroxide is often used in industry, and is often coupled with ferrous iron to induce Fenton's reaction and cause the production of hydroxyl radicals and hydroperoxyl radicals[22].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \qquad \text{(Hydroxyl Radical)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^{+} \qquad \text{(Hydroperoxyl Radical)}$$

Hydrogen peroxide with a ferrous iron catalyst added at a 12:1 ratio of hydrogen peroxide to 1,4-dioxane has been seen to degrade 97% of 1,4-dioxane after 10 hours incubation[22]. UV added to hydrogen peroxide degradation has shown to accelerate this reaction, resulting in a 90% reduction of 1,4-dioxane in 5 minutes[23]. While advanced oxidation can be effective as a means for 1,4-dioxane degradation, much of the oxidant reacts with soil, so *in situ* applications can be limited by the ability for the oxidant to distribute in the subsurface. Oxidation is often done *ex situ*, using a pump and treat method, making the system prohibitively expensive.

1.5 1,4-Dioxane Biodegradation

Biodegradation of 1,4-dioxane, both metabolic and co-metabolic, is well-documented and established. Several microbes are known to have the ability to biodegrade 1,4-dioxane, either metabolically or co-metabolically[24-26]. Metabolism is a process of degradation in which the cell produces enzymes that can break down the target compound. This degradation and transformation of the target compound produces energy for the microbe and serves as its carbon or energy source. Co-metabolism is a process of degradation in which the cell produces enzymes that can break down the primary substrate, which is not the target compound. However, these enzymes also have the ability to break down the target compound in the absence or scarcity of the primary substrate. The degradation and transformation of the primary substrate produces energy for the microbe and serves as its carbon and energy source. The degradation and transformation of the target compound does not produce energy for the microbe and stresses the microbe. Remediation systems that co-metabolism rely either on continuous low concentration

feeds of the primary substrate or periodic pulses of the primary substrate in order to sustain the microbes.

Biodegradation is very versatile, as many other treatment systems can be bioaugmented with degrading bacteria. Much research focus on the direct application of degrading bacteria into the subsurface in order to achieve *in situ* degradation [27]. This system would be much cheaper than many other methods, as water would not have to be pumped from the subsurface, treated, and then pumped back into the subsurface. However, nutrient requirements of the bacteria are difficult to accommodate in the subsurface, and research into incorporating biodegradation into other treatment techniques is on-going [28-30].

1.6 Existing Bioaugmented Sorbent Research

Processes using bioaugmented sorbent have been established and are in use for other hydrophilic organic compounds with similar water solubility and partition coefficients. One of these established processes is the BioGAC system used for Methyl tert-Butyl Ether (MTBE) and tert-Butyl Alcohol (TBA). Because these compounds are often found as co-contaminants in effected groundwater systems, have similar physiochemical properties (as can be seen in Table 3), and can be biodegraded by the same bacteria, they are both targeted by the BioGAC approach[31-33].

Attached growth reactors have been used with other compounds, especially with the selection of an activated carbon solid particle surface. Bioaugmented granular activated carbon

research for the biodegradation of methyl tert-butyl ether and tert-butyl alcohol is a clear example of this line of research, turned to practice. These compounds are fuel oxygenates, and are present in areas of large fuel spills, often a result of leaking underground storage tanks. Many conventional methods of remediation are known to be largely ineffective, such as air stripping, abiotic adsorption, or advanced oxidation. Granular activated carbon beds are inoculated with a patented microbe blend prior to operations[32, 34]. A feed of contaminated water with nutrients, oxygen, and hydrogen peroxide is added to satisfy biological growth and microbial and sorbent oxygen requirements[34-35]. A comparison with a parallel abiotic granular activated carbon bed shows that abiotic adsorption of both methyl tert-butyl ether and tert-butyl alcohol is present, but breakthrough occurs rapidly for both compounds. In a bench-scale bioreactor system, methyl tert-butyl ether break-through occurred in 14 days and tert-butyl alcohol break-through occurred in 2 days, while bioaugmented-GAC showed no breakthrough of methyl tert-butyl ether or tert-butyl alcohol, disregarding a shock loading event where influent concentrations increase ten-fold, and the effluent concentration rose from below detection to 0.1mg/L[34]. This study and subsequent pilot and full-scale studies, show that bioaugmented sorbent can be an effective means for remediation of miscible or highly soluble compounds that do not sorb strongly on activated carbon.

Table 2 Physiochemical Properties of Methyl tert-Butyl Ether (MTBE) and tert-Butyl Alcohol (TBA).

Chemical and Physical Properties	1,4-Dioxane	Methyl tert-Butyl Ether (MTBE)	tert-Butyl Alcohol (TBA)
Molecular Weight (g/mol)	88.11 [a]	88.15 [f]	74.12 [f]
Density (g/mL at 20°C)	1.0329 [a]	0.744 [f]	0.791 [f]
Melting Point (°C at 760 mm Hg)	11.8 [a]	-109 [g]	25.8 [h]
Boiling Point (°C at 760 mm Hg)	101.1 [a]	55.2 [f]	82.4 [f]
Octanol-Water Partition Coefficient log(K_{ow})	-0.27 [b]	1.24 [f]	0.35 [f]
Water Solubility (g/L)	miscible [c]	48 [f]	miscible [f]
Henry's Law Coefficient K_H at 25°C (atm-m^3/mol)	4.80×10^{-6} [d]	5.9×10^{-4} [f]	1.4×10^{-5} [f]
Vapor Pressure (mm Hg at 25°C)	38.1 [e]	249 [f]	42 [f]

[a] (O'Niel et al, 2001)
[b] (Hansch et al, 1995)
[c] (Riddick et al, 1986)
[d] (Park et al, 1987)
[e] (Daubert et al, 1985)
[f] (Schmidt et al, 2004)
[g] (Budavari et al, 1989)
[h] (Haynes et al, 2013-2014)

2. Objectives

The purpose of this study was to assess the effectiveness and feasibility of bioaugmented granular activated carbon as an alternative to abiotic sorption or planktonic biodegradation treatment options for 1,4-dioxane contaminated groundwater, drinking water, or industrial wastewater.

Abiotic and bioaugmented batch reactors were constructed to assess the potential for bioaugmented sorbent processes to remove 1,4-dioxane from the system and to be able to compare this to the abiotic and planktonic counterparts. A 1,4-dioxane metabolizing bacterial strain, *Pseudonocardia dioxanivorans* CB1190, and a 1,4-dioxane metabolizing bacterial strain, *Mycobacterium austroafricanum* JOB5, were used to compare abiotic and bioaugmented sorption.

3. Materials and Methods

3.1 Sorbent Specifications and Preparation

The sorbents utilized in this study included Norit 1240 (Norit Americas Inc.), Ambersorb 560 (Rohm and Haas Chemicals LLC), US1078 (US Research Nanomaterials, Inc.), US1076 (US Research Nanomaterials, Inc.), and Bayoxide E33 (Severn Trent Services). The properties of the sorbents are summarized in Table 2.

Norit 1240, US1078, and US1076 are activated carbon sorbents, which are manufactured through the partial combustion and activation of the original carbon material, which is coal, wood, or coconut hull, respectively[43-44]. Norit1240 has a negative surface charge at pHs higher than 4. In the pH range used in this study, 6.8 to 7.3, a zeta potential of -26 to -32 mV for Norit 1240 in deionized water at a concentration of 0.1mg sorbent/mL would be expected[45]. US1078 and US1076 also have a negative charge, both having a reported 7150 negative-ions /cm^3 [44], and a measured mean zeta potential of -23.17 mV and -43.45 MV, respecively, in a 0.01 mg

sorbent/mL solution in AMS. Bayoxide E33 has a positive surface charge at pHs lower than 8.28. In the pH range used in this study, 6.8 to 7.3, a zeta potential of 14.5 to 9.6 mV for Bayoxide E33 in a solution of 3.0 mM ionic strength would be expected[46]. Ambersorb 560 is a sulfonated polystyrene crosslinked with divinylbenzene resin, and its sorption capacity is believed to be unaffected by pH, as the resin does not have surficial functional groups or properties affected by pH[47].

Table 3. Properties of sorbents used in study.

Sorbent	Norit 1240	Ambersorb 560	US1078	US1076	Bayoxide E33
Material	Coal Based [a]	Synthetic Carbonaceous Resin [c]	Charcoal Based [e]	Coconut Based [e]	Goethite (Iron Oxide) [f]
Particle Shape	Granular [a]	Spherical [c]	Spherical [e]	Spherical [e]	Granular [f]
Effective Size	0.65 mm [a]	0.45 mm [d]	< 100 nm [e]	< 100 nm [e]	0.5 – 2 mm [f]
Particle Density (g/mL)	0.497 [a]	0.849 [c]	0.45 [e]	0.42 [e]	3.6 [f]
Bulk Density (g/mL)	0.44 [a]	0.53 [c]	0.33 [e]	0.28 [e]	0.4 – 0.6 [f]
Specific Surface Area (m^2/g)	1175 [b]	550 [d]	300 [e]	1300 [e]	120 – 200 [f]
Pore Volume (cm^3/g)	0.83 [b]	0.60 [c]	1.1 – 1.3 [e]	1.1 – 1.3 [e]	0.583 [g]

[a] (Norit Americas, 2003)
[b] (Yapsakli et al, 2009)
[c] (Flores, 2000)
[d] (Rohm and Haas, 1999)
[e] (US Research Nanomaterials, 2011)
[f] (Servern Trent Services)
[g] (Lalley et al, 2015)

Before use in the studies, Norit 1240, Ambersorb 560, and Bayoxide E33 were washed with nanopure water to remove fines, then dried in a 105˚C oven. When dry, sorbents were measured into the experimental bottles and autoclaved at 121˚C and 15 psi for a hold time of 45 minutes in order to sterilize the sorbents. Nanopowders were measured as packaged, and then autoclaved. It was determined that the particle size was too small for biofilms to form, and sorption occurred near-instantaneously, not allowing time for bacteria to grow or cause the particles aggregation.

3.2 Culture Conditions

Pseudonocardia dioxanivorans CB1190 (CB1190) is an actinomycete that was previously isolated from 1,4-dioxane contaminated industrial sludge[25, 52]. CB1190 can grow in ammonium mineral salts (AMS) medium, using the 1,4-dioxane monooxygenase enzyme to break down 1,4-dioxane and use it as a sole energy and carbon source[25-26, 53]. CB1190 can completely degrade 1,4-dioxane with a bacterial yield of 0.09 g protein (g dioxane)$^{-1}$, producing carbon dioxide as a byproduct. Other aerobic bacteria, such as *Pseudonocardia tetrahydrofuranoxydans*, *Methylosinus trichosporium* OB3b, or *Mycobacterium vaccae (austroafricanum)* JOB5 (JOB5), can degrade 1,4-dioxane after growth and enzyme induction by several linear and branched alkanes, alcohols, and ethers[54-55]. No degradation products were measured during the degradation of 1,4-dioxane, and it is believed that 1,4-dioxane is completely degraded, producing carbon dioxide as a terminal product[54]. Both CB1190 and JOB5 optimal growth temperatures in the laboratory are 30^{o}C.

Pure cultures of *Pseudonocardia dioxanivorans* CB1190 were grown in sterile 2 L conical flasks containing 400 mL ammonium mineral salts (AMS) media[26], with 100 mg/L 1,4-dioxane (Sigma-Aldrich, St. Louis, MO, USA), as the sole carbon and energy source. The bacterial cultures were incubated with 150 rpm of agitation at 30°C. When 1,4-dioxane became undetectable using a gas chromatograph equipped with a flame ionization detector (GC-FID), an additional 100 mg/L pulse of 1,4-dioxane was added. After a minimum of three 1,4-dioxane pulses, the required culture volume was centrifuged and added to the relevant experimental configuration.

Pure cultures of *Mycobacterium vaccae* JOB5 were grown in sterile 500 mL bottles fitted with septa. Nitrate mineral salts medium (NMS) medium[56] (100 mL) was added to the bottles, with a 12.5% headspace propane pulse administered as a sole carbon and energy source. The bacterial cultures were incubated with 150 rpm of agitation at 30°C. Propane concentrations were monitored using GC-FID measurements. When propane degradation plateaued, the bottles were aerated with filtered air for 15 minutes and an additional 12.5% headspace propane pulse was administered. After a minimum of three propane pulses, the required culture volume was separated and added to the relevant experimental configuration.

3.3 Abiotic Sorbent 1,4-Dioxane Batch Reactors

The abiotic sorption study was designed to assess the various 1,4-dioxane sorption capacities in order to identify the best suited sorbent for further bioaugmented 1,4-dioxane sorption studies. This study also helped to determine the isotherm model best suited to model

1,4-dioxane sorption. After equilibrium was achieved and verified, the aqueous volume was replaced with 1,4-dioxane-free medium to determine if desorption of used sorbents is significant and if it poses a threat to treatment processes using abiotic sorption in order to sequester 1,4-dioxane. Sorbents selected for this study included Norit 1240, Ambersorb 560, US1076, US1078, and Bayoxide E33. Sterile 100 mL Corning Pyrex bottles containing sterile sorbents were filled with 20 mL AMS medium and 1,4-dioxane was added in order to achieve concentrations between 12.5 and 800 mg/L. Samples of 100 µL were taken and filter sterilized using 0.2 µm-pore Fisherbrand nylon syringe filters. Theses samples were stored at -20˚C before analysis with a Hewlett-Packard 6890 Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID). Filter sterilization was both a means to ensure no bacteria was present within the samples and that minimal activated carbon remained in the sample. Initial, equilibrium, and equilibrium verification samples were taken and analyzed.

Concentration data and sorbent masses were then used to fit these sorbent-temperature conditions to select isotherm models. The main variables used in fitting these models are q_e and C_e, which are amount of adsorbate on the adsorbent at equilibrium (mg/g) and equilibrium concentration (mg/L), respectively. In using these variable and, for the Temkin and Dubinin-Radushkevich Isotherm Models, the universal gas constant and temperature, the data were plotted to the respective isotherm linearized equations (Table 4) in order to determine the values for the model constants and the R^2 values. The equations for the linear regression lines shown were used to solve for the unknown model constants, shown in Table 7. The equations, plots, and arithmetic explanations for this process can be found in Appendices A-E.

Table 4 Equations and linearized equations for sorption models.

Isotherm Model	Equation	Linearized Equation
Freundlich	$q_e = K_f C_e^{1/n}$	$\log(q_e) = \log(K_f) + \left(\frac{1}{n}\right)\log(C_e)$
Langmuir	$q_e = \frac{Q_o K_L C_e}{1 + K_L C_e}$	$\frac{C_e}{q_e} = \frac{C_e}{Q_o} + \frac{1}{Q_o K_L}$
Temkin	$q_e = \frac{RT}{b_T}\ln(A_T C_e)$	$q_e = \frac{RT}{b_T}\ln(A_T) + \frac{RT}{b_T}\ln(C_e)$
Dubinin-Radushkevich	$q_e = (q_s)e^{-K_{ad}\varepsilon^2}$ where $\varepsilon = (RT)\ln\left[1 + \frac{1}{C_e}\right]$	$\ln(q_e) = \ln(q_s) - K_{ad}\varepsilon^2$
Linear	$q_e = K_{lin} C_e$	$q_e = K_{lin} C_e$

Desorption of 1,4-dioxane was also measured. Bioreactor supernatant was removed after adsorption equilibrium was achieved and replaced with sterile dioxane-free AMS medium. Samples of 100 µl were taken at Day 1 to measure equilibrium concentration and at Day 2 to confirm equilibrium. A desorption percentage is then calculated using the desorption equilibrium concentration, the initial added concentration, and the adsorption equilibrium concentration.

$$\text{Desorption Percentage} = \frac{(\text{Desorption Equilibrium Concentration})}{(\text{Initial Concentration})(\text{Adsorption Equilibrium Concentration})}$$

3.4 Bioaugmented Sorbent Batch Reactors

Bioaugmented sorbent bioreactors were constructed to determine if it was an effective means of 1,4-dioxane removal. Corning Pyrex reusable bottles (100 mL) containing 0.4 g Norit 1240 or Ambersorb 560 were autoclaved at 121˚C and 15 psi for a hold time of 45 minutes in order to sterilize the sorbents and bottles. After cooling, 20 mL AMS medium containing 100 or 400 mg/L 1,4-dioxane was added, along with 200 µL CB1190 culture. These bioreactors were maintained in a 30^{o}C standing incubator with daily sampling. 1,4-Dioxane was monitored and initial 1,4-dioxane concentrations were restored when concentrations became below detection. While this configuration had the ability to test the ability of bioaugmented sorbent bioreactors, bioreactors contained high proportions of planktonic cells, which are less abundant in water treatment and groundwater systems.

Bioaugmented sorbent bioreactors were then constructed with distinct incubation, rinsing, and experimental phases to remove planktonic cells and create a system where degradation is a result of attached-cells or cells that have sloughed off of the sorbent. This configuration was determined to be more representative of water treatment and groundwater systems. Boston round bottles (250 mL) fitted with Mininert valve caps and containing 1 g Norit 1240 were autoclaved at 121˚C and 15 psi for a hold time of 45 minutes in order to sterilize the sorbents and bottles. After cooling, 50 mL CB1190 culture containing 100 mg/L 1,4-dioxane was added. These bioreactors were maintained in a 30^{o}C incubator at 150 rpm with daily sampling. 1,4-Dioxane was monitored and initial 1,4-dioxane concentrations were restored when concentrations became below detection. After 2 rounds of degradation, the sorbent is rinsed 3 times in AMS medium,

resuspended in AMS medium containing 100 mg/L 1,4-dioxane, and maintained in a 30°C incubator at 150 rpm with daily sampling. 1,4-Dioxane was monitored and initial 1,4-dioxane concentrations were restored when concentrations became below detection. This process was then repeated using a mixture of 90% industrial wastewater or groundwater and 10% AMS medium for the experimental phase.

In order to compare metabolic and cometabolic bioaugmented sorbent bioreactors, JOB5 was also used in the system with distinct incubation, rinsing, and experimental phases. Boston round bottles (250 mL) fitted with Mininert valve caps and containing 1 g Norit 1240 were autoclaved at 121˚C and 15 psi for a hold time of 45 minutes in order to sterilize the sorbents and bottles. After cooling, 50 mL JOB5 culture was added and fed 12.5% headspace filtered propane. These bioreactors were maintained in a 30°C incubator at 150 rpm with daily propane monitoring. When propane degradation ceased, propane was removed by heating bioreactors to 35°C and aerating three times for 15 minutes with filtered air, with 15 minute resting periods. Bioreactors were then injected with 12.5% filtered propane. After 2 rounds of propane degradation, the sorbent is rinsed 3 times in NMS medium, resuspended in NMS medium containing 100 mg/L 1,4-dioxane, and maintained in a 30°C incubator at 150 rpm with daily sampling. 1,4-Dioxane was monitored and initial 1,4-dioxane concentrations were restored when concentrations became below detection. When 1,4-dioxane degradation ceased, bioreactors were aerated and injected with 15% filtered propane. When headspace oxygen measurements fell below 10%, propane was removed by heating bioreactors to 35°C and aerating three times for 15 minutes with filtered air, with 15 minute resting periods. This process was then repeated using a

mixture of 90% industrial wastewater or groundwater and 10% NMS medium for the experimental phase.

1,4-Dioxane samples were taken throughout the experimental phase and filter sterilized by using 0.2 µm-pore Fisherbrand nylon syringe filter and stored at -20˚ C before being analyzed on a Hewlett-Packard 6890 Chromatograph equipped with a Flame Ionization Detector (GC-FID). Filter sterilization is both a means to ensure that no bacteria are present within the samples and that minimal activated carbon remains in the sample. Sorbent samples were taken at the beginning and end of the experimental phase. These consisted in a 500 mg sample for qPCR analysis, a 200 mg sample for ATP analysis, and a 100 mg sample for microscopy. Liquid samples were taken at the end of the experimental phase. This consisted of a 500 µL sample for qPCR analysis and a 100 µL sample for ATP analysis.

3.5 Analytical Methods for Concentration Measurements

Sample 1,4-dioxane concentrations were measured using a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (GC-FID) (Hewlett-Packard, Atlanta, GA) with a Restek® Stabilwax-DB capillary column (30 m x 0.53 mm ID x 1 µm; Restek, Bellefonte, PA). Liquid samples (100 µL) were collected and filtered through 0.2-µm-pore Fisherbrand nylon syringe filters. From the filtrate, 2 µl was directly injected into the GC-FID. The injector maintained a temperature of 220°C, while the detector maintained 250°C. The oven was programmed to begin at 80°C, then to rise to 140°C at a rate of 20°C/min, which was held for 1 minute. The 1,4-dioxane retention time was approximately 3.5 min. A signal intensity

verses time plot was derived using this method, with a correlation between the 1,4-dioxane concentrations and the area under the curve at the 1,4-dioxane retention time peak. A calibration curve was calculated through analysis of successive 1,4-dioxane dilutions in order to derive the sample concentrations.

3.6 DNA Extraction and Quantification

Total nucleic acids were extracted using a phenol/chloroform/isoamyl alcohol method. 500 μL of sample was taken from the batch reactors and flow through columns. Cells were lysed by incubating at 65°C for 2 minutes, bead beating for 2 minutes with a Mini Bead Beater (Biospec Products, Bartlesville, OK, USA), incubating for 8 minutes at 65°C, then 2 more minutes of bead beating. Phenol/chloroform/isoamyl alcohol reagent mixture was used for purification. Precipitation of nucleic acids was achieved with the addition of 0.1 volume of 3M sodium acetate and 1 volume isopropanol, then allowed to sit in -20°C overnight. The precipitate was isolated using centrifugation, and was washed with 70% ethanol. The precipitate was resuspended into 100 μl DNase and RNase-free water. Extracts were quantified using a Nanodrop 2000C Spectrophotometer (Thermo-Scientific, Wilmington, DE, USA), then stored at -80°C until ready for qPCR analysis.

