Adsorption Behaviours of Cobalt onto Saline Soil with/without Biosurfactants

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ABSTRACT

This thesis focuses on the investigation of behaviors of cobalt (Co) adsorption on saline soil with and without the existence of biosurfactants. Three biosurfactants, including surfactin, trehalose lipids and rhamnolipid products, were examined. The kinetics and isotherms of Co adsorption on saline soil were firstly examined. The impact of pH, contact time, initial Co concentration, and type/dose of each biosurfactant on Co adsorption were evaluated. The adsorption kinetic models were explored, and results indicated that the pseudo-second-order kinetic model would fit the experimental data the best. Four isotherms, including Langmuir, Freundlich, Temkin and Redlich-Peterson were used for regulating the Co adsorption with and without the addition of each biosurfactant. The interations among parameters impacting Co adsorption on saline soil were then explored. The experiment was carried out using 2^4 factorial design to examine the main effects and quantify interactions between size of soil particles, biosurfactant concentration, type of biosurfactant and initial Co concentration during Co adsorption. The research findings would facilitate understanding of Co adsorption mechanisms in saline soil and the Co-biosurfactant interactions during adsorption. The results would also help to screen a more appropriate method for conductng effective soil remediation.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
CMC	Critical micelle concentration
Co	Cobalt
DCM	Dichloromethane
DOE	Design of experiment
EC	Electrical conductivity
FAAS	Flame atomic absorption spectroscopy
GLU	Glucose
GLY	Glycerol
ICP-MS	Inductively coupled plasma mass spectrometry
IMSM	Improved mineral salt medium
NRPOP	Northern Region Persistent Organic Pollution Control
MSM	Mineral salt medium
OFAT	One-factor-at-a-time
RMSE	Root mean squared error
SS	Sum of squares

ST	Surface tension
SUC	Sucrose
XRF	X-ray fluorescence

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

Cobalt (Co) occurs naturally in the Earth's crust in carbonate and igneous rocks (Anjum et al., 2015; Coughtrey et al., 1983). It is used in making refractory metals, permanent magnets, and pigments as well as an additive in agriculture. Co can enter the environment from natural sources and human activities, and occurs in different chemical forms. It can be released into the environment from burning coal and oil, incinerators, vehicular exhausts and industrial processes such as mining and operation of Co ores. To form an alloy, Co is blended with other metals. Alloys are resistant and used in industrial activities such as making aircraft, grinding and cutting tools (Altintas, 2012). Ecotoxicity knowledge regarding Co has been established on various organizations using several environmental protection criteria. Co has played a role in the development of diffuse interstitial lung disease, hard metal disease and asthma (Linna et al., 2003). Exposure to Co can cause negative health effects such as asthma and pneumonia (Naqvi et al., 2008). However, Co is an essential trace element, high concentration levels of it would be toxic. Based on cobalt ecotoxicity data, protection criteria were defined for the ecosystems (Adam et al., 2010; Polechońska and Samecka-Cymerman, 2018). In some animal experiments (e.g. mice), the contamination status of cobalt was found to strongly associate with the radical activation and lung injury (Kim et al., 2013). The possibility of Co induced carcinogens in humans has been reported by the International Agency for Research on Cancer (WHO, 2010). Exposure to Co at work place would occur in some industries like paints, varnishes, linoleum and electroplating (Lin et al., 2018). In view of their ecotoxicity and the associated risks in the environment, USEPA (2005) has defined guideline values for Co concentration levels in environment in order to protect various target organisms. Co as a kind of heavy metals has considerable toxicity, and it is also non-degradable with significant bioaccumulation (Sayyad et al., 2010; Yin et al., 2010). However, their behaviors in soils have not been well studied.

Adsorption is the most important chemical process affecting the behaviour of heavy metals in a subsurface, and plays a key role in governing the mobility and fate of Co in soils (Jalali and Majeri, 2016). Soil properties and acidic conditions affect Co adsorption behavior (Substances and Registry, 2004). A pH dependence experiment of Co sorption on soil displayed that Co sorption was strongly affected by the pH of a solution (Chon et al., 2012). The adsorption of Co from an aqueous solution strongly depended on pH so that, as the pH turned to alkaline range (pH equal to 9), the removal efficiency increased (Hashemian et al., 2015). Clay has a high capacity of metal adsorption comprising silt and sand, which makes Co removal from clay quite challenging. Moreover, soil salinity might impact the Co adsorption behavior; however, the impact has not been reported previously.

1.2 COBALT ADSORPTION: KINETICS AND ISOTHERM STUDIES

Adsorption dynamics, especially the kinetic models, have been used to track the associated mechanisms and to determine the adsorption rate of heavy metals in soil (Sheela et al., 2012). Pseudo-first and pseudo-second-order models were used to kinetically study Cu^{2+} adsorption in soil minerals (Komy et al., 2014). The kinetics of divalent copper and Co cations from aqueous solutions on clay samples demonstrated that the second-order model can better express the kinetics of both metal adsorptions (Guerra and Airoldi, 2008). A nano hollow sphere was applied to remove heavy toxic metals (Hg^{2+}, Pb^{2+} and Cd^{2+})

from water samples. The sorption dynamics were established with the Lagergren pseudo-first-order, the pseudo-second-order and Elovich kinetic model (Rostamian et al., 2011). In order to predict adsorption process of Co from aqueous solutions, pseudo-first-order and pseudo-second-order kinetic models were applied to the data (Hashemian et al., 2015). Study of kinetic models on the adsorption rate of Co^{2+} in activated carbon indicated that the system was described by the pseudo-second-order kinetic model (Abbas et al., 2014). Although there were many previous studies regarding heavy metal adsorption kinetics, limited work has targeted Co kinetics in the soil.

To achieve equilibrium data, isotherm studies are important for describing a solid-liquid adsorption system. Isotherms are an equilibrium relation between the adsorbate concentration in the solid phase and liquid phase (Sheela et al., 2012). Hashemian et al. (2015) demonstrated that the equilibrium data for Co adsorption had a high correlation coefficient when fitted with a Langmuir model. Jalali and Majeri (2016) found that the Co sorption could be well fitted with Langmuir and Freundlich models. Chon et al. (2012) disclosed that for sorption isotherm, there is a good fit with the Freundlich equation for the experimental results for Co sorption in soil. Chen and Lu (2008) indicated that the adsorption isotherm of Co on montmorillonite soil was linear at both pH values including 7.3 and 7.7. The experimental data fitted the Freundlich model. Guerra and Airoldi (2008) used a Langmuir isotherm model with a nonlinear approach and yielded good fits with the experimental adsorption data for divalent copper and Co cations/clay interactions.

Soil washing is a cost effective physio-chemical method to decontaminate the soil efficiently and a reliable metal removal alternative (Siddiqui et al., 2015). Because of wide applicability and economic feasibility, soil washing has played a major role and it has been successfully applied in the field. Co extraction can be carried out with different additives, such as acid solutions, reducing and oxidizing agents (Ferraro et al., 2016). During soil washing, solutions of surface-active molecules (i.e., surfactants) have been widely applied as washing agents (Harendra and Vipulanandan, 2013). In recent years, biosurfactants have gradually replaced chemical surfactants in soil washing cases. Biosurfactants are promising due to their low toxicity, possibility of reuse and biodegradability (Cai et al., 2014). In heavy-metal contaminated soils, biosurfactants can form complexes with metals at the soil interface (Singh and Cameotra, 2013). The process is followed by desorption of the metal and removal from the soil surface, leading to the increase of metal ions' concentration and their bioavailability in the soil solution. However, when using it to treat Co in the subsurface, the removal rate could be low. For example, soil washing aided by a biosurfactant (i.e., lipopeptide biosurfactant consisting of surfactin and fengycin) removed just 35.4% of Co from a contaminated soil (Singh and Cameotra, 2013). In addition, a biosurfactant might not apply for soil washing due to its high adsorption capacity in soil. With a high percentage adsorbed onto soil particles, the biosurfactant would bring an extra chemical load to the environment. Till now, there is a lack of literature regarding the impact of biosurfactants on Co adsorption in soil, and nearly no associated kinetics and isotherm studies have been reported.

1.3 COBALT ADSORPTION: INTERACTIONS AMONG PARAMETERS

The understanding of the behavior of heavy metals in the environment facilitates the prediction of their associated risks and the remediation strategy. Adsorption is one of the key processes in the fate and transportation behaviors of heavy metals in the soil (Jalali and Majeri, 2016). During adsorption, the mobility of heavy metal depends on its concentration in the soil, as well as the properties of the metal and soil concerned (Covelo et al., 2007). The research efforts on Co transferability and mobility in the environment have been very limited. Papelis (1996) touched the issue and found that Co was not highly mobile in soil and its migration depended on soil characteristics, for example, the soil with high clay content was associated with little Co mobility. Further research is in need to fill the knowledge gap.

Several parameters, namely soil particle size/surface area and initial heavy metal concentrations play significant roles in the metal sorption. The size of soil particles affects the adsorption of heavy metals on the soil, and the distribution of soil particle size shape the transport of heavy metals (Liu et al., 2017). The larger the specific surface area (SSA), the finer the soil particles, which generally have stronger affinity for heavy metals than the coarse soil particles (Aşçı et al., 2008b; Gong et al., 2014; Huang et al., 2014; Zhang et al., 2013). However, no studies investigated the effects of soil particle size on the adsorption of Co. Initial concentration of the heavy metals also affects their adsorption in soil. The effect of initial Zn (II) concentration on the removal of Zn (II) from soil by rhamnolipid has been studied. When the concentration of Zn (II) in solution was raised, Zn (II) adsorption efficiencies to soil decreased (Aşçı et al., 2008c). Aşçı et al. (2008a) characterized the effect of initial Cd (II) concentration on sorption capacity on the soil. The sorption capacity for Ni(II) and Cu(II) onto kaolin were measured through the variation in initial concentration of the heavy metals (Chen et al., 2008). Aşçı et al. (2008b) loaded the soil with different doses of Cd (II) and used rhamnolipid biosurfactant for the recovery of the heavy metal. The sorbed Cd (II) amount onto the soil increased with increasing initial Cd (II) concentration (Aşçı et al., 2010). The adsorption of heavy metals with different initial concentrations varied between different studies and the type of heavy metals used. However, no studies have worked on the impact of initial concentration on Co adsorption in soil.