The comparative threshold cycle (*CT*) method was utilized in order to quantify the CB1190-specific 16S rRNA gene abundance [25]. CB1190-specific 16S primers were used in amplification, along with 2 μl of template DNA and 1× Luminaris Color HiGreen High ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA). The qPCR target gene

amplification efficiency was calculated through analysis of successive dilutions of genomic DNA extracted from pure CB1190 cultures. The 1,4-dioxane specific 16S gene abundance was measured from initial and final samples for the flow-through column and batch reactor experiments.

3.7 Microscopy

In order to visualize the cell growth, sorbent samples from the batch reactors were prepared in a 5 g Acridine Orange per liter of Phosphate Buffered Saline (PBS) solution in the absence of light. Sorbent samples were allowed to remain in this solution for one hour before being removed from the dye solution and placed on a glass slide[57]. An Olympus BX51W1 fluorescence microscope emitting blue (460-500nm) light was used in order to visualize and photograph the image of the sorbent.

3.8 ATP Analysis

ATP measurements from liquid and sorbent samples were obtained using standard protocols for BacTiter-Glo, provided by Promega corporation[58-59]

4. Results

4.1 Abiotic Sorbent 1,4-Dioxane Batch Reactors

Selected sorbents were introduced to a range of 1,4-dioxane concentrations (0-800 mg/L) that can be found in industrial wastewater and highly impacted environmental contamination sites. The sorbents selected for this study represent a wide range of available sorbent compositions: coal-based granular activated carbon, charcoal-based nanopowder activated carbon, coconut-based nanopowder activated carbon, synthetic carbonaceous resin, and synthetic goethite. The specific properties are summarized in Table 2 and discussed in Section 2.3. The sorbents were all tested at 20^{o}C initially to test sorption capacity. This study was used to select for sorbents with high sorption capacities for 1,4-dioxane. Ambersorb 560 was found to have the highest sorption capacity for 1,4-dioxane, with a range of 90-98% sorption for initial concentrations ranging from 49.5 – 602.6 mg/L. Norit 1240 also had high sorption, with a range of 67-88% sorption for initial concentrations ranging from 21.2 – 789.4 mg/L. The Norit 1240 and Ambersorb 560 experiments were conducted with 0.4 g of sorbent, whereas the nanopowder activated carbon sorbents, US1076 and US1078, were conducted with 1 g of sorbent. Preliminary experiments using 0.4 g US1076 and US1078 were inconclusive, so experiments were conducted with 2.5 times the sorbent mass in order to be able to fit isotherm models and derive isotherm parameters. Experiments using 1 g nanopowder yielded high adsorption percentages. US 1076 had a range of 83-98% sorption for initial concentrations ranging from 13.7 – 802.9 mg/L. US 1078 had lower sorption, with a range of 48-79% sorption for initial concentrations ranging from 9.3 – 794.9 mg/L (Table 5). The synthetic goethite sorbent, Bayoxide E33, showed no

measurable sorption using 0.4 g of sorbent. Experiments using larger quantities of Bayoxide E33 were not conducted, as this sorbent was found to grow large salt crystals in AMS growth medium, which would rule out use in a full-scale flow-through column bioreactor system.

Table 5 A summary of abiotic sorption for selected sorbents.

US 1076 – Coconut Nanopowder (20°C)			US 1078 – Coal Nanopowder (20°C)			Norit 1240 GAC (20°C)		
Concentration (mg/L)		Percent Adsorbed	Concentration (mg/L)		Percent Adsorbed	Concentration (mg/L)		Percent Adsorbed
Initial	Final		Initial	Final		Initial	Final	
13.7	0.2	98%	9.3	2.0	79%	21.2	3.7	82%
24.9	0.8	97%	20.1	4.9	76%	32.6	4.0	88%
57.2	1.7	97%	40.0	10.8	73%	65.8	12.4	81%
103.1	6.5	94%	107.2	31.9	70%	110.1	20.8	81%
185.2	9.5	95%	202.9	64.2	68%	196.6	46.3	76%
400.1	37.3	91%	408.7	181.8	56%	377.5	117.2	69%
802.9	139.8	83%	794.9	411.8	48%	789.4	263.1	67%

Norit 1240 GAC (30°C)			Ambersorb 560 (20°C)			Ambersorb 560 (30°C)		
Concentration (mg/L)		Percent Adsorbed	Concentration		Percent Adsorbed	Concentration		Percent Adsorbed
Initial	Final		Initial	Final		Initial	Final	
13.0 ± 1.0	1.6 ± 0.2	88%	--	--	--	13.0 ± 1.0	0.1	99%
24.3 ± 0.7	4.1 ± 0.6	83%	49.5	1.1	98%	24.3 ± 0.7	0.2	99%
53.9 ± 1.2	9.7 ± 0.7	82%	85.4	1.2	99%	53.9 ± 1.2	0.5	99%
96.8 ± 1.0	21.4 ± 3.7	78%	94.7	2.5	97%	96.8 ± 1.0	1.3	99%
189.3 ± 3.9	54.2 ± 11.5	71%	177.3	5.8	97%	189.3 ± 3.9	3.3	98%
398.9 ± 2.2	126.7 ± 15.9	68%	370.9	16.8	95%	398.9 ± 2.2	16.1 ± 0.6	96%
800.0 ± 20.0	326.1 ± 25.3	59%	602.6	59.5	90%	800.0 ± 20.0	45.1 ± 8.9	94%

Norit 1240 and Ambersorb 560 were determined to be good candidates for bioaugmentation studies, as they exhibited high sorption capacities, and had a particle size that would support flow-through column bioreactors. While larger quantities of nanopowder activated carbon, US 1076 and US 1078, resulted in measurable sorption, the small particle diameters would be too small to support biofilm growth, as the effective size of the particles are smaller than the size of one CB1190 or JOB5 bacterium, assuming no aggregation of the nanoparticles. These nanoparticles would also be very difficult to use in future flow-through column bioreactor, as the pore size of the column supports would need to be smaller than 100 nm, which would result in a slow flow and high pressure system that would likely foul easily. Bayoxide E33 was not chosen for further studies, as it showed no 1,4-dioxane sorption and the use of this sorbent resulted in the growth of large salt crystals.

1,4-Dioxane sorption on Norit 1240 and Ambersorb 560 was then analyzed at 30^{o}C, the optimal temperature for CB1190 and JOB5 microbial growth and the temperature at which the bioaugmented reactors would run. The sorbents both performed well in the adsorption segment of this experiment. Ambersorb 560 once again had the highest sorption capacity for 1,4-dioxane, with a range of 94-99% sorption for initial concentrations ranging from 13 – 800 mg/L. Norit 1240 also had high sorption, with a range of 59-88% sorption for initial concentrations ranging from 13 – 800 mg/L (Table 5).

Table 6 A summary of isotherm parameters for selected sorbents at 20°C and 30°C.

Isotherm	Parameter	US1076 20°C	US1078 20°C	Norit 1240 20°C	Norit 1240 30°C	Ambersorb 560 20°C	Ambersorb 560 30°C
Freundlich	K_f (L/g)	0.69	0.10	0.43	0.4	3.01	3.69
	n	1.60	1.33	1.39	1.4	1.76	1.58
	R^2	0.99	0.99	0.99	1	0.96	0.99
Langmuir	K_L (L/mg)	0.03	0.01	0	0.01	0.07	0.1
	Q_o (mg/g)	15.61	10.43	58.9	32.79	33.51	43.06
	R^2	0.95	0.96	0.92	0.9	0.98	0.91
Temkin	T (K)	293.15	293.15	293.15	303.15	293.15	303.15
	A_T (L/g)	1.54	0.23	0.14	0.27	1.18	4.85
	b_T	1263.67	1841.44	331.93	619.22	404.57	454.97
	R^2	0.82	0.86	0.84	0.82	0.95	0.82
Dubinin–Radushkevich	K_{ad} (mol^2/kJ^2)	$1.65x10^{-7}$	$2.99x10^{-6}$	$7.18\ x10^{-6}$	$1.85\ x10^{-6}$	$6.57\ x10^{-7}$	$8.45\ x10^{-8}$
	q_s (mg/g)	3.29	2.07	9.63	6.06	14.34	11.4
	R^2	0.56	0.57	0.54	0.53	0.72	0.74
Linear	K_{lin} (L/mg)	0.10	0.02	0.07	0.08	0.52	0.9
	R^2	0.84	0.91	0.9	0.93	0.69	0.92

Using the R^2 values (Table 7), the Dubinin-Radushkevich isotherm was found to be a poor representation of the kinetics of the sorbent-temperature conditions presented, with R^2 values ranging from 0.53-0.74. The Linear and Temkin isotherms were unreliable representations of the sorbent-temperature conditions. Neither model consistently accurately represented all sorbent-temperature condition. Also, in every condition, both isotherm models had lower R^2 values than the Freundlich isotherm model. The Langmuir isotherm model had high R^2 values, but had near-consistently lower values than that of the Freundlich isotherm model. The Langmuir isotherm model was a better fit for one sorbent-temperature condition: Ambersorb 560 at 20°C. As the Langmuir R^2 values were close to those of the Freundlich model, and the Freundlich model was more accurate for all other conditions, the larger Langmuir model R^2 value was

determined to be due to GC-FID measurement error. The Freundlich isotherm model was determined to be the most accurate isotherm model of the 5 tested. This can be visually confirmed in Figure 3, where observed concentrations are plotted against isotherm models, which are plotted using the variables found in Table 7. The Freundlich adsorption capacity parameter (K_f), confirms that adsorption is greatest for Ambersorb 560.

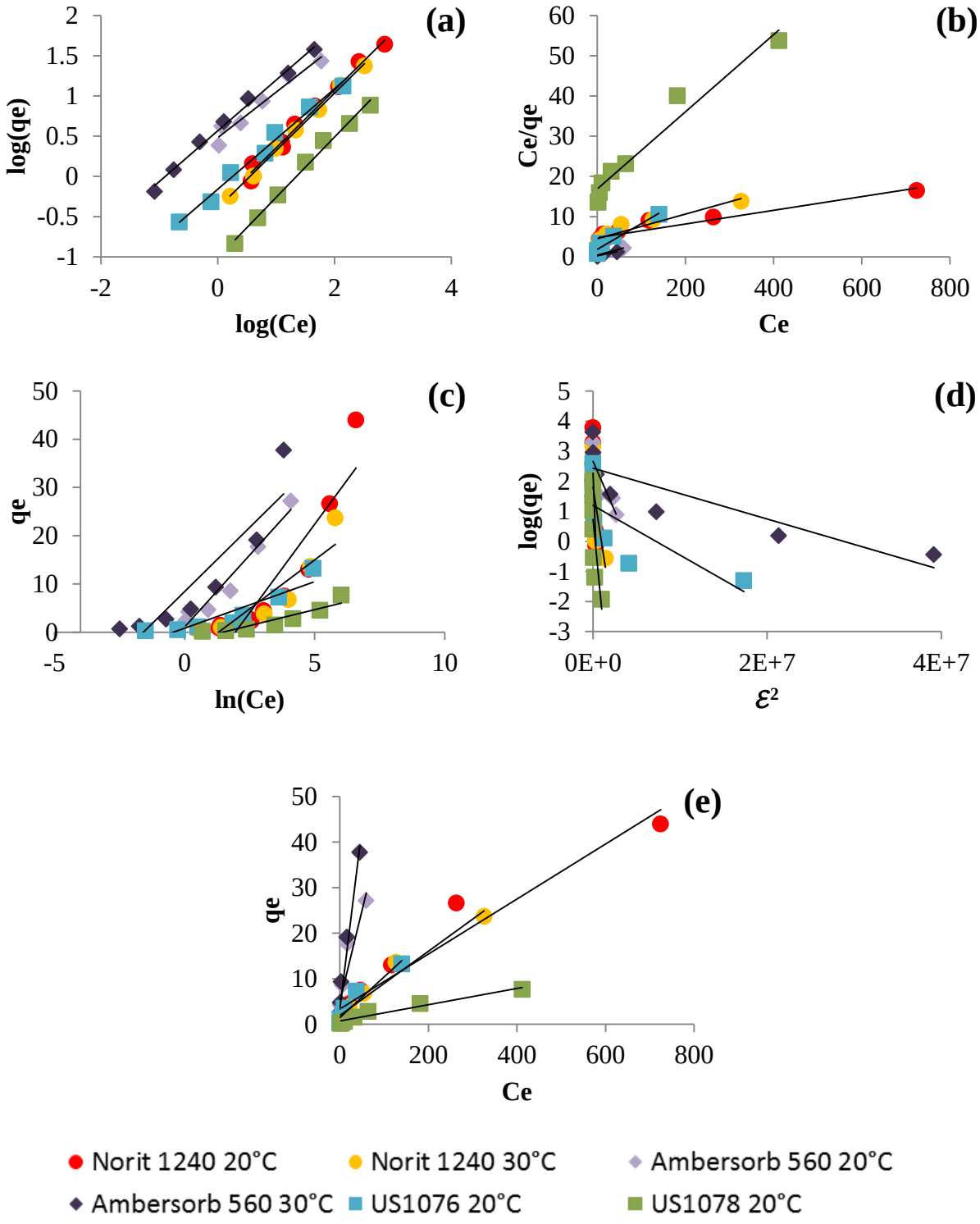


Figure 2 Linearized equations used to fit observed data to isotherm models. (a) Freundlich Isotherm (b) Langmuir Isotherm (c) Temkin Isotherm (d) Dubinin-Radushkevich Isotherm (e) Linear Isotherm.

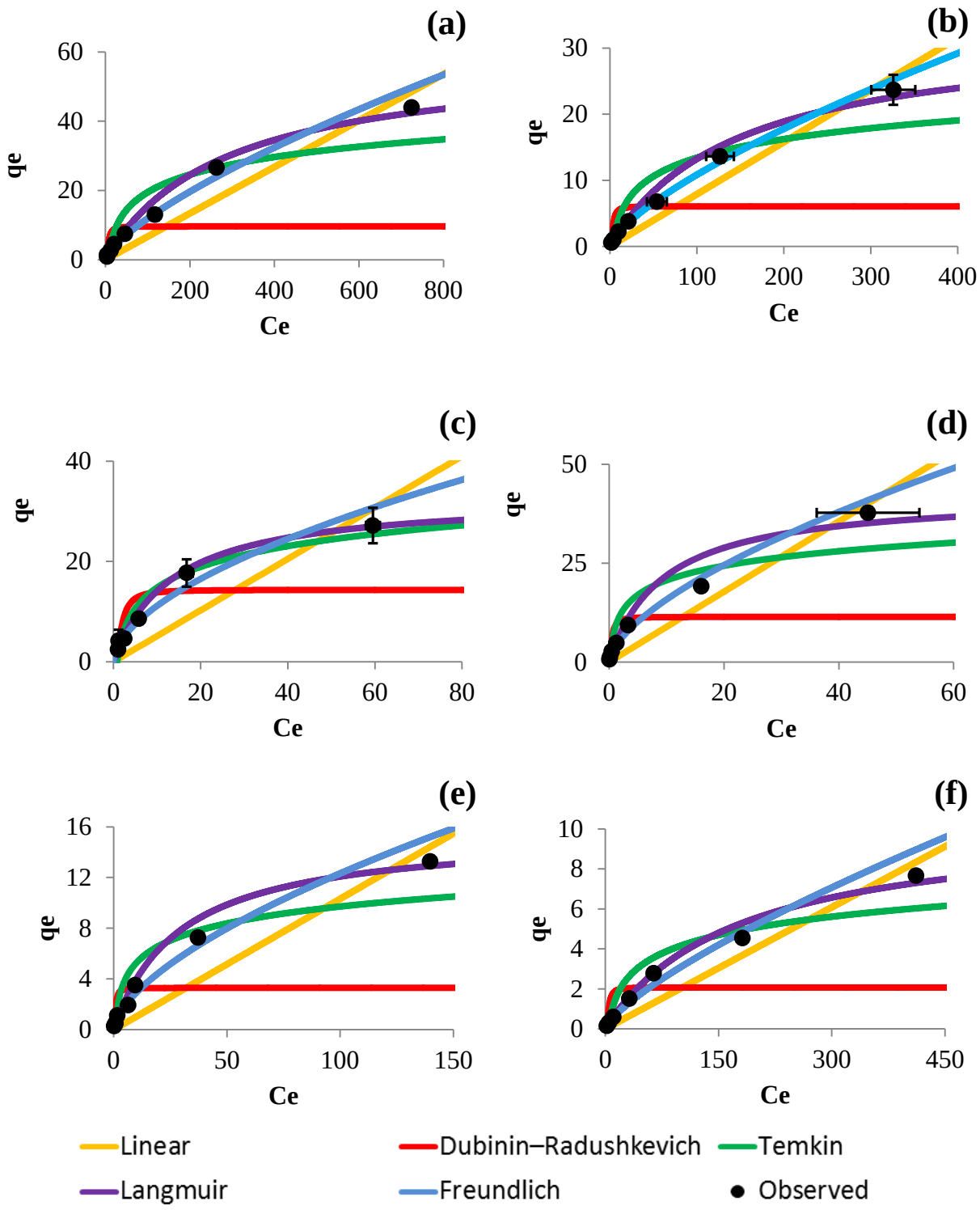


Figure 3 Observed adsorption values plotted with fitted sorption models. Isotherm parameters were used to plot isotherms. (a) Norit 1240 at 20^{o}C (b) Norit 1240 at 30^{o}C (c) Ambersorb 560 at 20^{o}C (d) Ambersorb at 560 30^{o}C (e) US 1076 at 20^{o}C (f) US1078 at 20^{o}C**.**

Table 7 A summary of abiotic desorption for selected sorbents at 30°C.

	Norit 1240			Ambersorb 560		
Initial Concentration (mg/L)	Final Concentration (mg/L)	Desorbed Concentration (mg/L)	Percent Desorbed	Final Concentration (mg/L)	Desorbed Concentration (mg/L)	Percent Desorbed
13.0 ± 1.0	1.6 ± 0.2	1.2	11%	0.1	0.1	1%
24.3 ± 0.7	4.1 ± 0.6	3.0 ± 0.1	15%	0.2	0.2	1%
53.9 ± 1.2	9.7 ± 0.7	7.3 ± 0.1	17%	0.5	0.5	1%
96.8 ± 1.0	21.4 ± 3.7	15.5 ± 0.3	20%	1.3	1.1 ± 0.1	1%
189.3 ± 3.9	54.2 ± 11.5	37.7 ± 3.2	28%	3.3	1.6	1%
398.9 ± 2.2	126.7 ± 15.9	91.7 ± 3.8	34%	16.1 ± 0.6	17.1 ± 3.6	4%
800.0 ± 20.0	326.1 ± 25.3	197.2 ± 6.8	42%	45.1 ± 8.9	49.7 ± 9.9	7%

Desorption of 1,4-dioxane from Norit 1240 and Ambersorb 560 at 30°C was then conducted. Norit 1240 experienced desorption percentages ranging between 11-42%, which amounted to a 1.2-197.2 mg/L increase in concentration in the previously 1,4-dioxane-free medium. Ambersorb 560 experienced much less desorption, with desorption percentages ranging between 1-7%. This amounted to a 0.1-47.7 mg/L increase in concentration in the previously 1,4-dioxane-free medium.

4.2 Bioaugmented 1,4-Dioxane Batch Reactors

1,4-Dioxane in the CB1190-bioaugmented Norit 1240 bioreactors was rapidly adsorbed, that decreased concentrations to 25-28% for 100 mg/L initial concentrations and to 27-39% for 400 mg/L initial concentrations. Bioaugmented Ambersorb 560 had an initial drop to 3-4% of initial 400 mg/L 1,4-dioxane (Figure 4). Initially there was no difference in concentration between bioaugmented sorbents and their respective abiotic sorbent, as bacteria was growing from the 1% transfer. Bioaugmented Norit 1240 bioreactors with starting concentrations of 100 mg/L surpassed abiotic GAC reactors in terms of 1,4-dioxane removal at Day 4, where bioaugmented Norit 1240 concentrations dropped to 45.8% of that of the abiotic Norit 1240. Near-complete 1,4-dioxane removal (3%) for bioaugmented Norit 1240 was observed at Day 5, whereas the CB1190 planktonic control was observed to fall to 7% at Day 6. After Day 7, both CB1190-bioaugmented Norit 1240 and the CB1190 planktonic control demonstrate greater than 85% 1,4-dioxane removal in one day. Bioaugmented Norit 1240 bioreactors with starting concentrations of 400 mg/L surpassed abiotic GAC reactors in terms of 1,4-dioxane removal at Day 14.5, where bioaugmented Norit 1240 concentrations drop to 27.3% of that of the abiotic Norit 1240. Near-complete 1,4-dioxane removal (5%) for bioaugmented Norit 1240 was observed at Day 15.5. Bioaugmented Ambersorb 560 bioreactors with starting concentrations of 400 mg/L surpassed abiotic Ambersorb 560 reactors in terms of 1,4-dioxane removal at Day 7, where bioaugmented Ambersorb 560 concentrations drop to 33.3% of that of the abiotic Ambersorb 560. Near-complete 1,4-dioxane removal (0.4%) for bioaugmented Ambersorb 560 was observed at Day 8.5. Abiotic sorbent reactors, which were spiked at the same time as the bioreactors, demonstrated the limited 1,4-dioxane capacity of each sorbent. In a full-scale

filtration process, the sorbent would be able to adsorb less 1,4-dioxane over the life of the reactor. CB1190 cell concentrations in the bioreactors measured using the CB1190-specific 16S sequence show a 14.8-fold increase in aqueous CB1190 concentration (Figure 5), indicating that degradation and growth were occurring within the bioreactors.

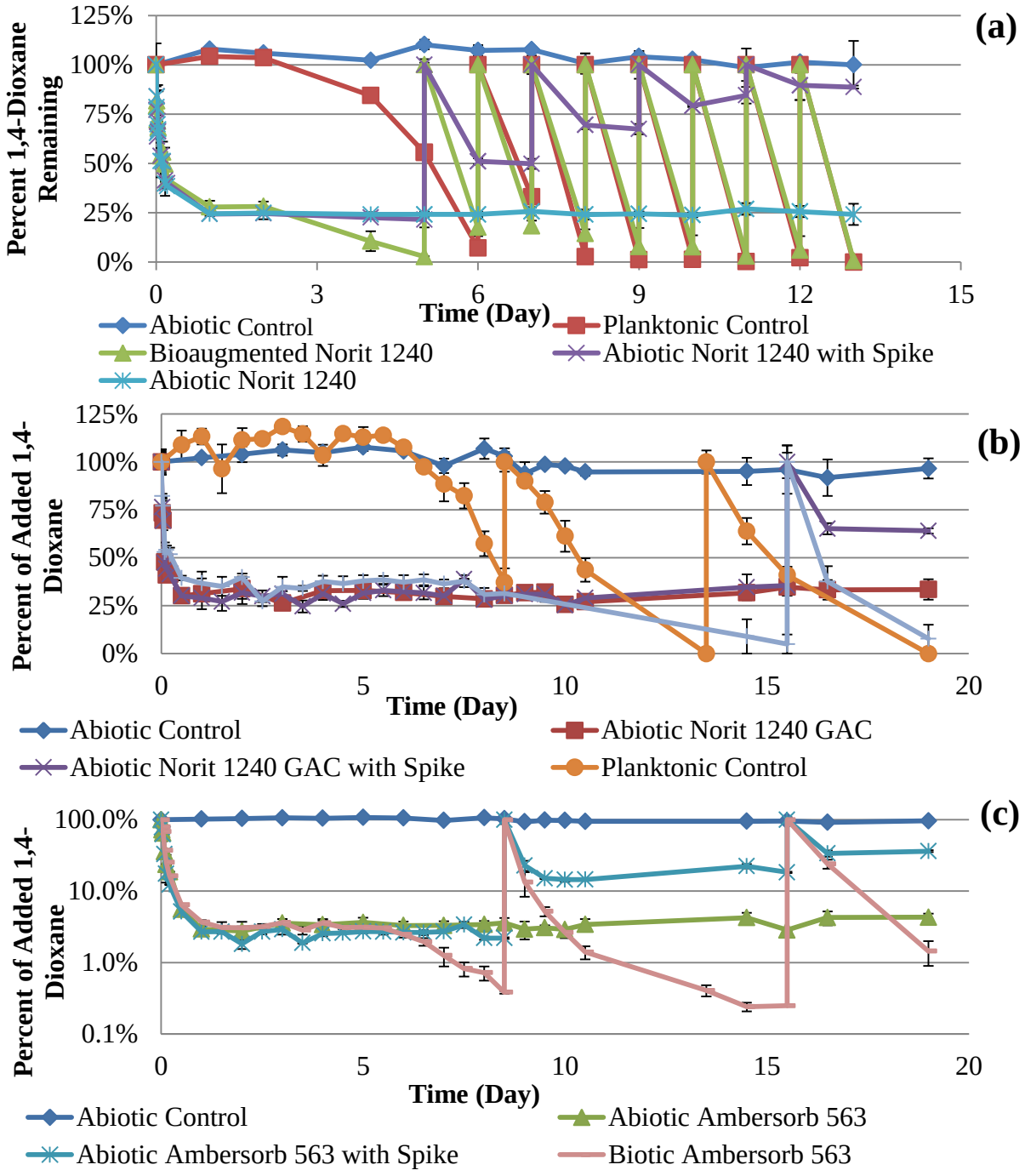


Figure 4 CB1190 bioaugmented sorbent batch reactors. Abiotic sorbent with spike received 1,4-dioxane concurrently with the bioaugmented sorbent reactors. Abiotic and planktonic CB1190 controls were added for reference. (a) Bioaugmented Norit 1240 with 100 mg/L initial concentration. Biological reactors spiked after reaching less than 5%. (b) Bioaugmented Norit 1240 with 400 mg/L initial concentration. Biological reactors spiked after reaching less than 5%. (c) Bioaugmented Ambersorb 560 with 400 mg/L initial concentration. Biological reactors spiked after reaching less than 0.5%.

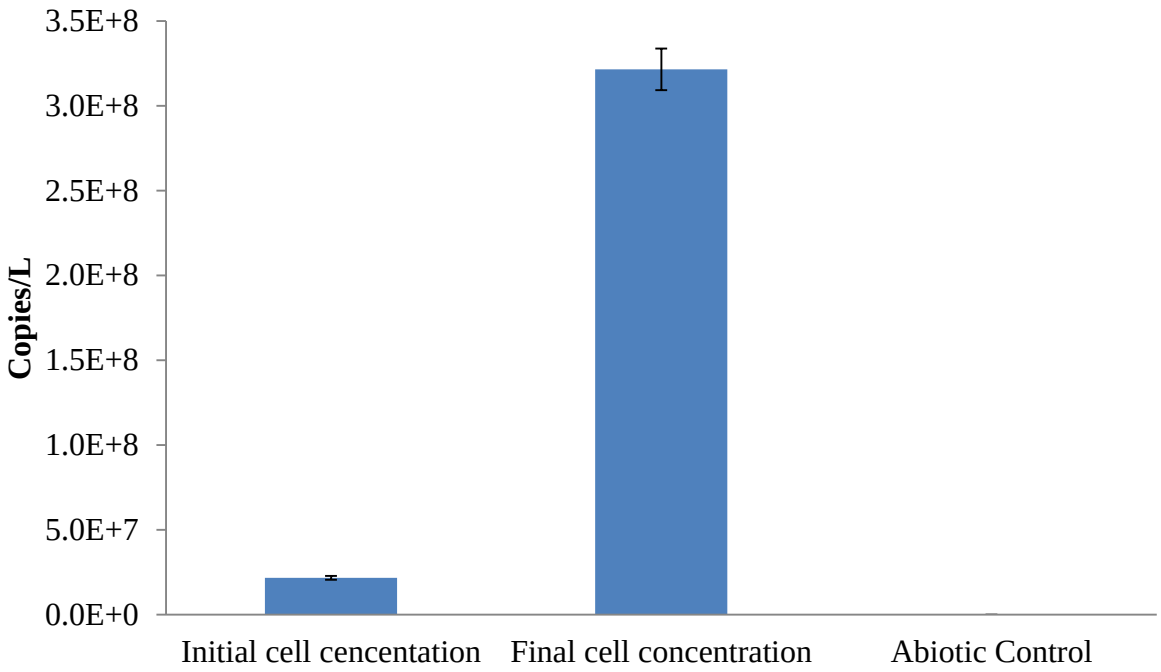


Figure 5 Measured aqueous CB1190 concentration in CB1190-bioaugmented Norit 1240 batch reactors with 100 mg/L starting concentrations.

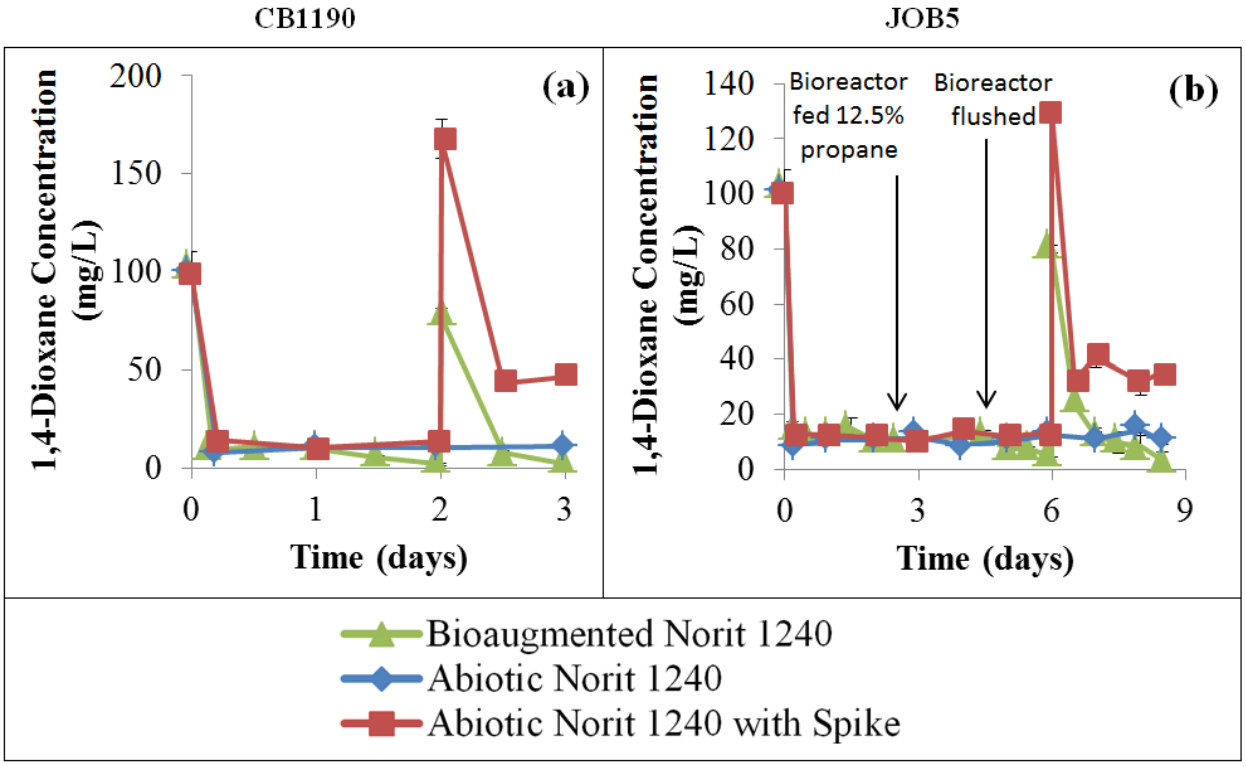


Figure 6 CB1190 and JOB5 bioaugmented Norit 1240 batch reactors. Abiotic sorbent with spike received 1,4-dioxane concurrently with the respective bioaugmented sorbent reactors. (a) CB1190 bioaugmented Norit 1240 bioreactor. 1,4-Dioxane removal in CB1190 bioreactors surpassed abiotic reactors at Day 1.5. (b) Job5 bioaugmented Norit 1240. Bioreactor was fed 12.5% propane on Day 2.5 after no observed degradation occurred. After increasing levels of CO_2 were detected at Day 4.5, bioreactors were heated and flushed with filtered air to remove propane. 1,4-Dioxane removal in JOB5 bioreactors surpassed abiotic reactors at Day 5.

These bioreactors demonstrate the ability for adsorption and 1,4-dioxane degrading bacteria have the ability to rapidly remove 1,4-dioxane and degrade to below detection. However, because the cells started predominantly in the aqueous phase, the contribution from attached growth cells is hard to distinguish. CB1190- and JOB5-bioaugmented Norit 1240 bioreactors were then constructed with a rinsing step prior to the start of the experiment in order to remove planktonic cells. Both CB1190- and JOB5-bioaugmented Norit 1240 reactors, as well as the abiotic bioreactors, experienced initial adsorption that decreased concentrations to 10-15%

of the 100 mg/L initial concentration (Figure 5). The CB1190-bioaugmented bioreactors surpassed abiotic GAC reactors in terms of 1,4-dioxane removal, starting at Day 1.5. CB1190-bioreactor concentrations fall under 2 mg/L at Day 2 and were subsequently spiked with the initial volume of 1,4-dioxane and fall to a concentration of 2.2 mg/L within one day, once again diverging from the abiotic controls. This represented a 98% removal of 1,4-dioxane in the reactor liquid within 2 days, and an increase in degradation rates in the second round of degradation, due to an increase in both solid phase and liquid phase CB1190 (Figure 6b,c). JOB5-bioaugmented bioreactors experienced much slower degradation and displayed greater removal than the abiotic sorbent reactors on Day 5. Concentrations remained unchanged for the first 2.5 days of the experiment and monitoring of the headspace O_2 and CO_2 levels showed no evidence of degradation occurring. The system was fed 12.5% headspace propane from day 2.5 to day 4.5, when the headspace O_2 and CO_2 levels showed degradation occurring. The bioreactors were then flushed and degradation below 5mg/L was observed at Day 6. The bioreactors were subsequently spiked with the initial volume of 1,4-dioxane and degraded to a concentration of 3.1 mg/L within 2.5 days, once again removing more 1,4-dioxane than the abiotic controls.

Planktonic microbes, which can be seen in the final liquid samples (Figure 6c,d) were the resultsloughing of cells from the granular activated carbon particles, as well as possible desorption of adsorbed cells. These planktonic cells represented an integral part of the bioaugmented granular activated carbon system, as full-scale processes would experience this sloughing and desorption as well, and would experience degradation from these active cells, as well as the attached-growth cells.

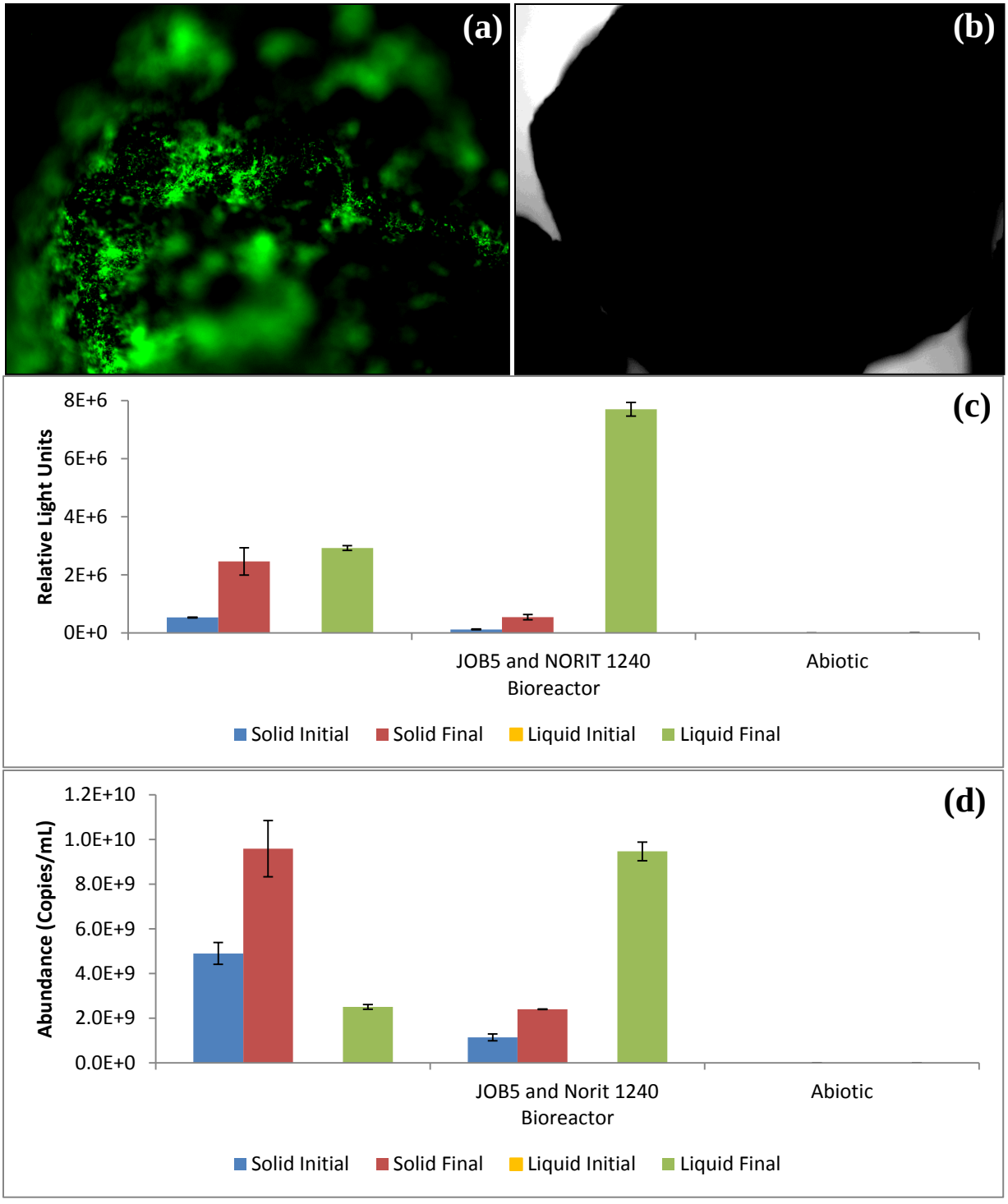


Figure 7 Evidence of attached-cell growth (a) CB1190-Bioaugmented Norit 1240. Green represents bacterial cells colonizing on the GAC. (b) Abiotic Norit 1240. Green bars represent bacterial cells colonizing on the GAC. (c) ATP measurements for solid and liquid samples. (d) Cell abundance using 16S rRNA qPCR measurements.

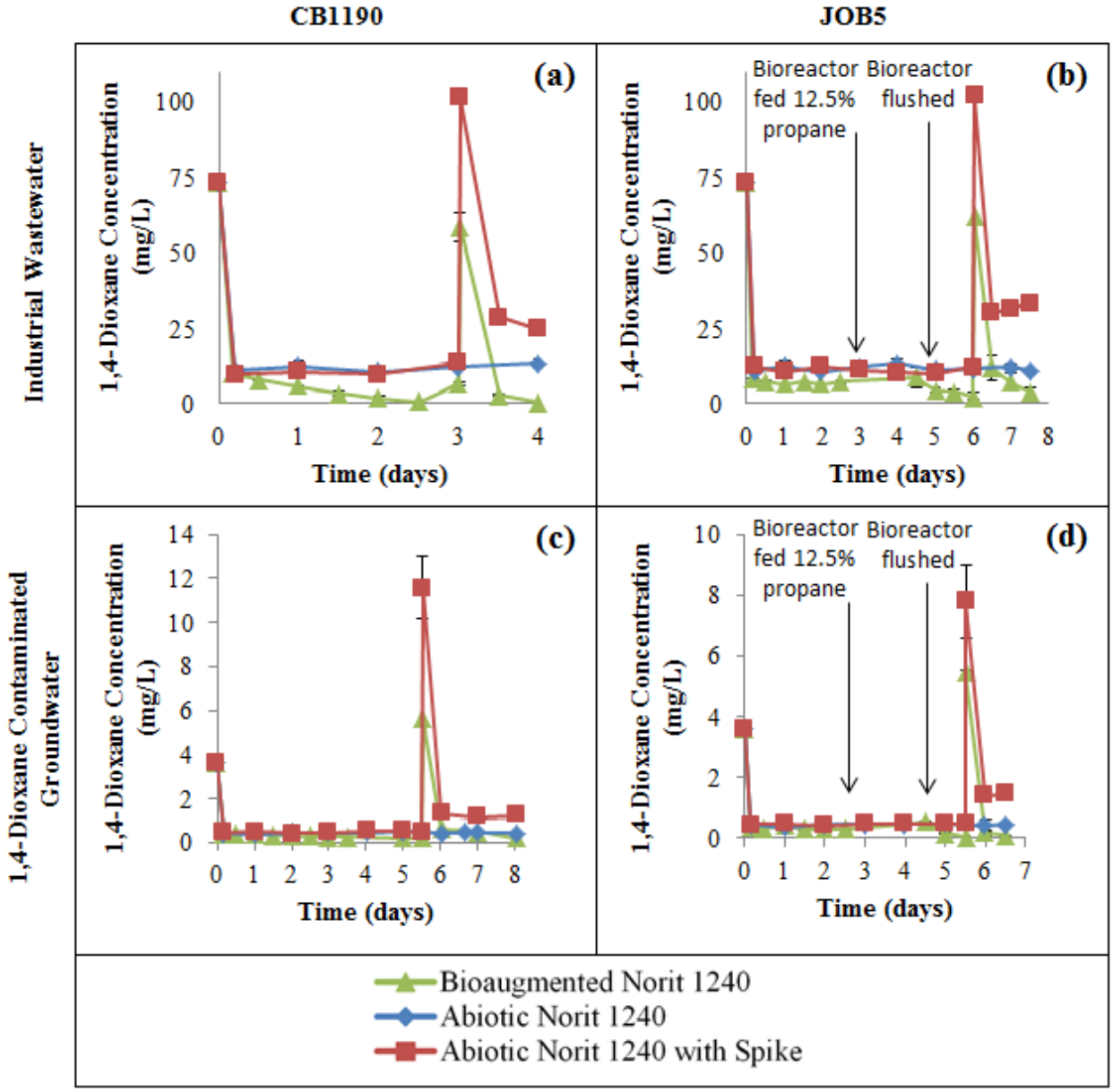


Figure 8 **Bioaugmented sorbent bioreactors with environmental water samples.** (a) CB1190 bioaugmented Norit 1240 bioreactors with industrial wastewater removed two rounds of 1,4-dioxane, 73.4 and 58.6 mg/L, from the process water in 4 days. (b) JOB5 bioaugmented Norit 1240 bioreactors with industrial wastewater removed two rounds of 1,4-dioxane, 73.4 and 62.0 mg/L, from the process water in 7.5 days. (c) CB1190 bioaugmented Norit 1240 bioreactors with contaminated groundwater removed two rounds of 1,4-dioxane, 3.6 and 5.6 mg/L, from the process water in 8 days. (d) JOB5 bioaugmented Norit 1240 bioreactors with contaminated groundwater removed two rounds of 1,4-dioxane, 3.6 and 5.4 mg/L, from the process water in 6.5 days.

Bioreactors using these environmental samples in the place of synthetic growth media were constructed, using identical methods as the bioreactors with distinct rinsing steps to remove planktonic cells. At Day 0.5, the CB1190-bioaugmented Norit 1240 bioreactor with industrial wastewater showed greater 1,4-dioxane removal than the abiotic GAC control (Figure 7a), 1 day sooner than the CB1190 bioreactor with synthetic growth medium. Two rounds of 1,4-dioxane, 73.4 and 58.6 mg/L, were removed to below detection within 4 days. JOB5-bioaugmented Norit 1240 with industrial wastewater showed greater 1,4-dioxane removal than the abiotic GAC control at Day 5 The CB1190-and JOB5-bioaugmented Norit 1240 with 1,4-dioxane contaminated groundwater showed greater removal than the abiotic GAC controls at day 2 and 5, respectively. JOB5-bioreactors, both with industrial wastewater and with contaminated groundwater, required a propane pulse after initial rinsing, but otherwise needed no inputs for the remainder of the experiment.