Biosurfactants, biologically produced surface-active agents, can potentially be used to influent the sorption of some heavy metals through remediation techniques such as soil washing or to predict removal efficiencies (Mulligan et al., 2001b). Soil washing, or flushing is a popular technology to remediate heavy metals contaminated soil as it increases fate and transport of heavy metals to the liquid phase by desorption (Kim et al., 2013). Biosurfactants have been added during soil washing to facilitate the process (Mulligan, 2009; Shekhar et al., 2015). They also have advantages of low toxicity, biodegradability, possibility of reuse and great surface active properties when compared with chemically synthesized surfactants (Cai et al., 2014). A biosurfactant works as a bridge between the liquid interface and air, so that the surface tension is reduced. Decreasing surface tension of water increases the heavy metals mobilization from soil, and makes the heavy metals more available in the liquid phase (Ron and Rosenberg, 2001), thus easier to be recovered by washing, pumping or flushing (Mulligan, 2005). Aşçı et al. (2007) studied the potential of rhamnolipid to recovery of Cd from kaolin in different concentrations of a biosurfactant (0-100 mM). Cd recovery efficiency increased with increasing rhamnolipid concentration. Wang and Mulligan (2009) found that a 0.1% rhamnolipid solution improves heavy metals removal from mine tailing. After exposing the soil to a 1mM concentration of Cd(II), 73% of Cd(II) adsorbed onto the soil and 55.9% of the Cd(II) was reported to be desorbed by a 80 mM solution of rhamnolipid (Aşçı et al., 2007). Mulligan et al. (2001b) used surfactin, rhamnolipid and sophorolipid to remove Cu (II) and Zn (II) from metal contaminated sediment and found 65%, 25%, 15% of the Cu (II) and 18%, 60%, and 6% of the Zn (II) were removed by rhamnolipid, sophorolipid and surfactin, respectively. Hong et al. (2002) used saponin to effectively enhance recovery of heavy metals from soils, showing 90–100% of Cd removal and 85–98% of Zn removal. Filipkowska and Kuczajowska-Zadrożna (2016a) investigated the adsorption and desorption characteristics of Cd (II), Zn (II) and Cu (II) ions on/from digested sludge. They studied the effect of type and dose of biosurfactant on desorption with saponin and JBR 515 at initial concentrations of 0–250 g/L. Singh and Cameotra (2013) studied the application of lipopeptide biosurfactants including, surfactin and fengycin at different concentration (i.e., 0, 0.5, 1, 10, and 50 CMC) to remove heavy metals (i.e., Fe, Pb, Ni, Cd, Cu, Co and Zn) from the soil. From these studies, it was found that the type and dosage of biosurfactants have significant impacts on the mobility of heavy metals. However, very little information is also available concerning the influence of dose and type of a biosurfactant on the Co adsorption process on the soil. Additionally, as all of these studies investigated the desorption behavior of the accumulated heavy metals generally differ from that of sorption (Aşçı et al., 2010), there is a knowledge gap in the heavy metal adsorption behavior (specially Co) on the soil with the varied type and dosage of biosurfactants.

There may be interactions among size of soil particles, biosurfactant concentration, type of biosurfactant and initial concentration of heavy metals. The existence of interactions would complicate the understanding of the individual effects. For example, the soil with fine particle size (such as clay) have an intricate impact on the heavy metals sorption/desorption in soil when interacted with biosurfactants at varied concentrations (Aşçı et al., 2008c; Frazer, 2000), but this has not been well studied (Frazer, 2000). The existing studies working on the abovementioned 4 factors with heavy metals sorption in soil have been focused on individual effects. No study ever considered them and their interactions in a systematic manner using mathematically and statistically supported approach, instead one-factor-at-a-time approach (OFAT) has been designed to analysis effects. OFAT is incompetent when considering interactions, which can be significant in this scenario (Anderson and Whitcomb, 2016). To explore the interactions between operating factors, the method of statistical design for experimental (DOE) work (e.g., response surface methodology) have been used for modeling and optimization (Khuri and Mukhopadhyay, 2010). It has been widely known in industry to modify performance of products and procedure sufficiency. This mathematical and statistical approach save time to run experiment when compared with OFAT (Heiderscheidt et al., 2015). As one type of response surface methodology, factorial design is a well-established approach to study interactions between factors with less runs (Anderson and Whitcomb, 2016). In such statistical design experiments, adequent data can be derived with a minimum number of experiment runs, because the factors are simultaneously changed at their levels (Rocak et al., 2002). In the full factorial experiments, not only the effects of more than one factor on results are investigated but also their relative significance in the process are obtained and that the interactional impacts of two or more factors can also be generated (Regti et al., 2017). No study thus far has attempted to use DOE methodology to investigate how these four factors, including the size of soil particles, biosurfactant concentration, the type of biosurfactant and initial Co concentration, affect cobalt adsorption in soil.

Moreover, the sorption can be affected by various environmental conditions such as soil salinity (Acosta et al., 2011; Aşçı et al., 2008b). It was found that increasing soil salinity lead to higher heavy mental mobility, and the extent of increased mobility varied with the type of heavy metals (Acosta et al., 2011; Hatje et al., 2003). However, no studies have investigated the effects of soil salinity on Co adsorption in the soil so far, despite the prevalence of saline or sodic soils with shallow saline or sodic groundwater levels (Rengasamy, 2006).

1.4 OBJECTIVE OF THE THESIS

To fill the knowledge gap as described above, this thesis focuses on the investigation of behaviors of Co adsorption on saline soil with and without the existence of biosurfactants. Three biosurfactants, including surfactin, trehalose lipids and rhamnolipid products, were examined. It entails two research tasks.

The first task is to examine the kinetics and isotherms of Co adsorption on saline soil. The impact of pH, contact time, initial Co concentration, and type/dose of biosurfactant on Co adsorption were evaluated. The research results will help to better understand Co adsorption mechanisms in saline soil and Co-biosurfactant interaction during adsorption.

The second task is to explore the interactions among parameters impacting Co adsorption on saline soil. The experiment is carried out using 2^4 factorial design to examine the main effects and quantify interactions between size of soil particles, biosurfactant concentration, type of biosurfactant and initial Co concentration during Co adsorption. The obtained results would facilitate the understanding of Co transport/fate in soil and their potential associated risks. Moreover, by understanding the environmental behavior of Co in the soil, the more appropriate methods for soil remediation can be determined.