5. Discussion

Adsorption of 1,4-dioxane was previously thought to be largely ineffectual, as the compound has a low octanol water partition coefficient (K_{ow}) value[7] and a high water solubility[37], which suggests that the compound has a tendency to stay in solution. However, studies using granular activated carbon (GAC) and activated carbon derived from hard nut shells have shown success with adsorption of 1,4-dioxane[60-61]. This adsorption was found to be limited, resulting in lower adsorption rates and capacities with prolonged usage. More recent studies have focused on the use expensive synthetic carbonaceous sorbents to remove 1,4-dioxane to low levels in groundwater following pump-and-treat operations[62]. This study found that 1,4-dioxane

sorption to Norit 1240 GAC, Ambersorb 560, US1076, and US1028 fit the Freundlich isotherm model, similar to that previously reported for carbonaceous sorbent 1,4-dioxane adsorption[63]. The Freundlich isotherm model assumes variable energy at the active adsorption sites on the surface of the sorbent, with multi-layer adsorption of the adsorbate[64]. This adsorption profile is characteristic of activated carbon[64], which is consistent with the findings of our modeling.

This study demonstrated significant sorption using inexpensive granular activated carbon (Norit 1240), as well as expensive synthetic carbonaceous sorbents (Ambersorb 560). Both the granular activated carbon and the synthetic sorbents demonstrated significant desorption into water without the need for elevated temperatures or organic eluents. This approach could have significant implications for public health in drinking water systems or process water quality issues in industrial applications, but it is not discussed in literature about abiotic adsorption of 1,4-dioxane onto synthetic carbonaceous sorbents. In an abiotic sorbent filtration facility, rapid desorption of the target contaminant is liability, which could result in a situation where the target contaminant could desorb into influent process water if the influent concentration were decreased. Norit 1240 exhibits a large percentage of desorption, resulting in aqueous concentrations that were much higher than the target 0.35 μg/L advisory concentration for 1,4-dioxane. While Ambersorb 560 desorbed relatively lower 1,4-dioxane percentages, many of the equilibrium desorption concentrations still exceeded the 0.35 μg/L health advisory concentration for 1,4-dioxane. This desorption represents the possibility that an abiotic sorbent filtration facility could potentially contaminate process water for sensitive industrial or biological applications.

Physical-chemical sorption does not alter the structure of the compound or render it less toxic; it simply changes the phase from aqueous to solid. Due to the reversibility of this adsorption, a technology that permanently degrades 1,4-dioxane is required. While there are abiotic methods for destruction of 1,4-dioxane[19-22], many of these result in toxic byproducts, some with even higher toxicities than 1,4-dioxane[19-22]. Alternatively, 1,4-dioxane biodegradation, via both metabolic and co-metabolic mechanisms, have been shown to completely degrade 1,4-dioxane to CO_2[54, 65-66]. Bioaugmented sorbents experience rapid initial adsorption, which likely helped concentrated 1,4-dioxane during the bacterial lag phase. Irreversible microbial degradation of 1,4-dioxane reduced the possibility of desorption in a system with a variable influent concentration. The long-term performance of bioaugmented sorbent bioreactors should be superior to that of abiotic batch reactors, as the biomass increases and continue to remove 1,4-dioxane, and abiotic sorbents have a limited capacity dictated by the Freundlich isotherm parameters. However, biofouling would be a concern in these processes, if substrate concentrations were sufficiently high. This should be evaluated in future flow-through column studies.

While many current studies of 1,4-dioxane degrading microbes focus on planktonic cultures, biofilms and attached growth microbes are significant in the environment[67]. In subsurface aquifers and even in reservoirs systems, attached-growth bacteria are more common than planktonic bacteria[67]. Microbial biofilms are often characterized as containing extracellular polymeric substances (EPS), which anchor the cells to the solid surface, as well as provide some adsorption capabilities that can sequester toxic chemicals, reducing the bioavailability to the cells[68-69]. Planktonic studies could be poor representations of environmental or treatment

systems. This study explored the presence of attached-growth bacteria, presumably attached through a combination of direct adsorption of the cells and production of EPS. Further laboratory research will be necessary to determine the effect attached growth might have on 1,4-dioxane degradation or the expression of 1,4-dioxane monooxygenase encoding genes and effects EPS may have in the reduction of inhibition by toxic co-contaminants. CVOCs in particular, are common co-contaminants in 1,4-dioxane contaminated waters that have known inhibitory effects on 1,4-dioxane metabolizing bacteria, such as CB1190, and 1,4-dioxane co-metabolizing bacteria, such as JOB5 or *Pseudomonas mendocina* KR1[55, 70]. A broader study regarding the effect of 1,4-dioxane degrading bacteria release in the environment and into premise plumbing will also be necessary.

The selection of microbes in an engineered remediation system is crucial, as different microbes have different nutrient requirements and different levels of inhibition for common co-contaminants[55, 70-71]. Studies of both 1,4-dioxane metabolizing and cometabolizing bacteria are crucial for this reason, as these studies allow a wider range of growth substrates and geochemical conditions. For instance, 1,4-dioxane metabolizing bacteria, such as CB1190, may be better suited for conditions with higher 1,4-dioxane concentrations, as 1,4-dioxane is used as the sole carbon and energy source. 1,4-Dioxane cometabolizing bacteria may be suited for lower 1,4-dioxane concentration systems, as another compound is used as the sole carbon and energy source for powering bacterial growth and inducing enzymes relevant for cometabolism. This was demonstrated in the CB1190- and JOB5-bioaugmented Norit 1240 bioreactors tested with environmental samples in this study. The industrial wastewater bioreactors showed faster 1,4-dioxane removal in the CB1190 bioreactor, while in the contaminated groundwater, 1,4-dioxane

removal was much faster and complete in the JOB5 bioreactor. When selecting the microbe best suited for low-1,4-dioxane environmental conditions, certain 1,4-dioxane cometabolizing bacteria could be selected for their ability to metabolize present organic compounds, thus reducing the need for nutrient augmentation.

Many of the current technologies for 1,4-dioxane groundwater remediation rely either on expensive pump-and-treat methods or have limited dispersion abilities in the subsurface. Bioaugmented sorbent technologies can be a cheaper alternative to these technologies, when used as permeable reactive barriers *in situ* with an inexpensive sorbent. This technology can also be implemented in existing treatment facilities where sorbent filters are already in use. Bioaugmented sorbent reactors can be used as an inexpensive way to create more effective 1,4-dioxane removal systems. Additionally, bioremediation, both metabolic and cometabolic, display complete degradation of the 1,4-dioxane molecule, without the toxic byproducts that can result from many other treatment methods[21]. Bioaugmentation studies such as this can result in future environmentally friendly 1,4-dioxane treatment methods.

6. Summary and Future Work

In this study, the 1,4-dioxane affinities of 5 different sorbents were investigated: a granular activated carbon (Norit 1240), a synthetic carbonaceous sorbent (Ambersorb 560), a synthetic goethite (Bayoxide E33), a coconut-based nanopowder activated carbon (US1076), and a coal-based nanopowder activated carbon (US1078). Norit 1240 and Ambersorb 560 were found to have the highest adsorption capacity of these sorbents. Bayoxide E33 showed no measurable sorption and grew salt crystals in AMS medium. US1076 and US1078 required higher masses of sorbent than Norit 1240 or Ambersorb 560 in order to show measurable 1,4-dioxane sorption.

Norit 1240 adsorbed 59-88% for concentrations 13-800 mg/L at 20^{o}C and 30^{o}C. Ambersorb 560 adsorbed 90-99% for concentrations 13-800 mg/L at 20^{o}C and 30^{o}C. Isotherm models were fitted to these data in order to be able to predict equilibria for untested conditions. Freundlich was found to be the most accurate isotherm model for these data. Parameters were obtained and are available in Table 6.

Once these reactors reached equilibrium, the supernatant was removed and replaced with 1,4-dioxane-free water to test desorption. Norit 1240 displayed 11-42% desorption of sorbed 1,4-dioxane, while Ambersorb 560 displayed only 1-7% desorption of sorbed 1,4-dioxane. This desorption demonstrates the issues with using abiotic sorption. 1,4-Dioxane influent water could desorb 1,4-dioxane from the sorbents, thus contaminating the water. While Ambersorb 560 desorption was low, higher quantities of adsorbed 1,4-dioxane result in higher desorption

percentages, and Ambersorb 560 reactors that have treated more empty bed volumes have the potential to desorb more dioxane.

In order to reduce the risk for 1,4-dioxane desorption and increase the amount of 1,4-dioxane a reactor can permanently remove, bioaugmentation of these sorbents was explored with the 1,4-dioxane metabolizing bacterial strain, *Pseudonocardia dioxanivorans* CB1190 and 1,4-dioxane co-metabolizing propanotroph, *Mycobacterium austroafricanum* JOB5. CB1190-bioaugmented sorbents demonstrated greater 1,4-dioxane removal than the abiotic sorbent control between 4 and 14.5 days. These degradation rates were faster with each subsequent spike, as the biomass grew. This system also showed an advantage over the planktonic controls, as the initial sorption removed 62-98% of the aqueous 1,4-dioxane within the first day. JOB5 bioaugmentation was then tested. 1,4-Dioxane degradation was observed only after thermal desorption of propane from the sorbent. These bioreactors demonstrated the ability of bioaugmented sorbents to remove 1,4-dioxane. In order to better model the bioaugmented-sorbent systems as flow-through treatment systems, future work must include long-term column reactors, with 1,4-dioxane in realistic concentrations and matrices.

Appendix A. Freundlich Isotherm Model

$$q_e = K_f C_e^{1/n} \quad (1)$$

where

q_e = amount of adsorbate on the adsorbent at equilibrium(mg/g)

C_e = equilibrium concentration (mg/L)

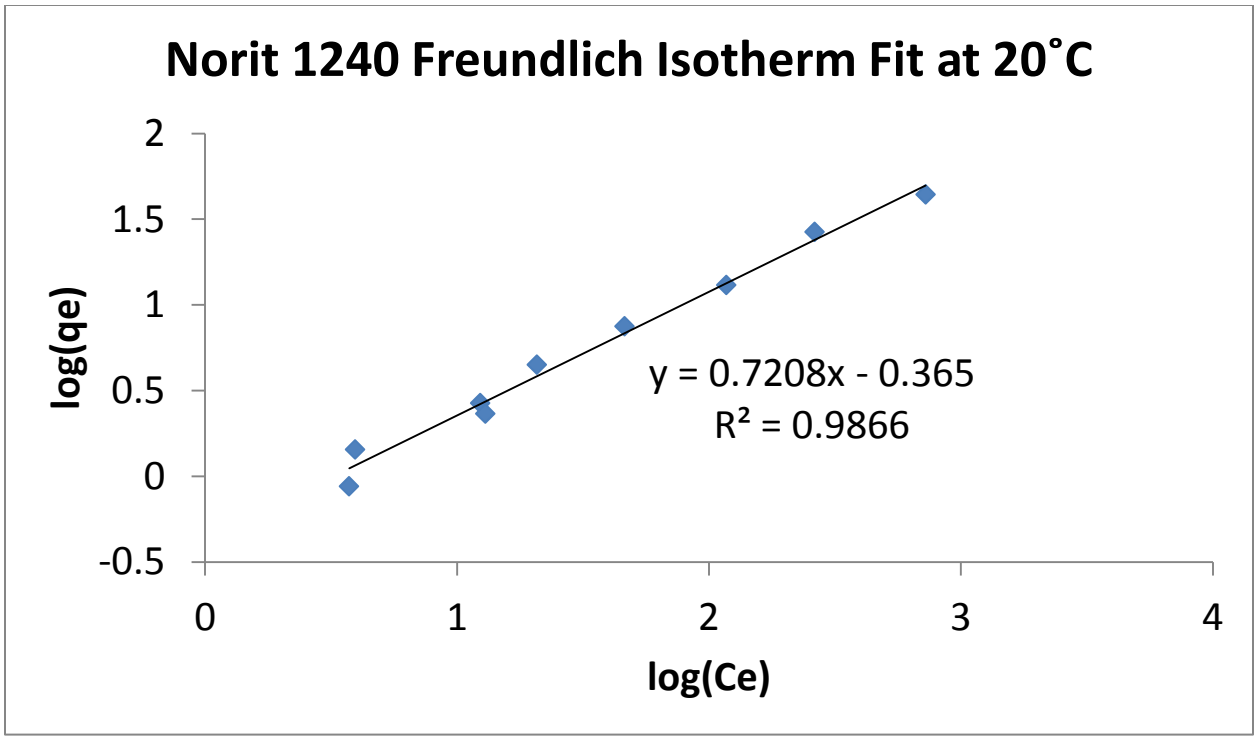
n = Freundlich adsorption intensity parameter, unitless

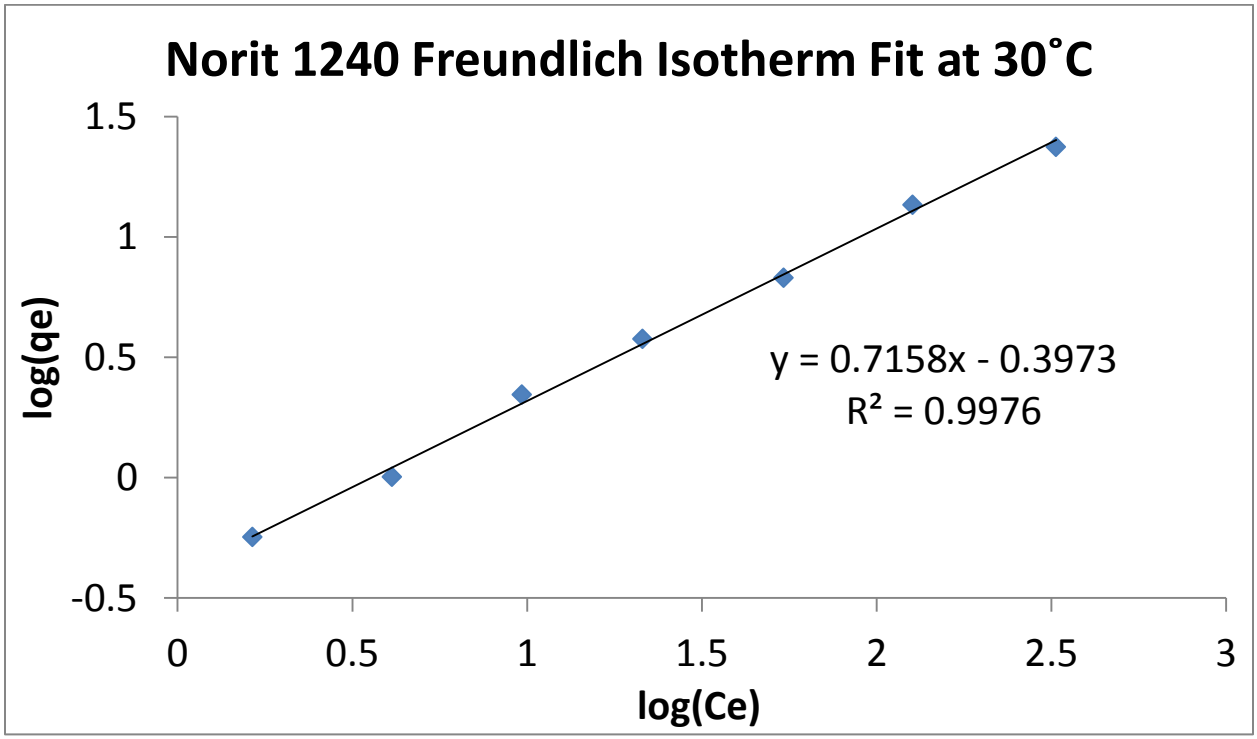
K_f = Freundlich adsorption capacity parameter (L/g)

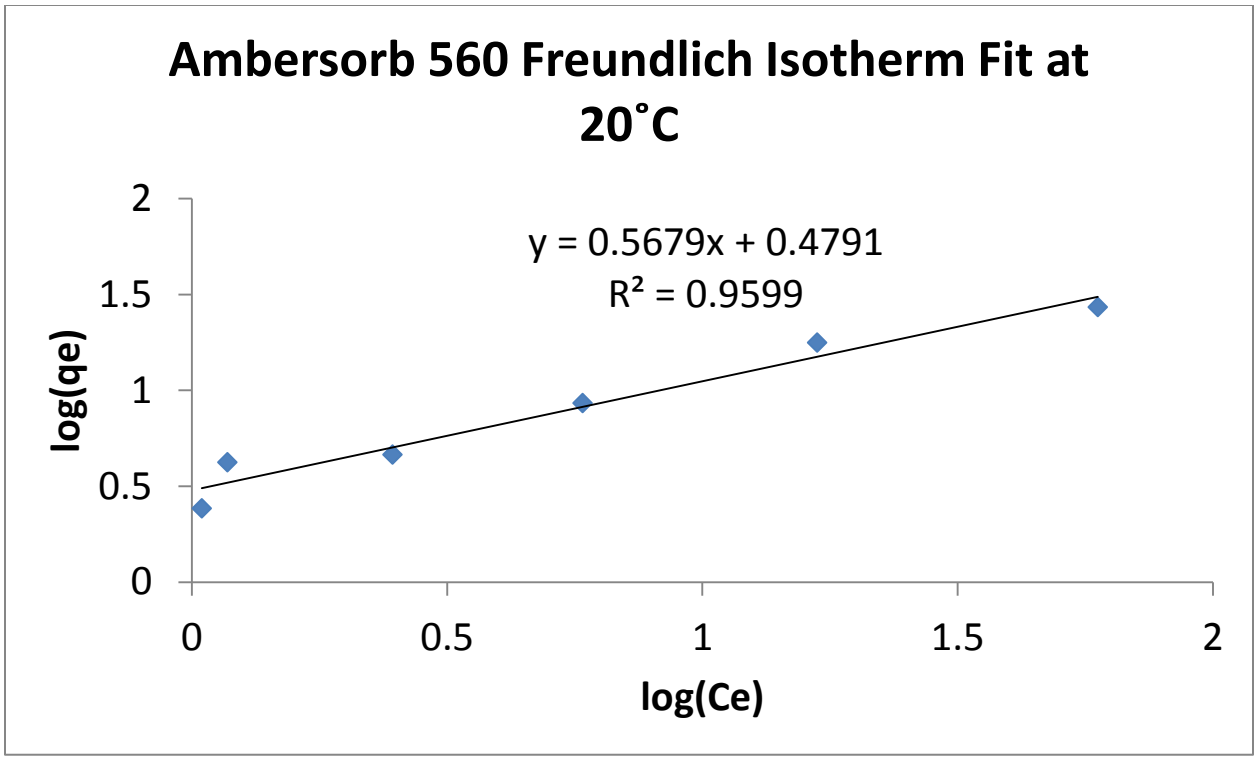
In order to use the Freundlich Isotherm Model, equation (1) is linearized.

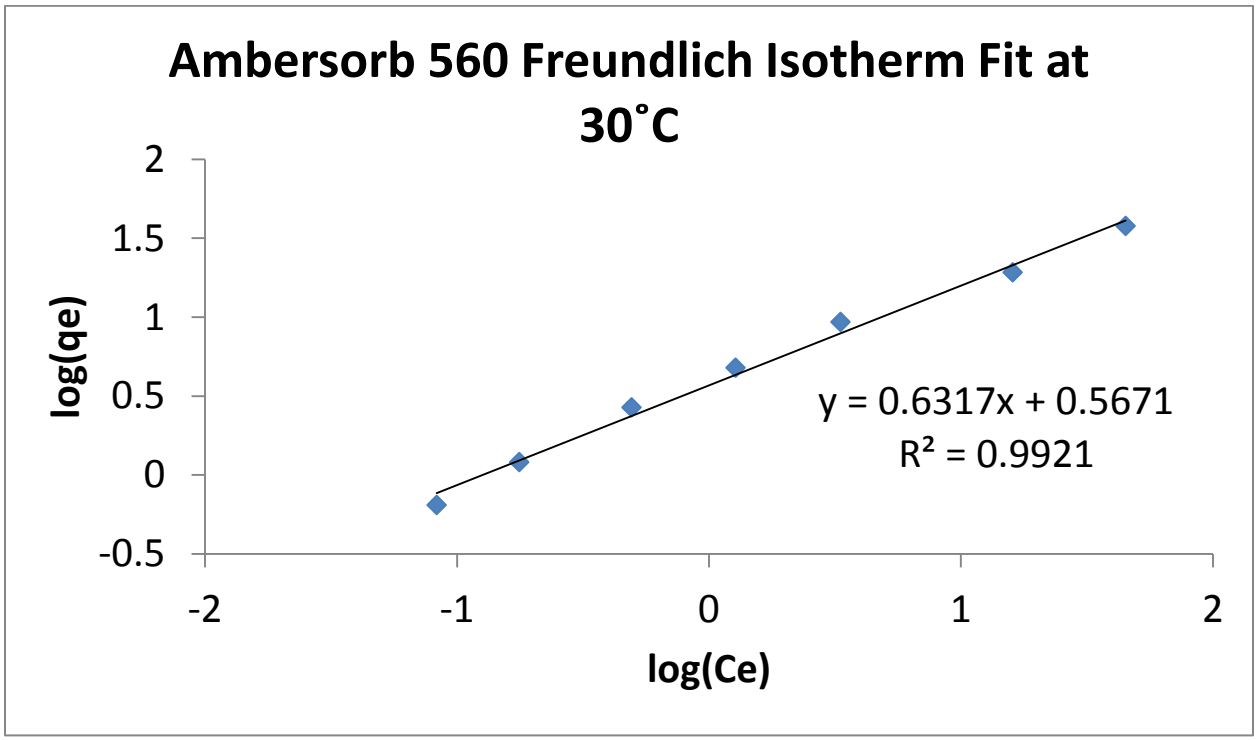
$$\log(q_e) = \log(K_f) + \left(\frac{1}{n}\right)\log(C_e) \quad (2)$$

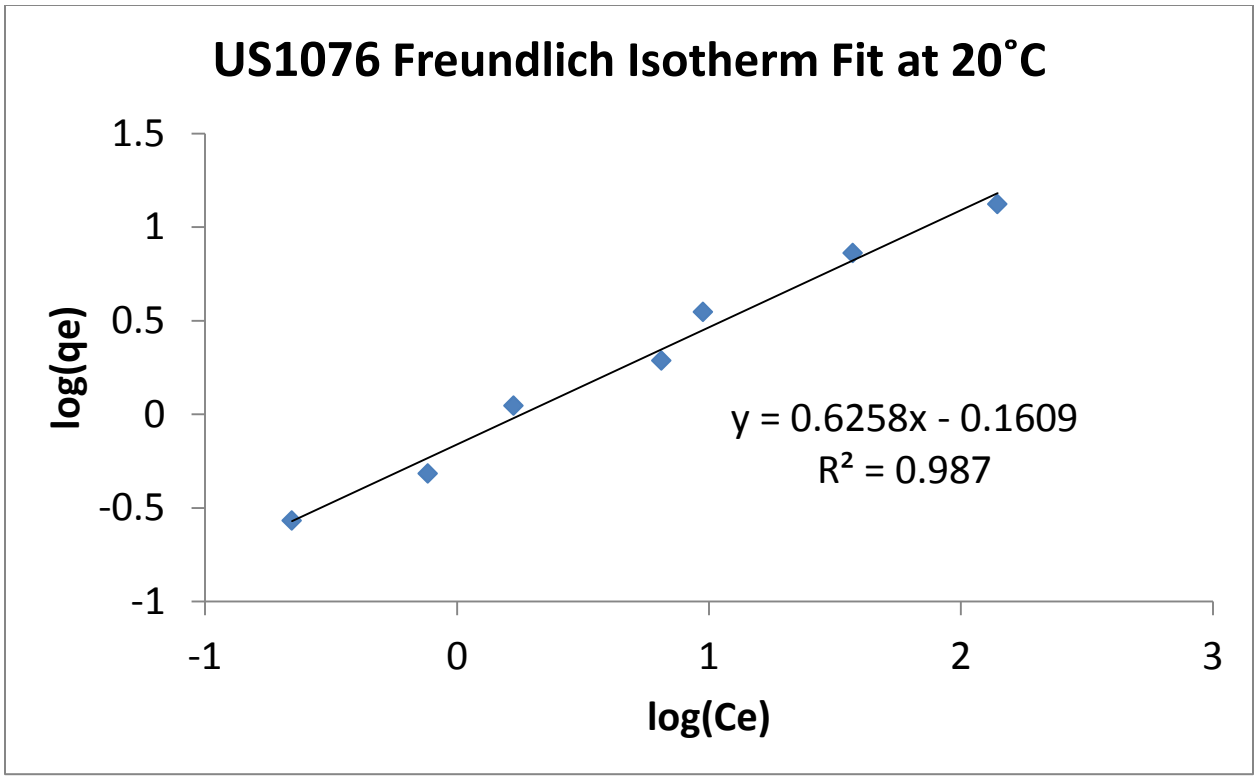
Using equation (2), the data from the adsorption experiments are plotted on a $\log(q_e)$ vs $\log(C_e)$ plot, where $\left(\frac{1}{n}\right)$ is the slope and $\ln(K_f)$ is the y-intercept [64].

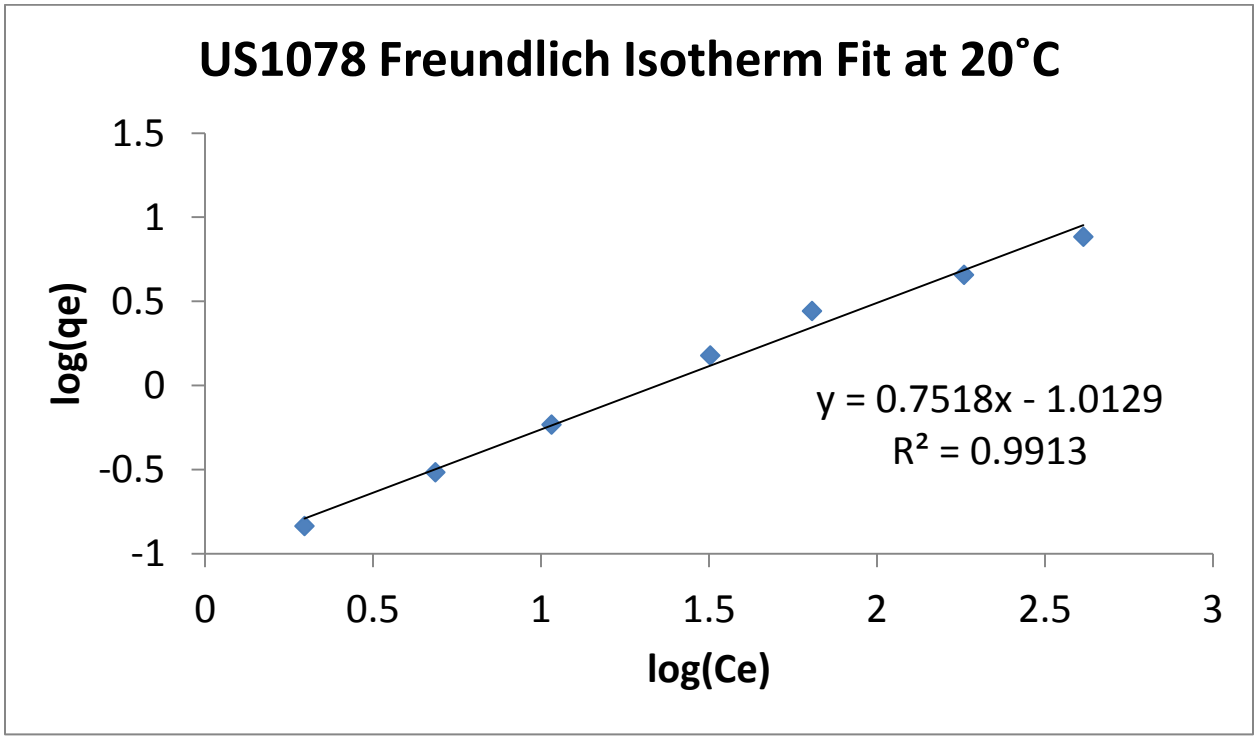

Norit 1240 Freundlich Isotherm Fit at 20˚C
log(qe)
log(Ce)
y = 0.7208x - 0.365
R² = 0.9866
2
1.5
1
0.5
0
-0.5
0
1
2
3
4


Norit 1240 Freundlich Isotherm Fit at 30˚C
log(qe)
log(Ce)
y = 0.7158x - 0.3973
R² = 0.9976
1.5
1
0.5
0
-0.5
0
0.5
1
1.5
2
2.5
3


Ambersorb 560 Freundlich Isotherm Fit at 20˚C
y = 0.5679x + 0.4791
R² = 0.9599
log(qe)
log(Ce)
2
1.5
1
0.5
0
0
0.5
1
1.5
2


Ambersorb 560 Freundlich Isotherm Fit at 30˚C
y = 0.6317x + 0.5671
R² = 0.9921
log(qe)
log(Ce)
2
1.5
1
0.5
0
-0.5
-2
-1
0
1
2


US1076 Freundlich Isotherm Fit at 20˚C
1.5
1
0.5
0
-0.5
-1
log(qe)
-1
0
1
2
3
log(Ce)
y = 0.6258x - 0.1609
R² = 0.987


US1078 Freundlich Isotherm Fit at 20˚C
1.5
1
0.5
0
-0.5
-1
log(qe)
0
0.5
1
1.5
2
2.5
3
log(Ce)
y = 0.7518x - 1.0129
R² = 0.9913

Appendix B. Langmuir Isotherm Model

$$q_e = \frac{Q_o K_L C_e}{1 + K_L C_e} \quad (3)$$

where

q_e= amount of adsorbate on the adsorbent at equilibrium(mg/g)

C_e= equilibrium concentration (mg/L)

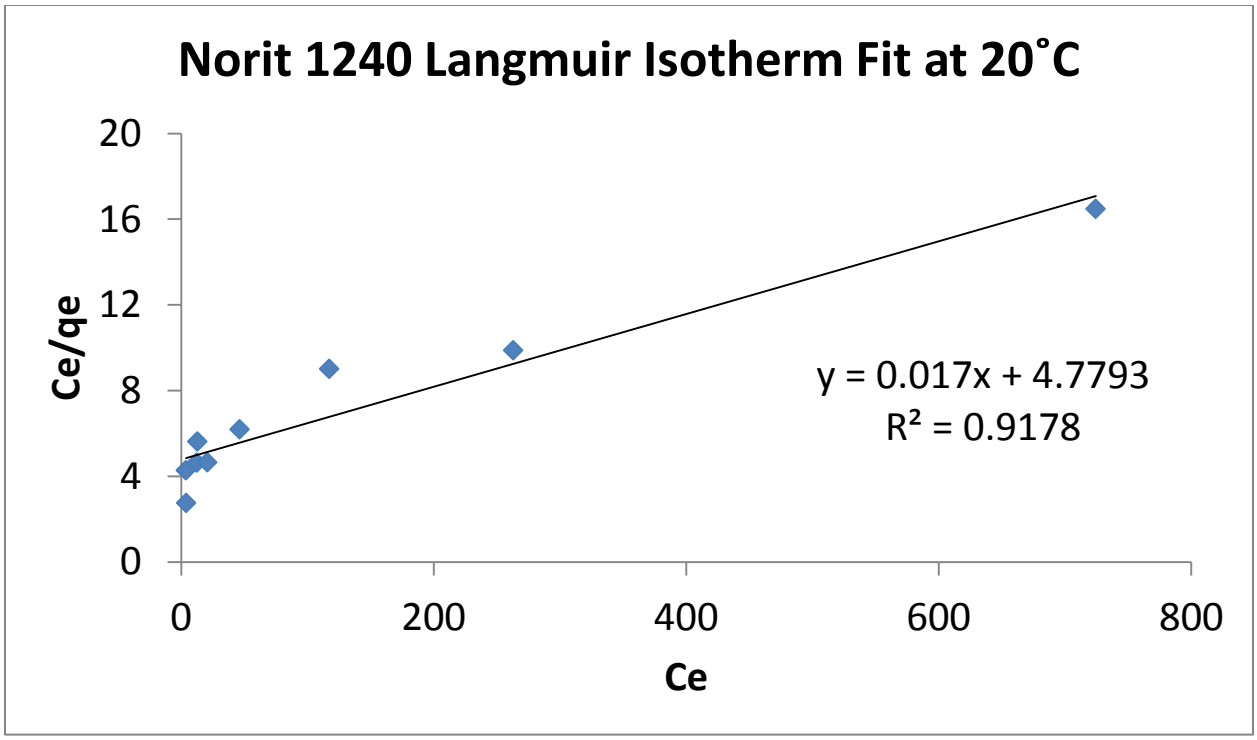
Q_o=theoretical maximum adsorbate on the adsorbent with saturated surface sites (mg /g)

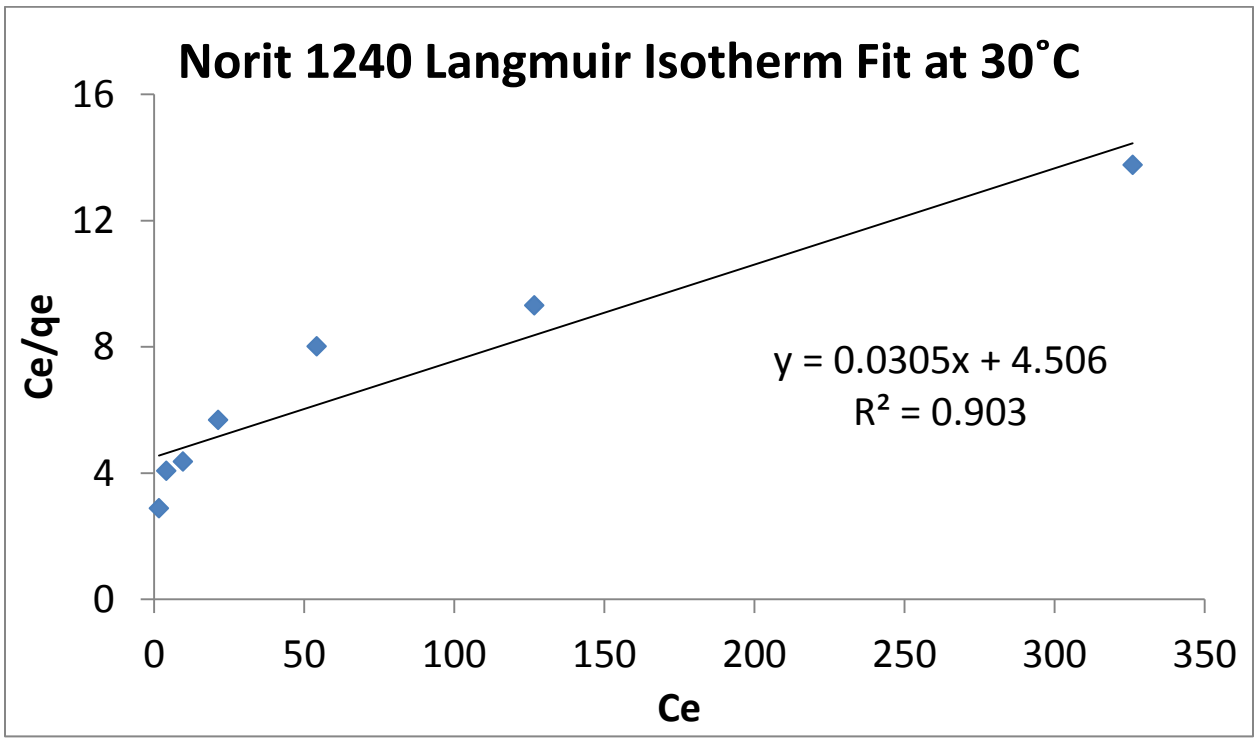
K_L=Langmuir adsorption constant of adsorbate (L/mg)

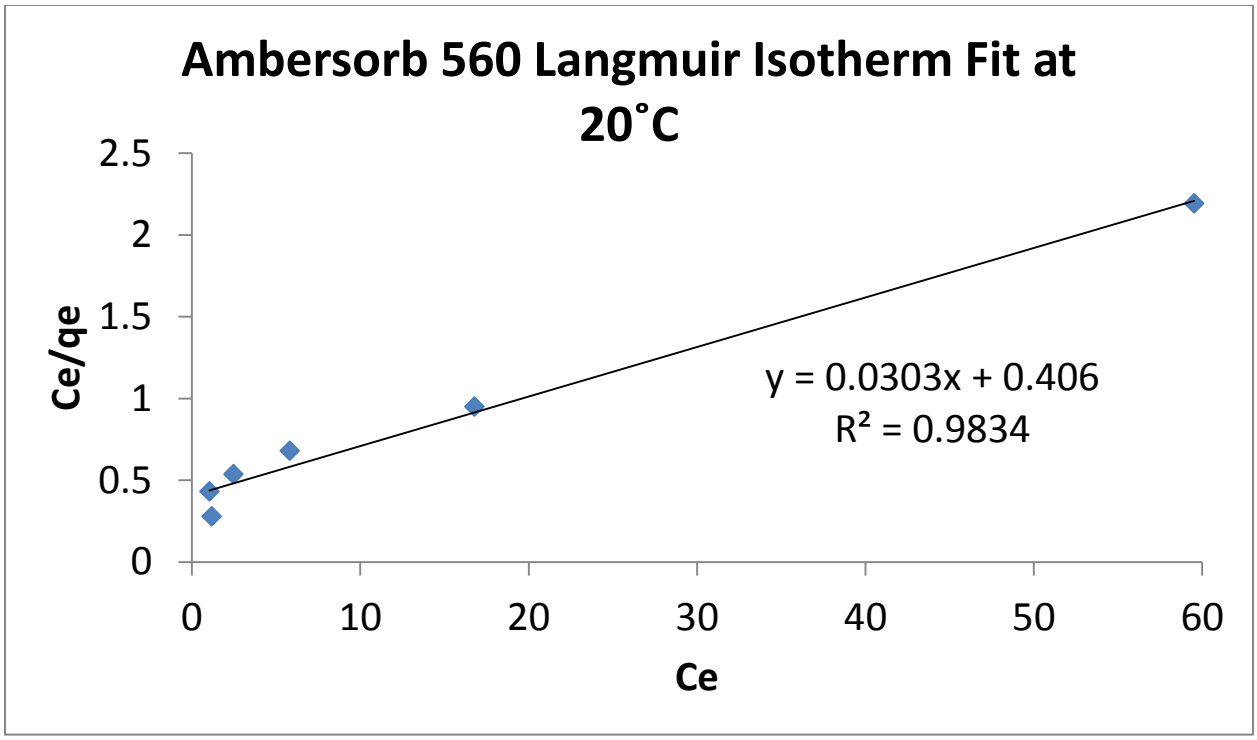
In order to use the Langmuir Isotherm Model, equation (3) is linearized.

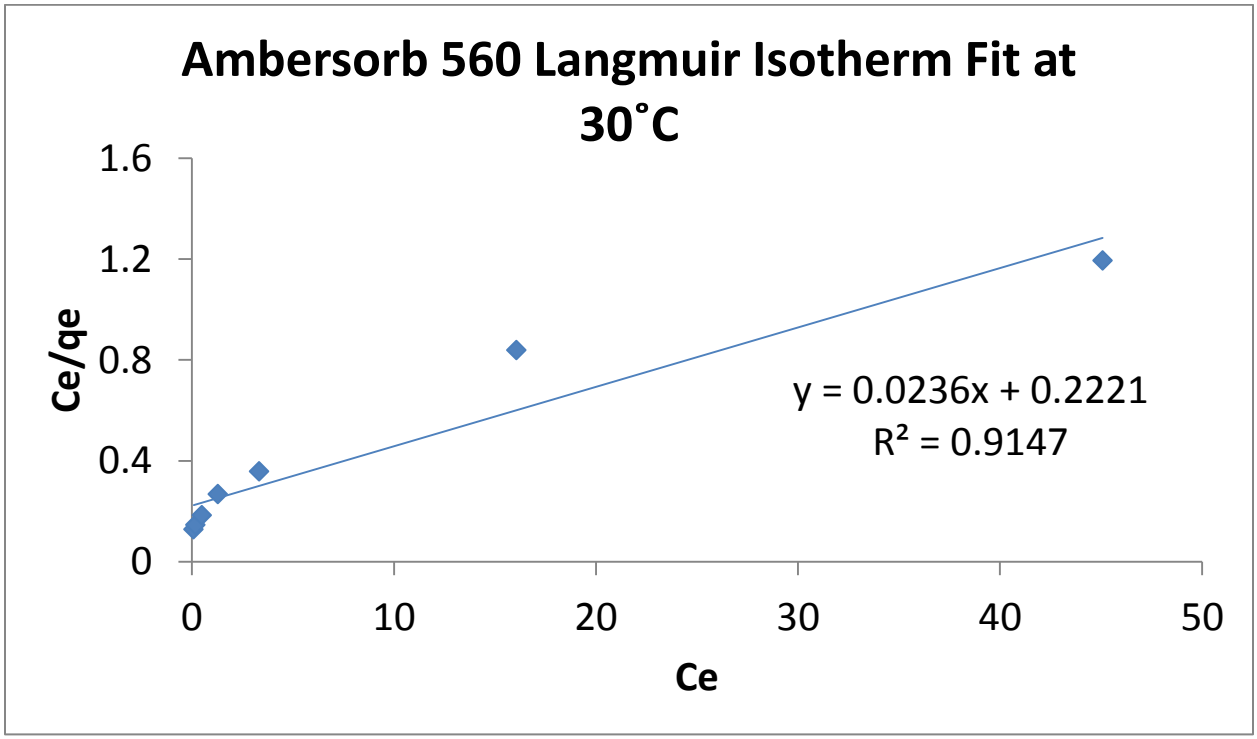
$$\frac{C_e}{q_e} = \frac{C_e}{Q_o} + \frac{1}{Q_o K_L} \quad (4)$$

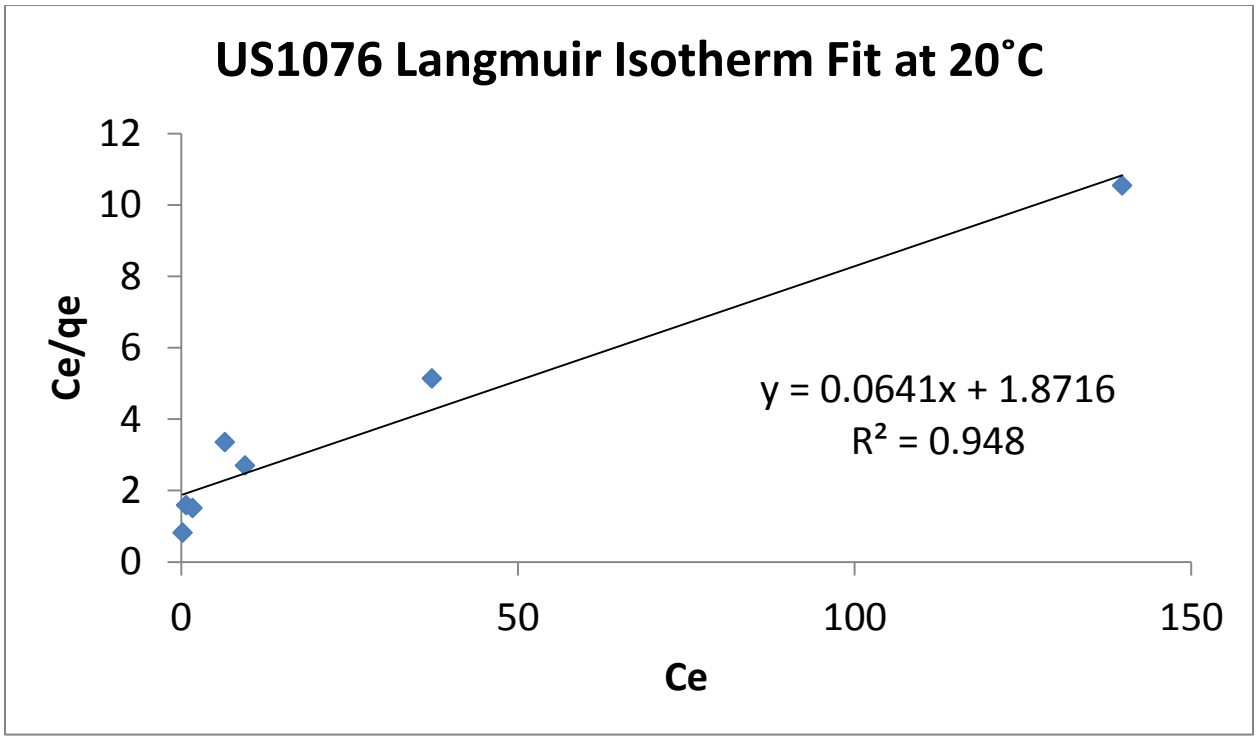
Using equation (4), the data from the adsorption experiments are plotted on a $\frac{C_e}{q_e}$ vs C_e plot, where $\frac{1}{Q_o}$is the slope and $\frac{1}{Q_o K_L}$is the y-intercept [64].