1.5 STRUCTURE OF THE THESIS

This thesis consists of four chapters. Chapter 1 includes the background introduction and literature review so as to identify the resaeerch gaps, and the thesis objectives. Chapter 2 is a study regarding Co adsorption onto saline soil with/without the existence of biosurfactants using the one-factor-at-a-time approach, and a study regarding the associated adsorption kinetics and isotherms. Chapter 3 investigates interactive parameters on the adsorption behaviors of Co onto saline soil with different biosurfactants using the full factorial design. Finally, the conclusions are drawn up with the research achievements and the recommendations for future work in Chapter 4.

CHAPTER 2 ADSORPTION BEHAVIOUR OF COBALT ONTO SALINE SOIL WITH/WITHOUT A BIOSURFACTANT: KINETIC AND ISOTHERM STUDIES

This chapter is based on and expanded from the following paper:

Narimannejad, S., Zhang, B., Lye, L. (2018) Adsorption Behaviour of Cobalt onto Saline Soil with/without a Biosurfactant: Kinetic and Isotherm Studies (Submitted to *Water, Air and Soil Pollution journal*).

Roles: Narimannejad designed and conducted the study under the guidance of Dr. Baiyu Zhang and Dr. Leonard Lye and acted as the first author of the manuscript. Most contents of this paper were written by Narimannejad and further reviewed and refined by the other co-authors.

2.1 BACKGROUND

Co can enter the soil from natural sources and human activities. Adsorption behavior of Co in the soil aid the screening of proper soil remediation technologies. Surface-active molecules (i.e., surfactants) can impact the Co adsorption onto the soil. Besides, Adsorption behavior of Co in the soil has been never studied widely. The aim of this study is thus to fill the knowledge gap by studying the impact of pH, contact time, initial Co concentration and type/dose of biosurfactant on adsorption, exploring adsorption kinetics and isotherms.

2.2 MATERIAL AND METHODS

2.2.1 Soil Characterization

Soil including 50% natural clay and 50% sand was applied for conducting each experimental run. The samples were taken using a soil sampling tube, trowel, and a clean pail. Thin slices or borings of soil were obtained from different locations. The soil taken was mixed into one composite sample, then spread on paper in a warm room to air dry overnight. One cup of representative sample was taken to conduct the experiment. The soil was kept in an oven at 105° C overnight. The soil was then sieved to pass through a 1.18 mm opening size stainless-steel mesh (No. 16). The 3% saline soil was obtained through adding NaCl. Soil physical properties, including pH (ASTM International, 2016a), soil bulk density (ASTM International, 2016b), and soil water content (ASTM International, 2016c) were characterized using standard methods. The soil cation-exchange capacity was measured using the protocol generated by (Bower et al., 1952). The results are presented in Table 2.1.

Table 2.1 Soil physical properties

Soil properties		**Value**
Particle size distribution (%)	Clay (< 0.002 mm)	50
	Sand (< 1.18 mm)	50
Salt (%)		3
Water content (%)		13.79
Bulk density (g/cm^3)		1.97
pH (soil materials suspended in water)		7.91
pH (soil materials suspended in a 0.01 M calcium chloride solution)		7.68
pH (saline soil (3%) suspended in water)		7.59
pH (saline soil (3%) suspended in a 0.01 M calcium chloride solution)		7.35
CEC (cmol (+) / kg)		135

Major element oxides in the soil were determined by X-ray fluorescence (XRF) spectrometry analysis. Sand and clay in the soil were crushed using a mortar and pestle, then mixed together before testing. For each test, 20 mg of the mixture was used, with results shown in Table 2.2.

Trace element concentrations in the soil were obtained using inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer Elan DRC II ICP-MS), with results presented in Table 2.3. Before analysis, sample preparation was conducted through a full digest of each sand/clay mixture. The findings show that there is no Co element in the soil used in the experiment.

2.2.2 Biosurfactant Production

2.2.2.1 Biosurfactant-Producing Microorganisms

Three different lab-generated biosurfactants were applied. The impact of each biosurfactant on Co adsorption in soil was evaluated. Two types of the biosurfactants were generated in the Northern Region Persistent Organic Pollution Control (NRPOP) Laboratory at Memorial University of Newfoundland. The first one was produced by *Bacillus subtilis* N3-1P isolated from oily seawater (Cai et al., 2014). The product, Surfactin, is a lipopeptide biosurfactant which can reduce water surface tension significantly (Zhu et al., 2016).The hyper-production strain, Rhodococcus erythropolis Mutant M36, was obtained through UV mutagenesis of a wild strain isolated from produced water samples from offshore Newfoundland, Canada (Cai et al., 2016) and used to generate the second biosurfactant product containing trehalose lipids. The third biosurfactant applied in this study was crude rhamnolipid produced by a research partner's lab.

Table 2.2 Soil major element oxides

Element oxides	Na_2O Wt%	MgO Wt%	Al_2O_3 Wt%	SiO_2 Wt%	P_2O_5 Wt%	K_2O Wt%	CaO Wt%
Clay / Sand Mixture	1.42	2.52	12.74	47.98	0.1	2.99	3.04

Table 2.3 Soil trace element concentrations

Element	Value (mg/l)	Element	Value (mg/l)	Element	Value (mg/l)	Element	Value (mg/l)
Li	9.22	Cs	1.13	Nd	28.6	Dy	6.35
Rb	48.4	Ba	621	Sm	6.53	Ho	1.32
Sr	351	La	22.94	Eu	1.93	Er	3.76
Y	35.1	Ce	51.0	Gd	7.01	Tm	0.542
Zr	194	Pr	6.84	Tb	1.03	Yb	3.43
Lu	0.520	Tl	0.244	Pb	9.70	Pb	9.70

2.2.2.2 Biosurfactant Production by B. Subtilis N3-1P

The recipe for preparing the agar plate to grow B. subtilis N3-1P was as follows: Tryptic Soy Broth 30.0 g, NaCl 15 g and Agar Bacteriological 15 g in 1 L of distilled water. BD Difco™ Nutrient Broth 23400 (Fisher Scientific Company, Ottawa, Canada) 8.0 g and NaCl 5.0 g in 1 L of distilled water is used as the recipe for a inoculum broth (Zhu et al., 2016).