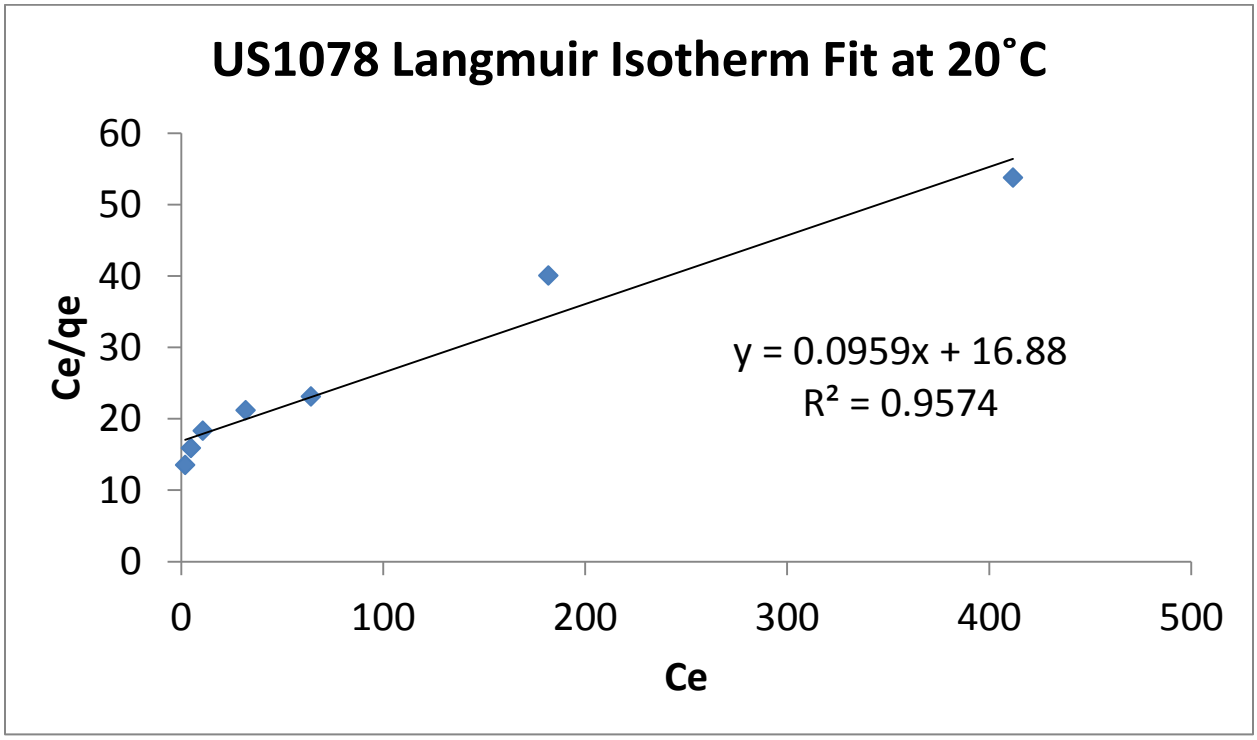

Norit 1240 Langmuir Isotherm Fit at 20˚C
Ce/qe
Ce
y = 0.017x + 4.7793
R² = 0.9178
0
4
8
12
16
20
0
200
400
600
800


Norit 1240 Langmuir Isotherm Fit at 30˚C
Ce/qe
Ce
y = 0.0305x + 4.506
R² = 0.903
0
4
8
12
16
0
50
100
150
200
250
300
350


Ambersorb 560 Langmuir Isotherm Fit at 20˚C
Ce/qe
Ce
y = 0.0303x + 0.406
R² = 0.9834
0
0.5
1
1.5
2
2.5
0
10
20
30
40
50
60


Ambersorb 560 Langmuir Isotherm Fit at 30˚C
Ce/qe
Ce
y = 0.0236x + 0.2221
R² = 0.9147
0
0.4
0.8
1.2
1.6
0
10
20
30
40
50


US1076 Langmuir Isotherm Fit at 20˚C
Ce/qe
Ce
y = 0.0641x + 1.8716
R² = 0.948
0
2
4
6
8
10
12
0
50
100
150


US1078 Langmuir Isotherm Fit at 20˚C
Ce/qe
Ce
y = 0.0959x + 16.88
R² = 0.9574
0
10
20
30
40
50
60
0
100
200
300
400
500

Appendix C. Temkin Isotherm Model

$$q_e = \frac{RT}{b_T}\ln(A_T C_e) \quad (5)$$

where

q_e= amount of adsorbate on the adsorbent at equilibrium(mg/g)

C_e=equilibrium concentration (mg/L)

R= universal gas constant (8.314J/mol/K)

T= Temperature (K)

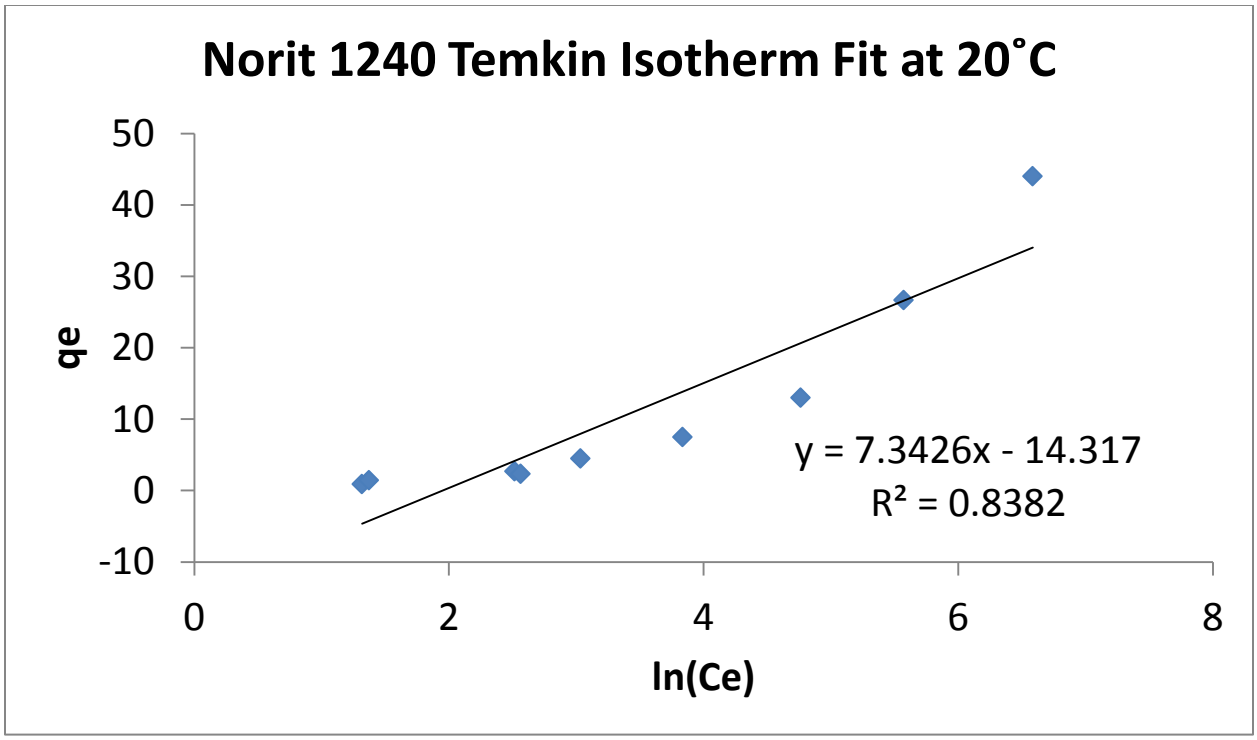
A_T =Temkin isotherm equilibrium binding constant (L/g)

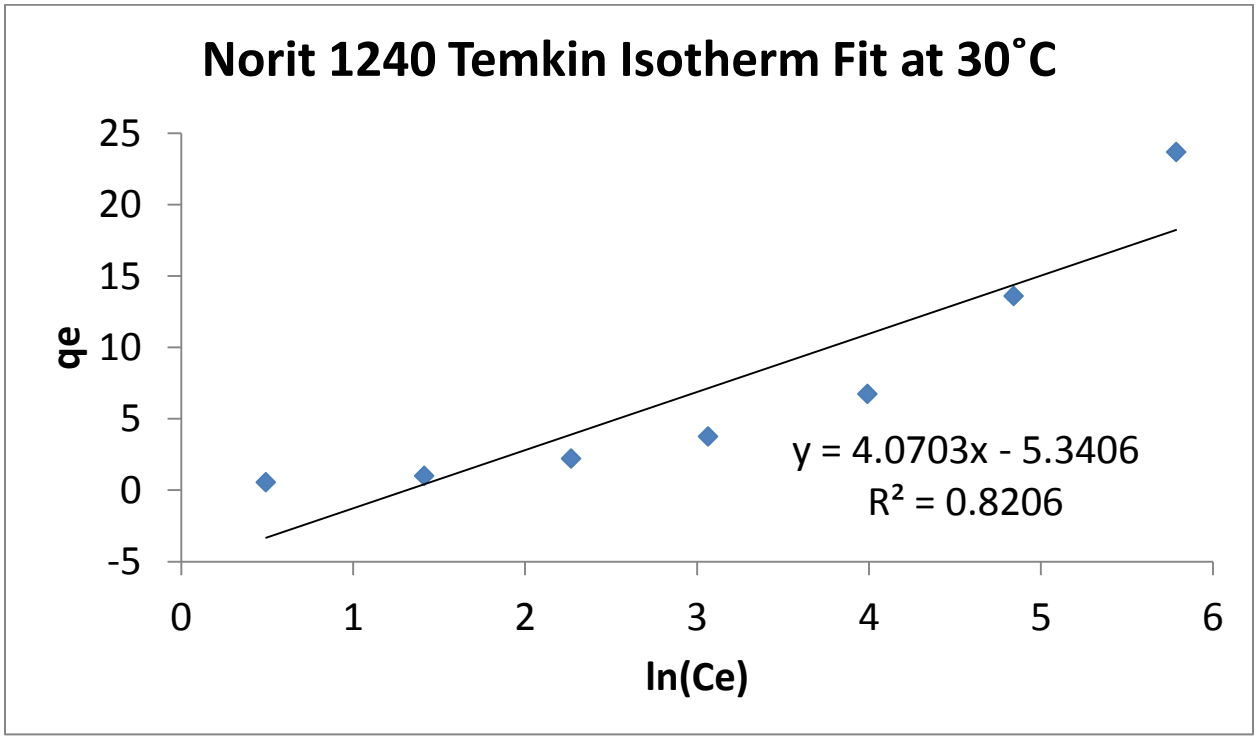
b_T = Temkin isotherm constant

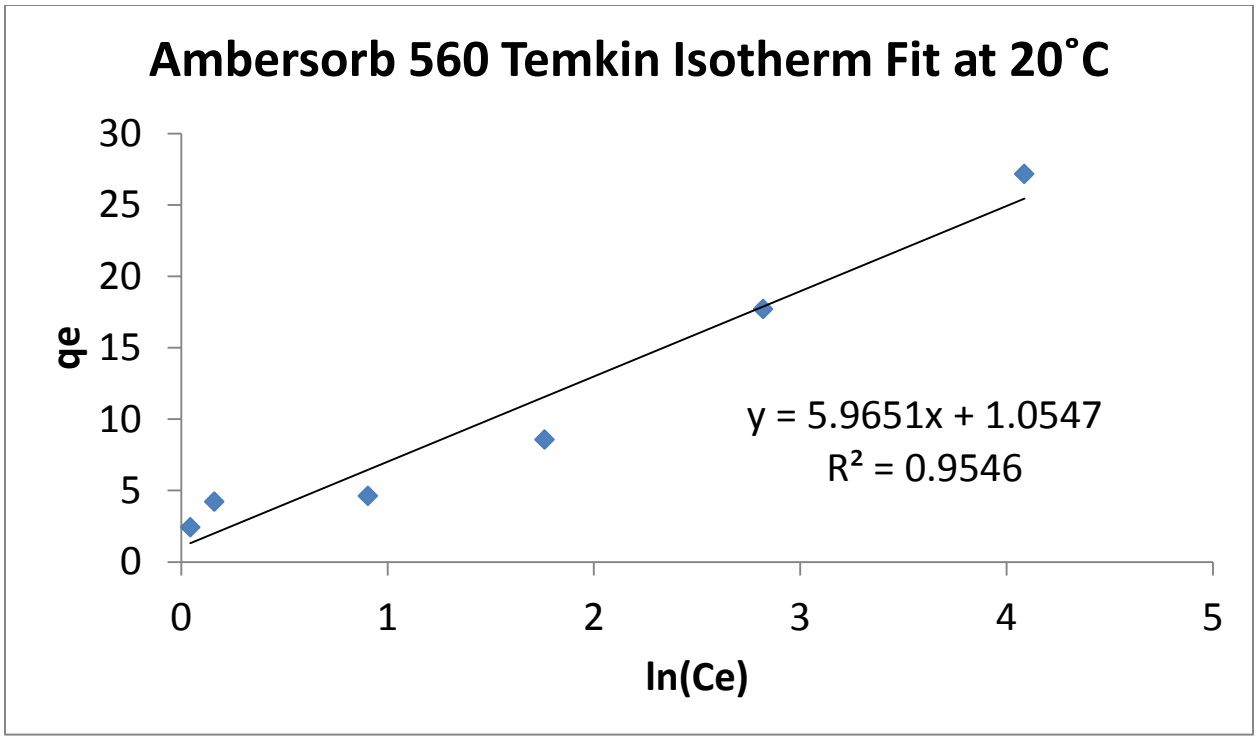
In order to use the Temkin Isotherm Model, equation (5) is linearized.

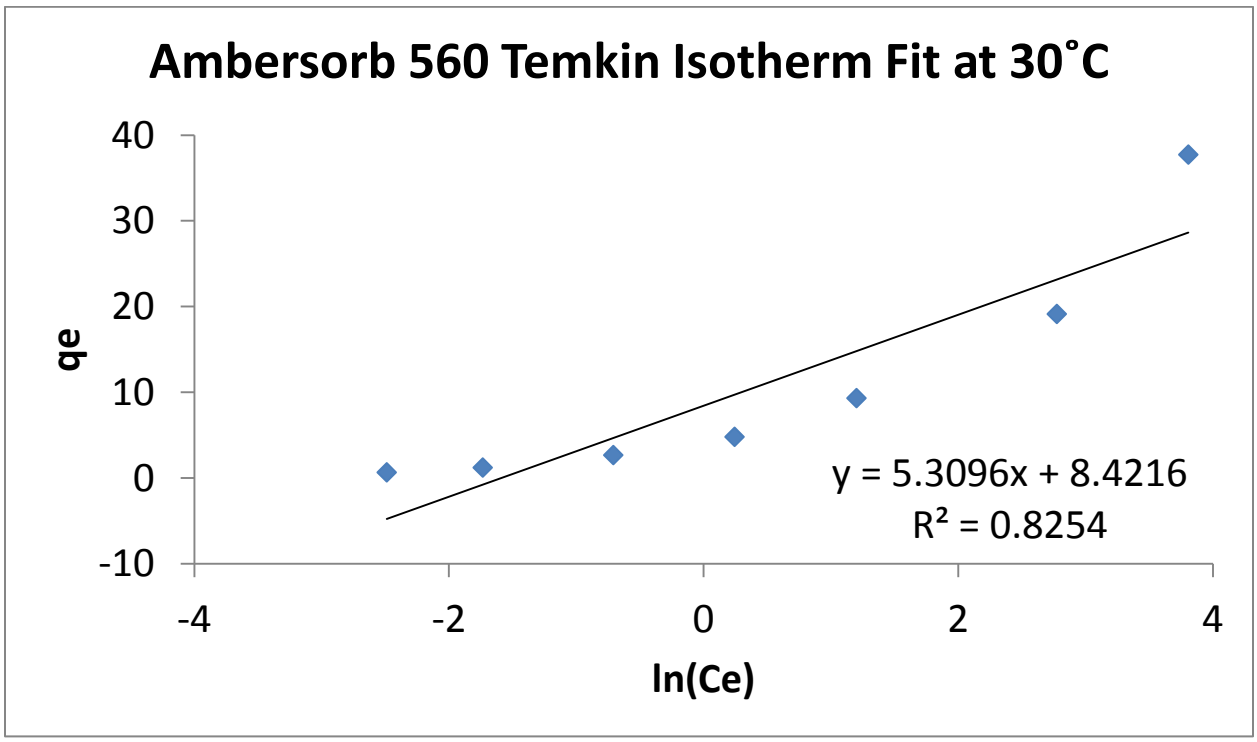
$$q_e = \frac{RT}{b_T}\ln(A_T) + \frac{RT}{b_T}\ln(C_e) \quad (6)$$

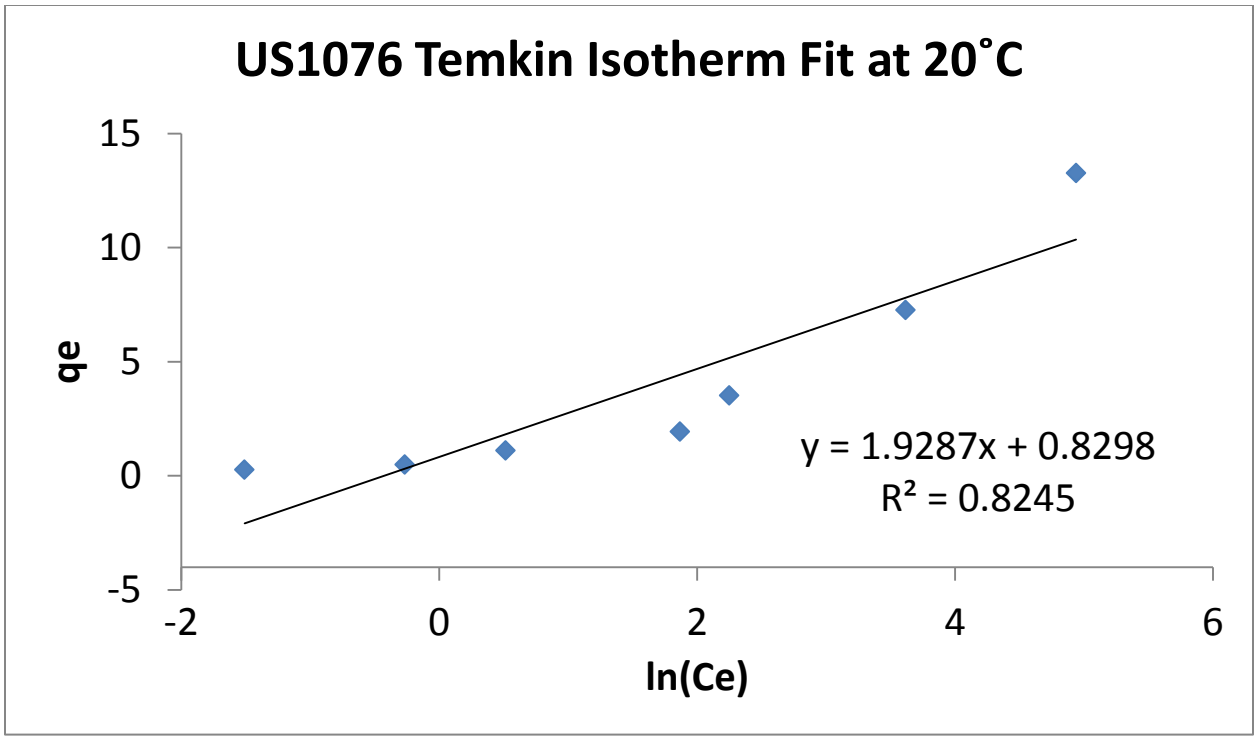
Using equation (6), the data from the adsorption experiments are plotted on a q_e vs $\ln(C_e)$ plot, where $\frac{RT}{b_T}$ is the slope and $\frac{RT}{b_T}\ln(A_T)$ is the y-intercept [72].