A loopful of bacteria was transferred from the agar plate to a 50-mL inoculum broth in a 125-mL Erlenmeyer flask. At first the culture in the flask was grown on a rotary incubator shaker (Thermo MaxQ 4000) at 200 rpm for 48 h at room temperature. The culture was applied as inoculum at the 1% (v/v) level. This means that 1 cc of the culture was used for biosurfactant production in the 100 cc mineral salt medium.

An improved mineral salt medium (MSM) for *B. subtilis* N3-1P fermentation (Zhu et al., 2016) was listed as follows (g / L): glycerol (10), nitrogen source $(NH_4)_2SO_4$ (10), NaCl (15), $FeSO_4$ $7H_2O$ (2.8×10^{-4}), KH_2PO_4 (3.4), K_2HPO_4 $3H_2O$ (4.4), $MgSO_4$ $7H_2O$ (1.02), yeast extract (0.5) and trace element solution 0.5 mL L^{-1} of distilled water. The composition of the trace element solution was as follows: $ZnSO_4$ (0.29), $CaCl_2$ (0.24), $CuSO_4$ (0.25), and $MnSO_4$ (0.17) g per 1 L of distilled water. It should be noted that the trace element solution was sterilized separately. In this growth media, the carbon source in the original recipe, glycerol (GLY), was changed by glucose (GLU) and sucrose (SUC) at a concentration of 15 g/L each. Also, Nitrogen sources ($(NH_4)_2SO_4$)) were replaced by NH_4NO_3 at a concentration of 10 g/L. The original concentration of $FeSO_4$ $7H_2O$ was changed to 0.001 g/L.

Fermentation of *B. subtilis* N3-1P took place in a sterilized fermenter containing 2 L MSM and inoculum at the 1% (v/v) level. The medium was incubated in the fermenter at 400 rpm for 7 days at 30˚C. To remove all the cells, the culture broth was centrifuged at 6,000 rpm for 20 min. Then the pH of the biosurfactant solution was adjusted to 2 using concentrated hydrochloric acid. The solution was kept in a cooler overnight so biosurfactant precipitated in the container. The biosurfactant was separated in the centrifuge at 4,000 rpm for 15 min. The produced biosurfactant was washed with dichloromethane (DCM) for further purification and left in the hood overnight. The powdered biosurfactant product was transferred to the freezer to prevent degradation.

2.2.2.3 Biosurfactant Production by Rhodococcus Erythropolis Mutant M36

To prepare agar plates to grow *Rhodococcus erythropolis* Mutant M36, BD Difco™ Nutrient Broth 23400 (Fisher Scientific Company, Ottawa, Canada) 8 g, NaCl 20 g and agar bacteriological 15 g were dissolved in 1 L of distilled water. The composition of the medium used for fermentation of the mutant was as follows (g/L): glycerol (1), NaCl (10), $MgCl_2$ (0.1), $CaCl_2$ (0.1), $FeCl_3$ (0.01), NH_4NO_3 (1), KH_2PO_4 (0.41), K_2HPO_4 (8.2) and Diesel 100 mL L^{-1} of distilled water.

Before the fermentation, the medium and the fermenter were both sterilized by autoclaving. The strains from the prepared agar plate were transferred into the medium, which was incubated in the magnetic shaker for 5-7 days. During the first 2 days, the temperature of the shaker was adjusted to 30˚C. Afterwards, room temperature was used.

After the fermentation, the culture broth was transferred to a separation beaker and the water phase was discarded. To remove diesel, an equal volume of petroleum ether was added, and the mixture was centrifuged at 4,000 rpm for 10 min. Then, the upper layer was discarded using a pipette. The diesel removal treatment was repeated 3 times to obtain a diesel-free product. The mixture containing biosurfactant was further purified through organic solvent extraction with a fivefold volume of methanol-chloroform (1:2 *v/v*) solvent. The solution was sonicated with an intensity level of 30% for 20 min. To remove the cells, the solution was centrifuged at 6,000 rpm for 20 min. The crude biosurfactant was concentrated by rotary evaporation and the final jelly product was frozen at 0˚C.

2.2.3 Co Adsorption onto Soil without Biosurfactant Addition

Co adsorption onto saline soil under various pH, initial Co concentrations and durations was conducted. Table 2.4 lists the number of runs when one factor at a time was examined.

2.2.3.1 Effect of pH on Co Adsorption

The soil was air dried, homogenized, and kept in an oven at 105˚C for 1 day. Each 2.9 g sample of 3% saline soil was equilibrated in a polypropylene centrifuge tube with 50 ml of solution including a 300 mg/L concentration of Co.

Table 2.4 Experimental runs and associated conditions

Effect of factor	Number of levels	Replication	Number of blanks	Replication	Total number of runs
pH	8	3	1	3	27
Time	12	3	12	3	72
Initial Co concentration	6	3	1	3	21

Samples before adsorption experiments were adjusted to a pH range of 3 to 9 (including pH 3, 4, 5, 6, 7, 7.5, 8, 9) by 1 M HCI or 1 M NaOH solution. During each adsorption run, the pH measurement was carried out by using a meter from METTLER TOLEDO Co. The pH level of each run was kept consistent by adding small volumes of 0.1 M HCI or 0.1 M NaOH solution. Blank runs were prepared without the adjustment of soil pH levels. Distilled water was applied instead of the Co solution during each blank run. Co adsorption was carried out by shaking each tube in a shaker at 200 rpm and at the room temperature for 48 hours. After reaching the ultimate equilibrium status, the tube was centrifuged at 12,000 rpm for 20 min. The supernatant was acidified to pH 2 using 2% HNO_3 for flame atomic absorption spectroscopy (FAAS) (Varian, version 1.133) measurement and the Co concentration remaining in the solution after each adsorption treatment was determined. A calibration curve for Co was constructed using standard solutions. Differences between each initial Co concentration in the solution and the remaining Co concentration in the supernatant after sorption indicated amount of Co that was adsorbed by the soil.

2.2.3.2 Effect of Adsorption Time

Adsorption was also carried out with 2.9 g saline soil and 50 mL of Co solution with the initial Co concentration of 300 mg/L. The effect of reaction time on Co adsorption was investigated at room temperature and the pH level selected based on a previous pH examination experiment.

The equilibration status of each run was investigated when shaking the tube at 200 rpm for various (i.e., 0, 10, 60, 120,180, 240, 300, 360, 420, 480, 540 and 600 min) times. Triplicate runs were conducted for each experimental setting. After adsorption, each tube was centrifuged at 12000 rpm for 20 min and the Co concentration remaining in the solution was measured using FAAS. In each blank run, no pH adjustment was conducted, and no Co was added to the soil.

2.2.3.3 Effect of Initial Co Concentration

Various concentrations of Co solution (i.e., 50 ppm, 100 ppm, 200 ppm, 250 ppm, 300 ppm and 400 ppm) were applied to investigate the consequences of initial Co concentration on adsorption. Each Co adsorption run was carried out with 2.9 g saline soil, at room temperature and the pH level selected based on previous pH examination experiments. During each run, the tube was shaken at 200 rpm to allow efficient mixing of the Co throughout the solution. After adsorption, each tube was centrifuged, and aliquots of the supernatant solutions were taken for FAAS analysis. In each blank run, no pH adjustment was conducted, and no Co was added to the soil. Triplicate runs were conducted for each experimental setting.

2.2.4 Co Adsorption onto Soil with Biosurfactant Addition

Solutions with different Co concentrations (i.e., 50 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm) were prepared. Three different types of biosurfactant products were applied at two concentrations (1 and 2 CMC), respectively. Co adsorption onto soil with added biosurfactant was examined using runs, indicated in Table 2.5.

Table 2.5 Number of runs for Co adsorption with biosurfactant

Biosurfactant	Concentration	Number of levels	Replication	Total number of runs
Surfactin	1 CMC	5	3	15
Surfactin	2 CMC	5	3	15
Trehalose lipids	1 CMC	5	3	15
Trehalose lipids	2 CMC	5	3	15
Rhamnolipid	1 CMC	5	3	15
Rhamnolipid	2 CMC	5	3	15

In each experimental run, 2.9 g of saline soil was placed into a 50 mL centrifuge tube. The Co solution was added to the tube followed by the addition of a biosurfactant product. The pH of the suspensions was adjusted to the desired level based on previous a pH examination experiment. Each tube was shaken in a shaker at 200 revolutions per minute at room temperature. The adsorption duration was selected based on previous examination, as stated in section 2.3. Once reaching equilibration status, each tube was centrifuged, and the supernatant solutions were engaged for FAAS analysis to obtain the Co concentration in the solution after adsorption. Surface tension analysis was applied to define the biosurfactant concentration in the supernatant solution. Both Co and biosurfactant adsorption in the soil were examined. In each blank run, pH adjustment was conducted and no biosurfactant was added to the soil. Triplicate runs were conducted for each experimental setting.

2.2.5 Biosurfactant Adsorption onto Soil without Existence of Co

To measure biosurfactant adsorption, 2.9 g of saline soil was placed into 50 mL centrifuge tubes. The soil was suspended in 50 mL of varying concentrations (1 and 2CMC) of 3 different types of biosurfactants (Table 2.6). Blank solutions contained the soil solution without the addition of any biosurfactant.

Table 2.6 Number of runs for biosurfactant adsorption without Co

Biosurfactant	Concentration	Replication	Total number of runs
Surfactin	1 CMC	3	3
Surfactin	2 CMC	3	3
Trehalose lipids	1 CMC	3	3
Trehalose lipids	2 CMC	3	3
Rhamnolipid	1 CMC	3	3
Rhamnolipid	2 CMC	3	3

The contact time and pH levels were selected based on the results of section 2.3. Each tube was incubated in a shaker at 200 rpm at room temperature. After reaching the ultimate equilibrium, the tube was centrifuged at 12,000 rpm for 20 min. Biosurfactant concentration in the supernatant was measured using surface tension analysis. The Du Nouy ring method of computing surface tension was applied to measure surface tension.