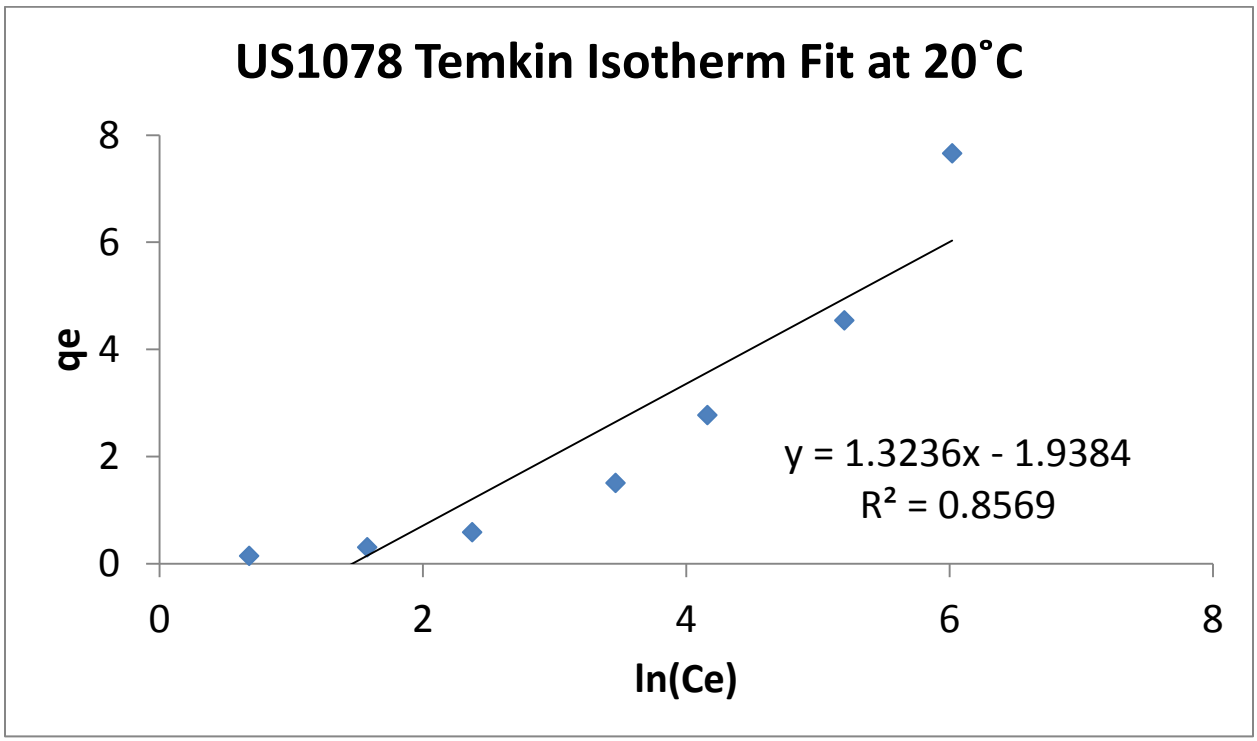

Norit 1240 Temkin Isotherm Fit at 20˚C
qe
ln(Ce)
y = 7.3426x - 14.317
R² = 0.8382
50
40
30
20
10
0
-10
0
2
4
6
8


Norit 1240 Temkin Isotherm Fit at 30˚C
qe
ln(Ce)
y = 4.0703x - 5.3406
R² = 0.8206
25
20
15
10
5
0
-5
0
1
2
3
4
5
6


Ambersorb 560 Temkin Isotherm Fit at 20˚C
qe
ln(Ce)
y = 5.9651x + 1.0547
R² = 0.9546
30
25
20
15
10
5
0
0
1
2
3
4
5


Ambersorb 560 Temkin Isotherm Fit at 30˚C
qe
ln(Ce)
y = 5.3096x + 8.4216
R² = 0.8254
40
30
20
10
0
-10
-4
-2
0
2
4


US1076 Temkin Isotherm Fit at 20˚C
15
10
5
0
-5
qe
-2
0
2
4
6
ln(Ce)
y = 1.9287x + 0.8298
R² = 0.8245


US1078 Temkin Isotherm Fit at 20˚C
8
6
4
2
0
qe
0
2
4
6
8
ln(Ce)
y = 1.3236x - 1.9384
R² = 0.8569

Appendix D. Dubinin-Radushkevich Isotherm Model

$$q_e = (q_s)e^{-K_{ad}\varepsilon^2} \quad (7)$$

where

q_e= amount of adsorbate on the adsorbent at equilibrium(mg/g)

q_s=theoretical maximum adsorbate on the adsorbent with saturated surface sites (mg /g)

K_{ad} = Dubinin–Radushkevich isotherm constant (mol^2/kJ^2)

ε =Dubinin–Radushkevich isotherm constant

$$\mathcal{E} = (RT)\ln\left[1 + \frac{1}{C_e}\right] \quad (8)$$

where

C_e=equilibrium concentration (mg/L)

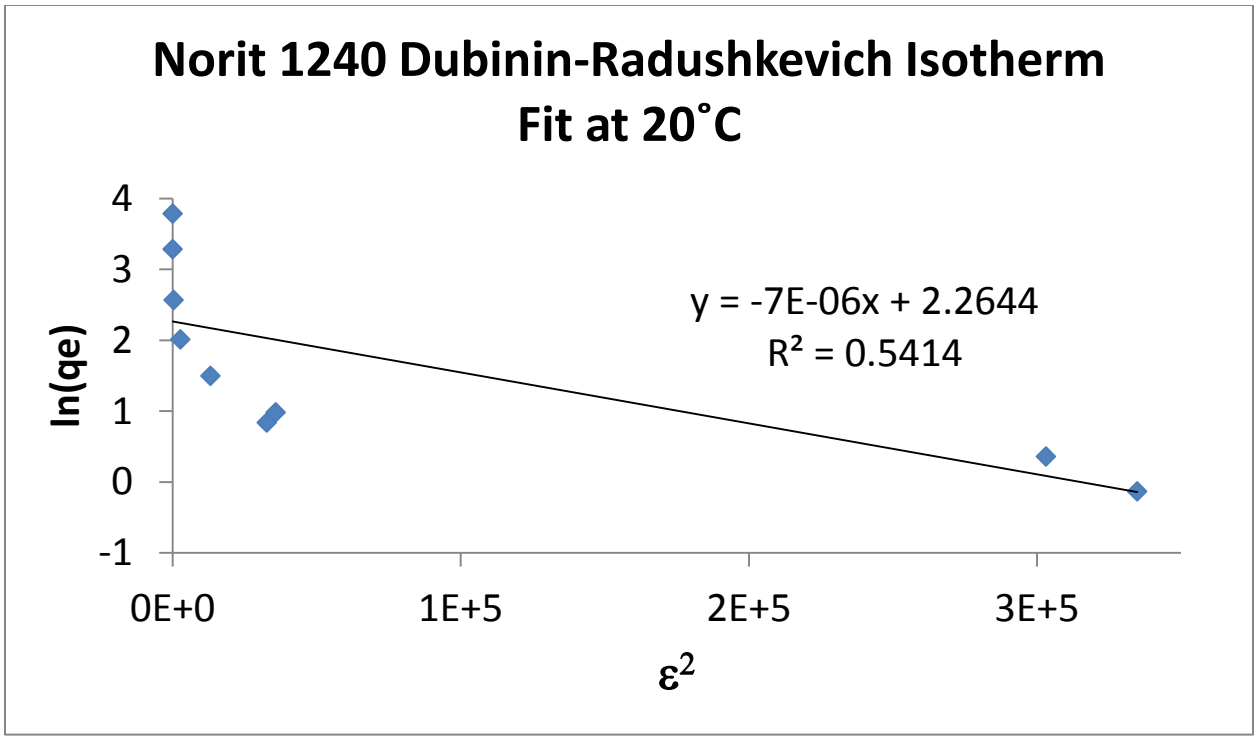
R= universal gas constant (8.314J/mol/K)

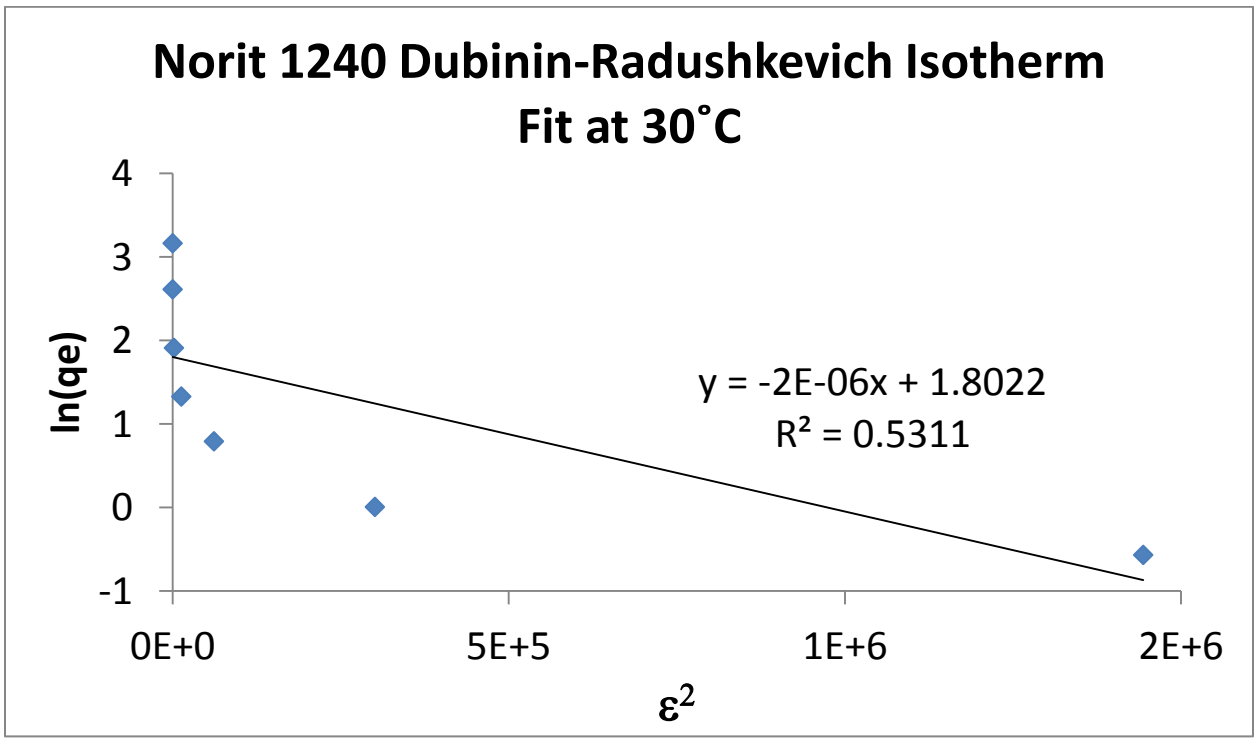
T= Temperature (K)

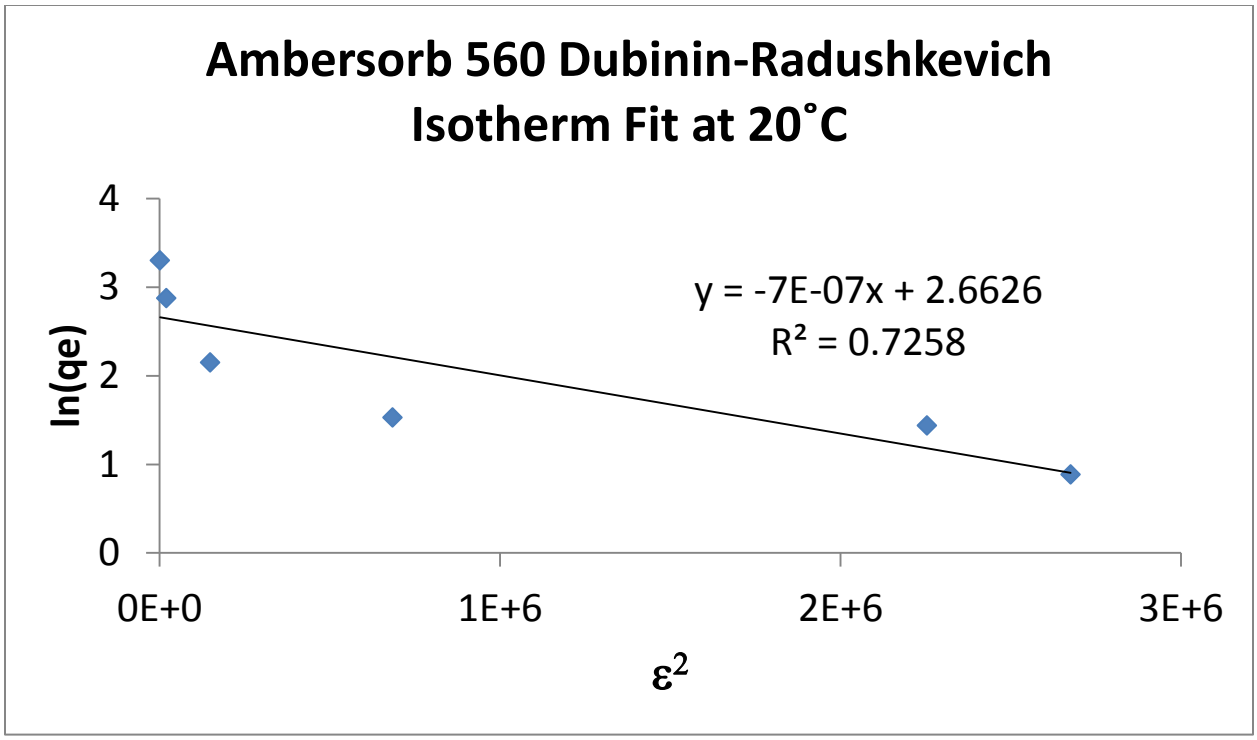
In order to use the Dubinin-Radushkevich Isotherm Model, equation (7) is linearized.

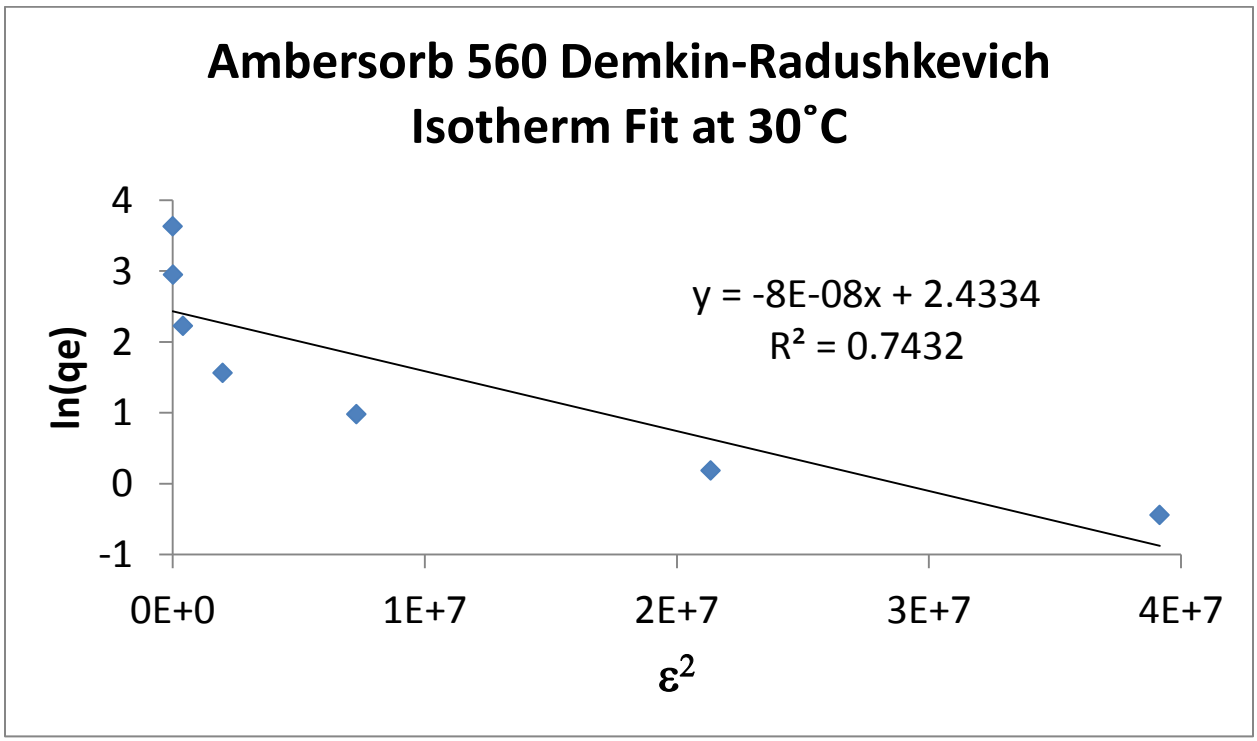
$$\ln(q_e) = \ln(q_s) - K_{ad}\boldsymbol{\varepsilon}^2 \quad (9)$$

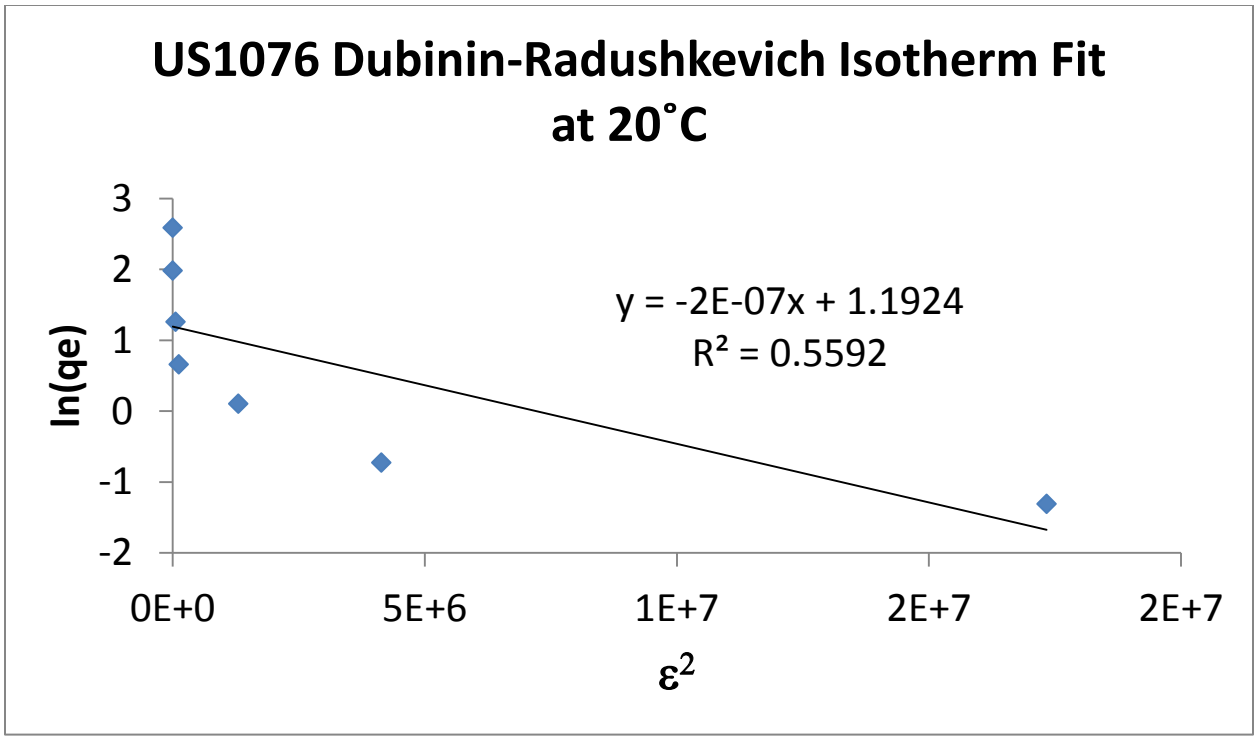
Using equation (9), the data from the adsorption experiments are plotted on a $\ln(q_e)$ vs $\boldsymbol{\varepsilon}^2$ plot, where $-K_{ad}$ is the slope and $\ln(q_s)$ is the y-intercept [72].