2.2.6 Kinetic Studies of Co Adsorption to Soil without Existence of Biosurfactants

The capacity of sorption q_t (mg g^{-1}) of soil was computed using (Najafi et al., 2011):

$$q_t = ((C_0 - C_t)/ w) . V \qquad (2.1)$$

where C_0 is the initial Co concentration (mg L^{-1}), C_t is the residual metal concentration (mg L^{-1}) at time t, V is the volume of Co solution (L), and W is the amount of soil (g). To present the Co sorption kinetics in the soil, pseudo-first-order, pseudo-second-order, Elovich and intra-particle diffusion kinetic models were examined. The linearized form of the pseudo-first-order equation (Lagergren) was stated as follows (Arabloo et al., 2016):

$$\ln (q_e - q_t) = \ln (q_e) - k_1 t \qquad (2.2)$$

where q_e and q_t are the adsorption capacity at equilibrium and at any time t, respectively (mg_{Co} g_{soil}^{-1}), and the value of k_1 (min^{-1}) is the rate constant of pseudo-first-order adsorption. The differential formula for the pseudo-second-order rate model was expressed as (Smičiklas et al., 2008):

$$t/q_t = (1/k_2 q_e^2) + (1/q_e)\, t \qquad (2.3)$$

where the value of k_2 (g $mg^{-1}min^{-1}$) is the overall rate constant of the pseudo-second-order adsorption rate constant. The simplified form of the Elovich equation was presented as follows (Pérez-Marín et al., 2007):

$$q_t = 1/ß \ln(\alpha ß) + 1/ß \ln(t) \quad (2.4)$$

where α is the initial adsorption rate constant (mg/g min) and ß is a measure of adsorption activation energy (g/mg). The possibility of intra-particle diffusion was expressed as (Sheela et al., 2012):

$$q_t = k_{id} t^{0.5} + I \quad (2.5)$$

where k_{id} is the intraparticle diffusion rate constant (mg/g $min^{0.5}$) and I is the thickness of film.

2.2.7 Isotherm Examination of Co Adsorption to Soil with and without Existence of Biosurfactants

To determine the adsorption isotherms, the graph relating equilibrium Co concentration and mass of Co adsorbed on per unit mass of the soil interface at a fixed temperature and pH was employed. The quantity of metal sorbed was calculated as the difference between the initial (C_0; mg/L) and final or equilibrium solution concentrations (C_e; mg/L). By using a mass balance equation, the mass of metal adsorbed by the soil (q_e; mg/g_{soil}) was calculated in every test tube as follows:

$$q_e = v/m (C_0 - C_e) \quad (2.6)$$

where v is the volume of the solution used (mL) and m is the dry mass of soil used (mg).

Four various sorption models, including Langmuir, Freundlich, Temkin and Redlich-Peterson, were analyzed for Co isotherm examination. When the extent of coverage of adsorbate was restricted to only one molecular layer, the Langmuir isotherm was applicable (Arabloo et al., 2016). The associated isotherm equation was expressed by Eq. (3.7):

$$q_e = Q_0 K_L C_e / 1 + K_L C_e \tag{2.7}$$

Here and in all isotherms analyzed, q_e (mg/g) is the amount of adsorbed Co per unit weight of the soil and C_e is the equilibrium Co concentration in the solution. K_L (L/mg) is the constant of the adsorption equilibrium and Q_0 (mg/g) is the maximum amount of adsorbed Co per unit weight of the soil. The empirical Freundlich model was generated based on multilayer adsorption on a heterogeneous surface (Arabloo et al., 2016). The model was stated by the following equation:

$$q_e = K_F \, C_e^{1/n_f} \tag{2.8}$$

where K_F is adsorption capacity and n_f is adsorption intensity (heterogeneity factor). The Temkin model, similar to the Freundlich isotherm, was used to present the increased linearly of the solid surface area with decreased adsorption heat (Arabloo et al., 2016). The Temkin model was first developed for investigating a solid/gas system, and then extended its applications to the solid/liquid system. Tong et al. (2011) applied the Temkin isotherm for modeling adsorption of copper ion's to a solid sorbent from an aqueous solution. Kumar et al. (2012) conducted equilibrium studies on biosorption of 2, 4, 6-trichlorophenol from aqueous solutions with Acacia leucocephala bark using the Temkin isotherm. The Temkin isotherm was expressed through the following form:

$$q_e = (RT / b_T) \ln A_T C_e \tag{2.9}$$

where $B = RT / b_T$ and is related to the heat of adsorption. A is the equilibrium binding constant related to the maximum binding energy (Rostamian et al., 2011).

A further empirical model, Redlich and Peterson's, was also adopted. It is a three-parameter adsorption isotherm and a combination of elements from Langmiur and Freundlich equations. This model does not follow ideal monolayer adsorption and the adsorption mechanism is a hybrid (Rostamian et al., 2011). The model was expressed as:

$$q_e = (K_R C_e) / (1 + a_R C_e^g) \tag{2.10}$$

where g, K_R (L/g), a_R (mg^{-1}) are the exponent, Redlich-Peterson isotherm constant and constant, respectively. When $a_R C_e^g$ is bigger than 1, the model befits the Freundlich equation. When $a_R C_e^g$ is smaller than 1, it fits a linear equation that occurs at low concentrations (Aşçı et al., 2007).

2.2.8 Sample Analysis

Surface tension (ST) was measured with a 20 mL solution by the ring method using a Du Nouy Tensiometer (CSC Scientific). The critical micelle concentration (CMC) is the concentration of biosurfactant at which micelle starts to form (Mulligan et al., 2001a). Surface tensions of lab generated biosurfactants as a function of concentration of biosurfactant were plotted. The CMC of each biosurfactant was obtained from the intercept of two straight lines extrapolated from the concentration-dependent and concentration-independent parts (de Oliveira et al., 2013). The CMC of three biosurfactants, including surfactin, trehalose lipids and rhamnolipid products, were 0.09 g/L, 3 ml/L (2.6 g/L) and 7 ml/L (7.05 g/L), respectively.

The sorption model constants were estimated from sorption data of Co on the soil using MATLAB (R2017a version) and Excel (2016) computer programs. Each test, including the blank runs in the study, was conducted three times. Each result was thus quoted using the mean ± standard division of the triplicated data.