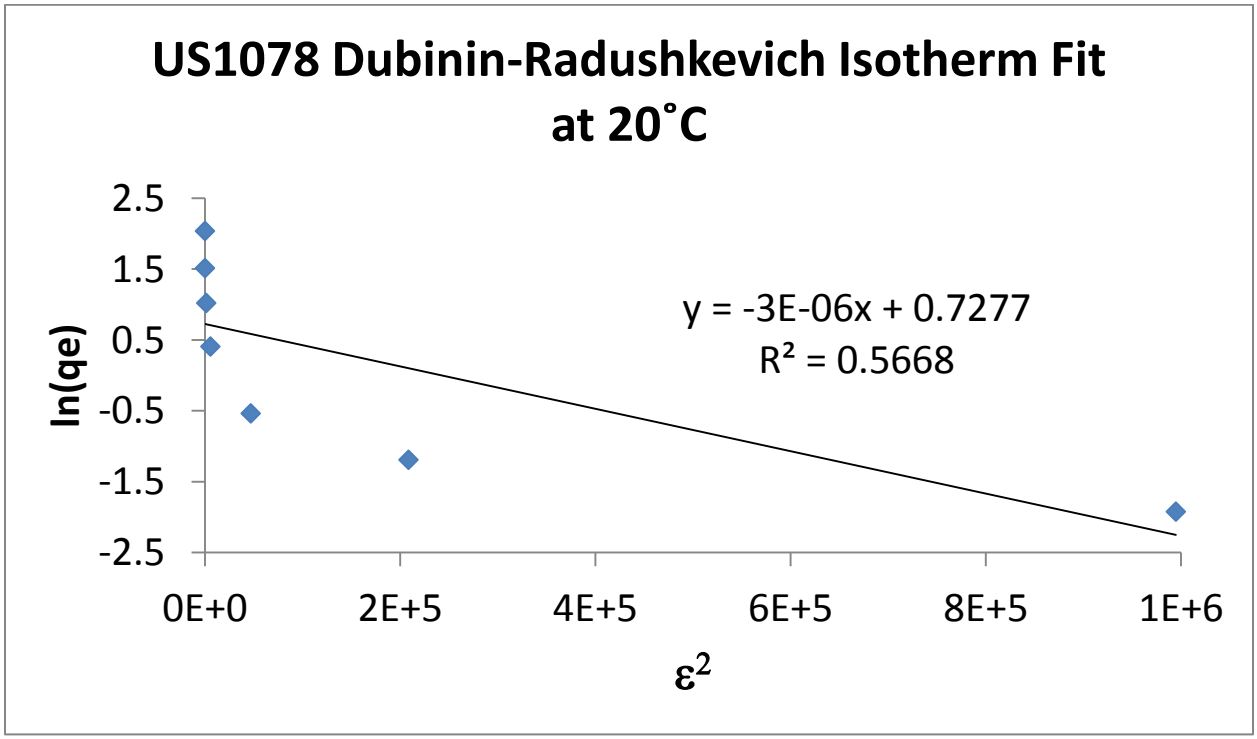

Norit 1240 Dubinin-Radushkevich Isotherm
Fit at 20˚C
y = -7E-06x + 2.2644
R² = 0.5414
ln(qe)
ε²
4
3
2
1
0
-1
0E+0
1E+5
2E+5
3E+5


Norit 1240 Dubinin-Radushkevich Isotherm
Fit at 30˚C
y = -2E-06x + 1.8022
R² = 0.5311
ln(qe)
ε²
4
3
2
1
0
-1
0E+0
5E+5
1E+6
2E+6


Ambersorb 560 Dubinin-Radushkevich
Isotherm Fit at 20˚C
y = -7E-07x + 2.6626
R² = 0.7258
ln(qe)
4
3
2
1
0
0E+0
1E+6
2E+6
3E+6
ε²


Ambersorb 560 Demkin-Radushkevich
Isotherm Fit at 30˚C
y = -8E-08x + 2.4334
R² = 0.7432
ln(qe)
4
3
2
1
0
-1
0E+0
1E+7
2E+7
3E+7
4E+7
ε²


US1076 Dubinin-Radushkevich Isotherm Fit at 20˚C
y = -2E-07x + 1.1924
R² = 0.5592
ln(qe)
3
2
1
0
-1
-2
0E+0
5E+6
1E+7
2E+7
2E+7
ε²


US1078 Dubinin-Radushkevich Isotherm Fit at 20˚C
y = -3E-06x + 0.7277
R² = 0.5668
ln(qe)
2.5
1.5
0.5
-0.5
-1.5
-2.5
0E+0
2E+5
4E+5
6E+5
8E+5
1E+6
ε²

Appendix E. Linear Isotherm Model

$$q_e = K_{lin} C_e \quad (10)$$

where

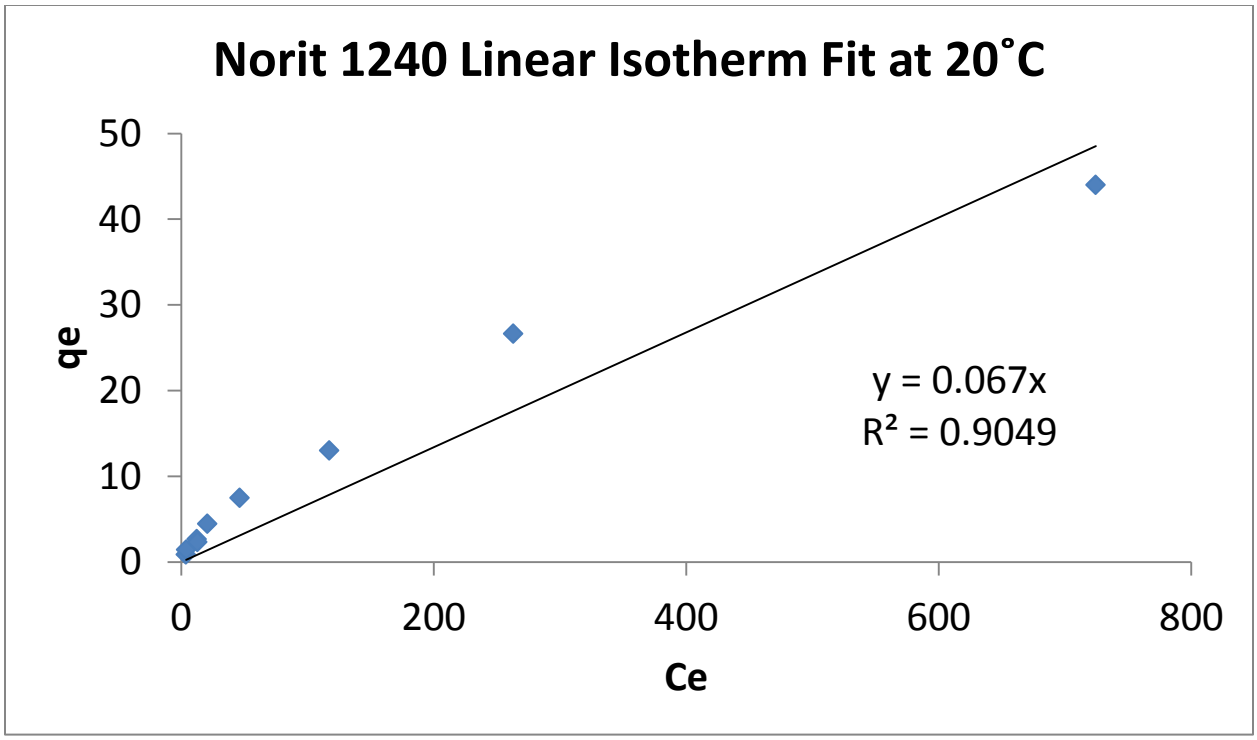
q_e= amount of adsorbate on the adsorbent at equilibrium(mg/g)

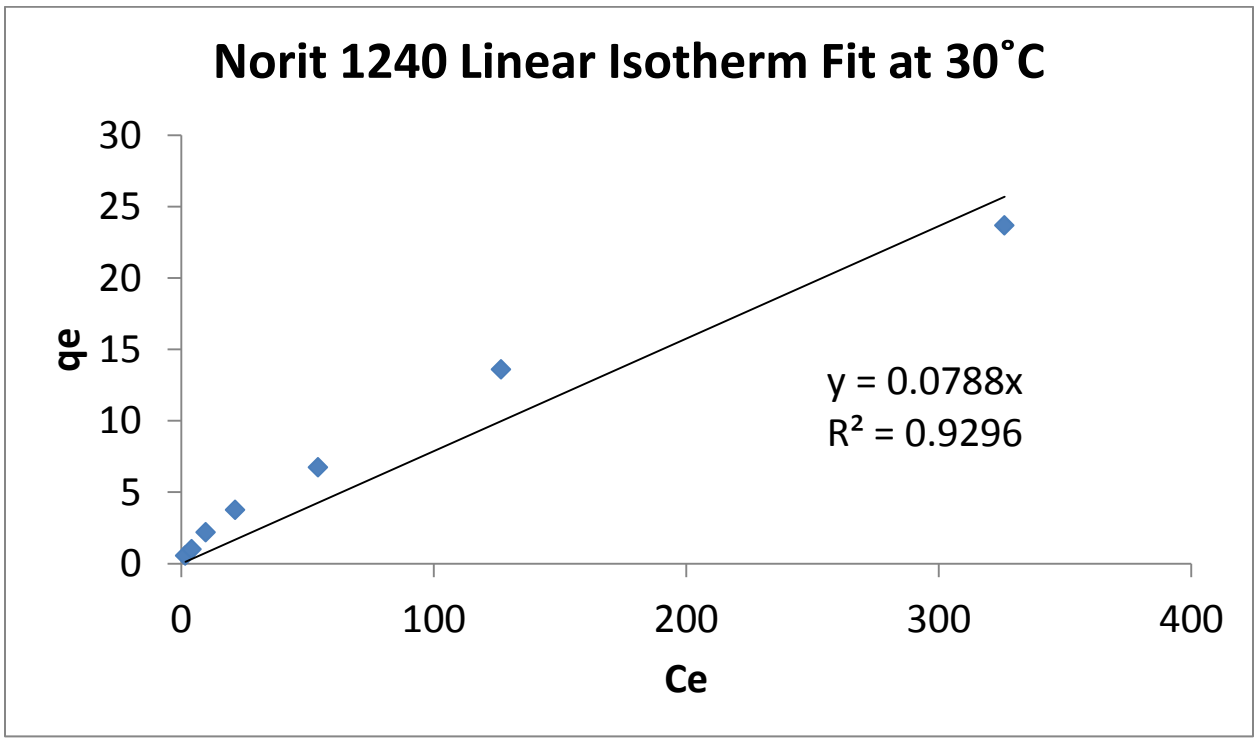
C_e= equilibrium concentration (mg/L)

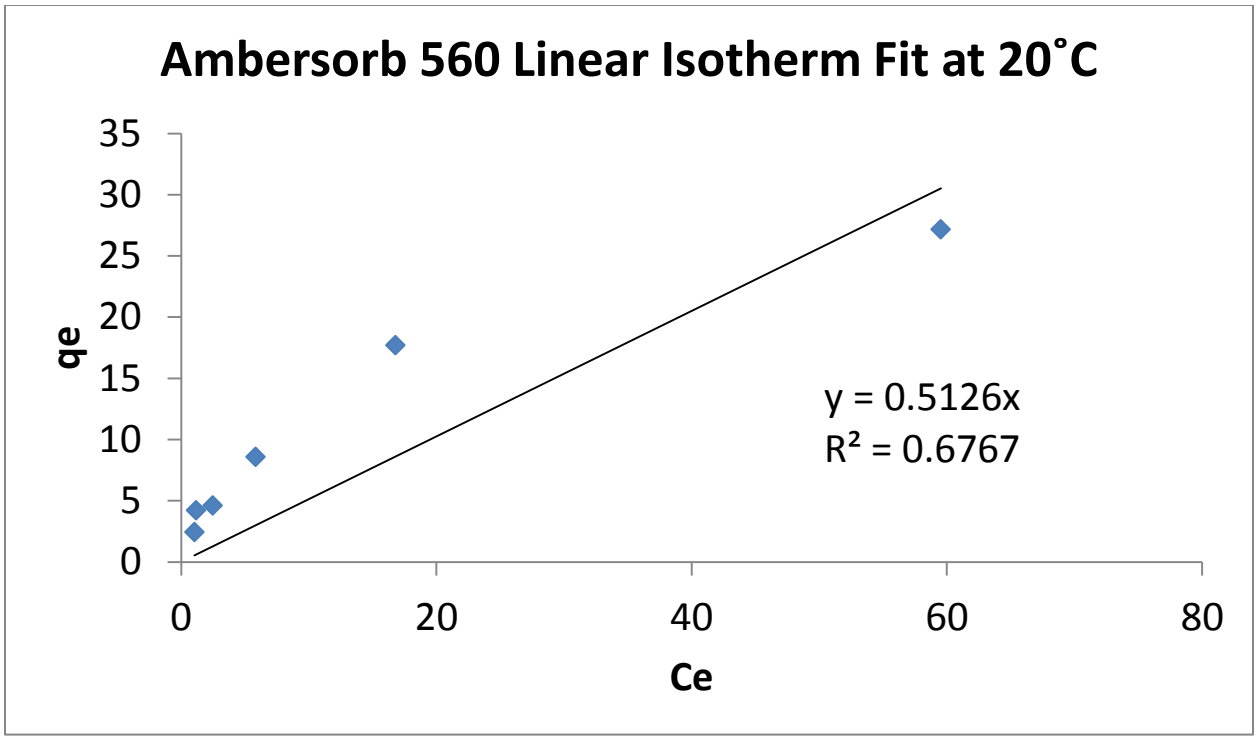
K_{lin}=Langmuir adsorption constant of adsorbate (L/mg)

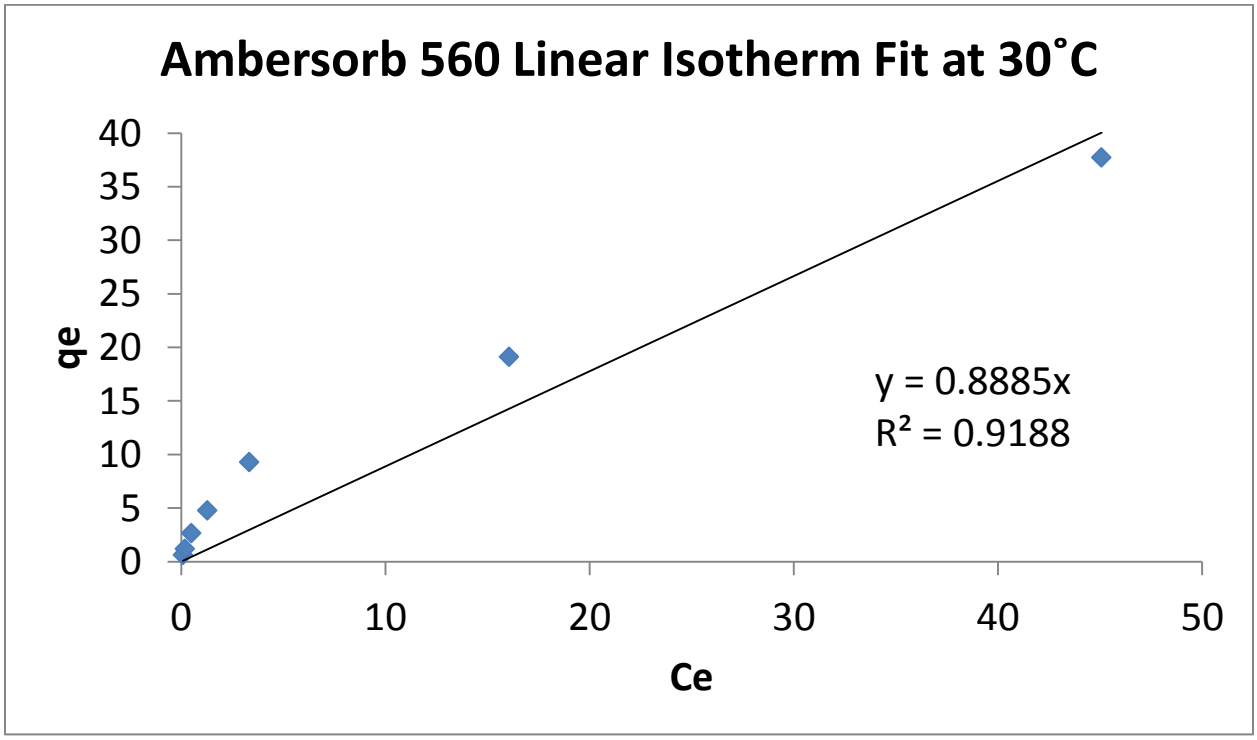
Equation (10) is linear, so no rearrangement is necessary for use.

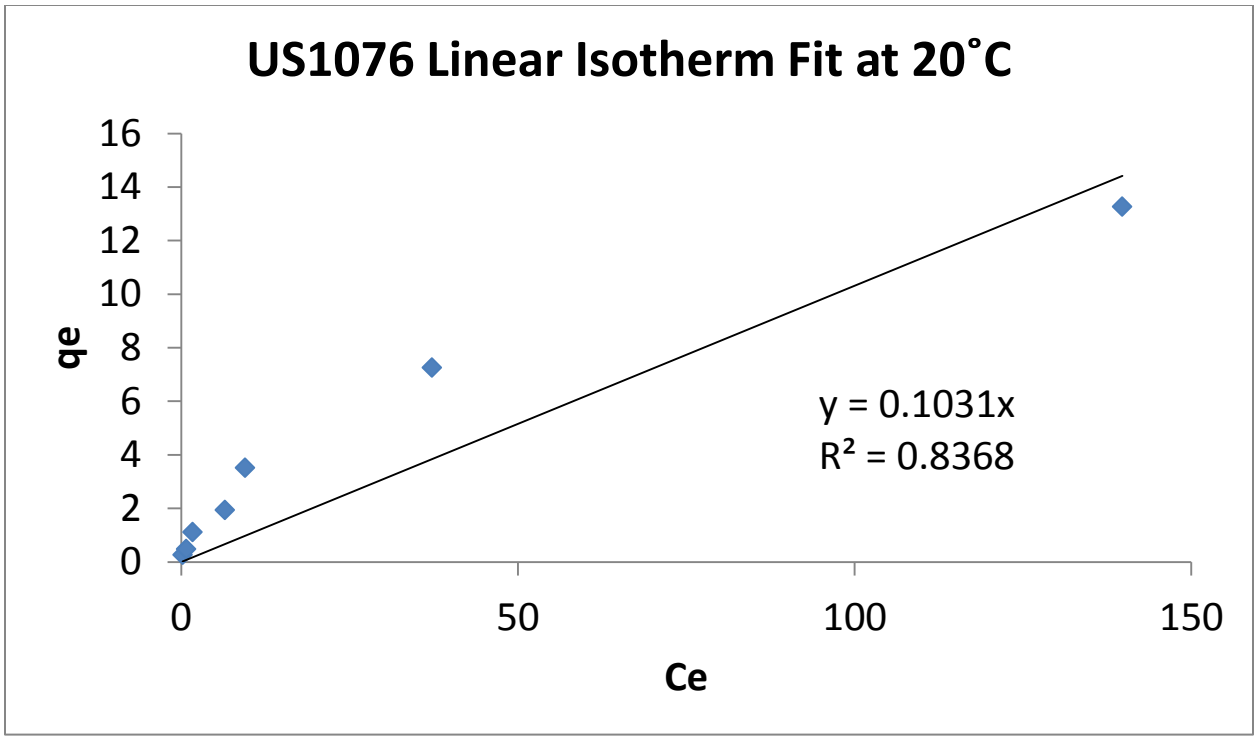
Using equation (10), the data from the adsorption experiments are plotted on a q_e vs C_e plot, where K_{lin} is the slope and the y-intercept is at (0,0) Dada, A.O.O., A.P.; Olatunya, A.M.; Dada, O. [72].

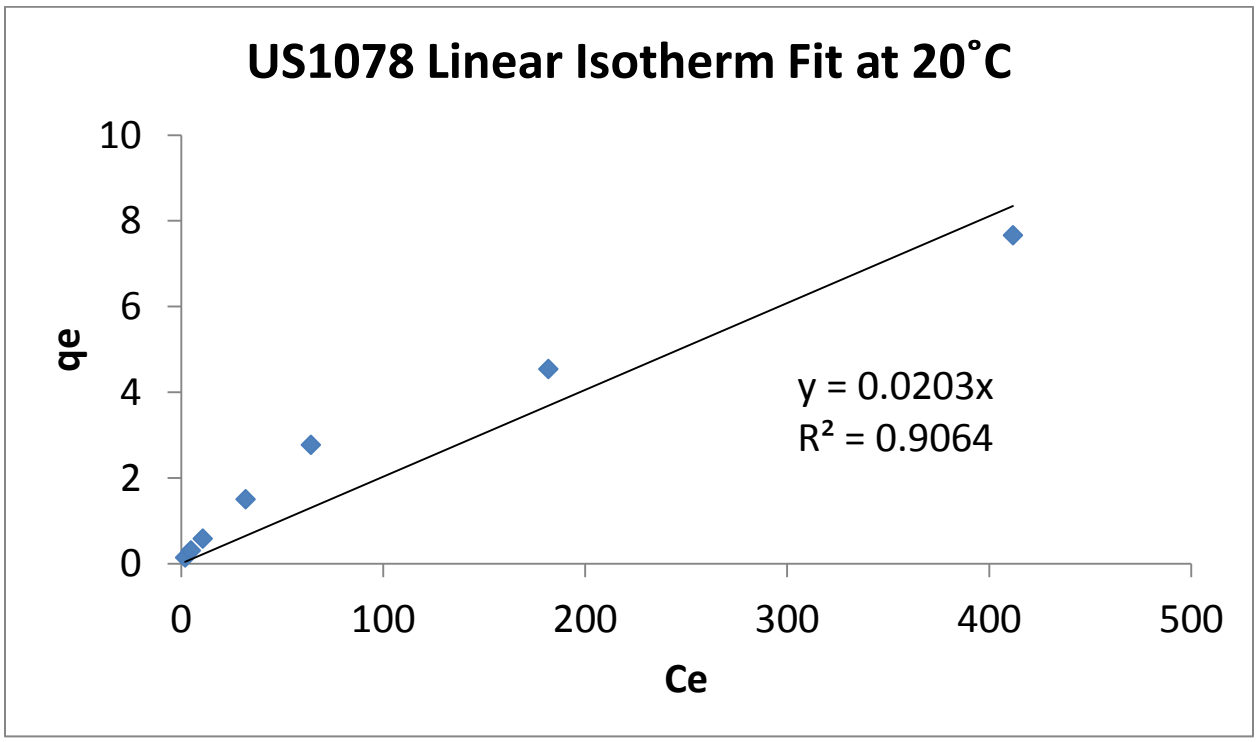

Norit 1240 Linear Isotherm Fit at 20˚C
qe
Ce
y = 0.067x
R² = 0.9049
0
10
20
30
40
50
0
200
400
600
800


Norit 1240 Linear Isotherm Fit at 30˚C
qe
Ce
y = 0.0788x
R² = 0.9296
0
5
10
15
20
25
30
0
100
200
300
400


Ambersorb 560 Linear Isotherm Fit at 20˚C
qe
Ce
y = 0.5126x
R² = 0.6767
0
5
10
15
20
25
30
35
0
20
40
60
80


Ambersorb 560 Linear Isotherm Fit at 30˚C
qe
Ce
y = 0.8885x
R² = 0.9188
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50


US1076 Linear Isotherm Fit at 20˚C
qe
Ce
y = 0.1031x
R² = 0.8368
0
2
4
6
8
10
12
14
16
0
50
100
150


US1078 Linear Isotherm Fit at 20˚C
qe
Ce
y = 0.0203x
R² = 0.9064
0
2
4
6
8
10
0
100
200
300
400
500

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