2.3 RESULTS AND DISCUSSION

2.3.1 Co Adsorption onto Soil without Biosurfactant Addition

2.3.1.1 Impact of pH on Co Adsorption

Previously studies were conducted to select the appropriate pH of an aqueous solution for achieving the maximum adsorption of metal ions, including Cu, Cd, and Zn, onto the adsorbent (Filipkowska and Kuczajowska-Zadrożna, 2016b; Meitei and Prasad, 2013; Rajaei et al., 2013). In this study, the pH value of Co solutions was varied over the range of 3 to 9 to assess the impact of pH on Co adsorption. Results indicated that Co adsorption by soil increased with increasing pH at a constant initial concentration of Co (300 mg/L) in all solutions. Therefore, the Co concentration in each solution at the equilibrium status decreased with increasing pH (Fig. 2.1). Reduction of the pH value to 3.0 led to Co adsorption by 77 mg/L. A pH 9 was selected in further experiments since it helped to achieve the maximum Co adsorption in the system.

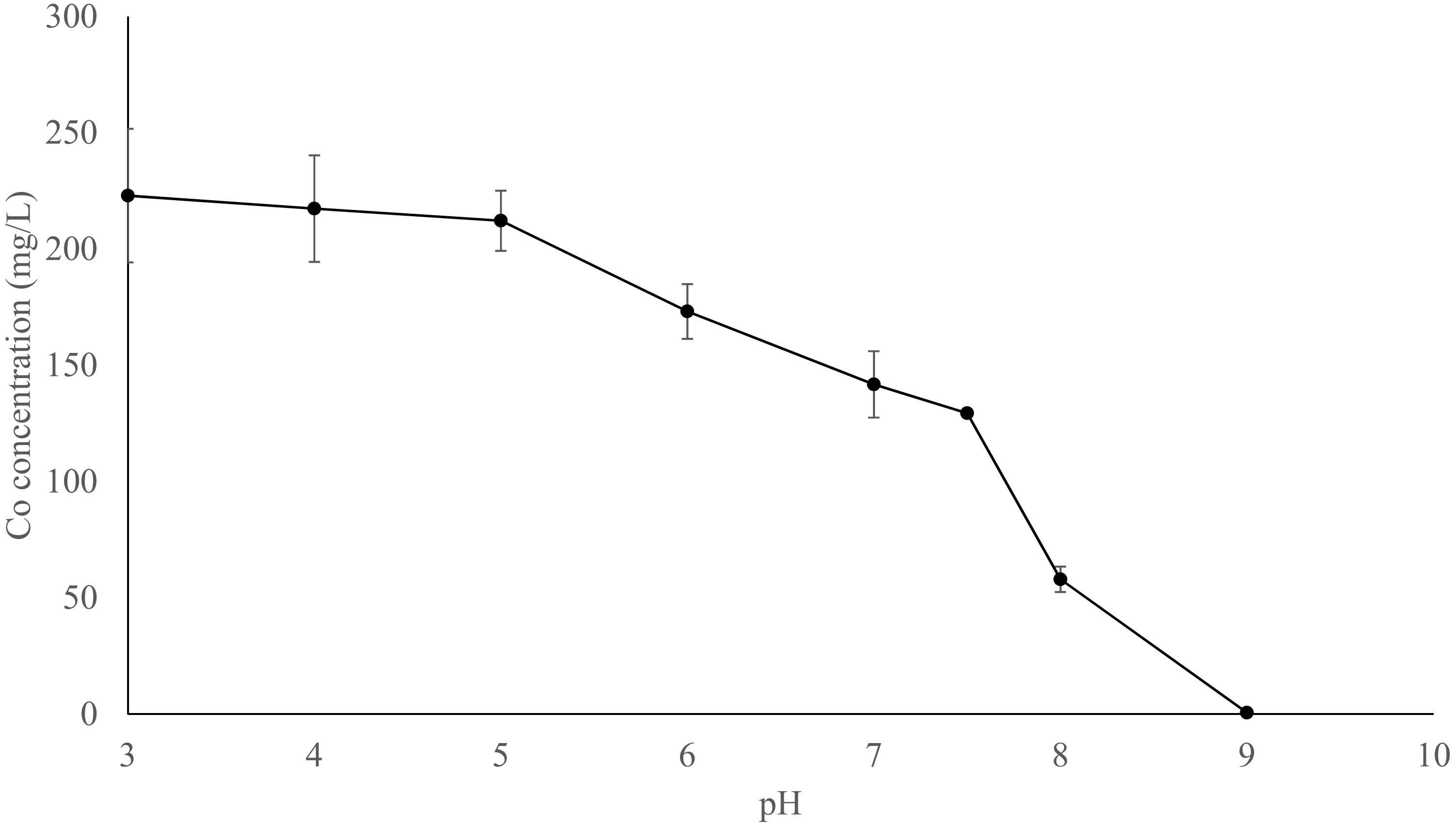

Figure 2.1 Co adsorption depending on the pH

2.3.1.2 Impact of Time on Co Adsorption

Fig. 2.2 presents the mass of Co adsorbed onto the soil particles. The bulk of the Co sorbed by soil increased significantly within 120 minutes. Consequently, the rate of Co uptake slowed down gently as available adsorption sites were occupied by the metal ions. The result is well aligned with work conducted by (Srivastava et al., 2006). After 420 minutes, the maximum Co adsorption was achieved and Co concentration in the solid phase became stable. The adsorption time (i.e., 420 min) was thus selected for conducting the following experiments.

2.3.1.3 Impact of Initial Concentration of Co on Adsorption

The impact of initial Co concentration in the solution on the capacity of Co adsorption onto soil is illustrated in Fig. 2.3. The amount of Co ions adsorbed onto soil at equilibrium status increased with raising the initial Co concentration in the solution.

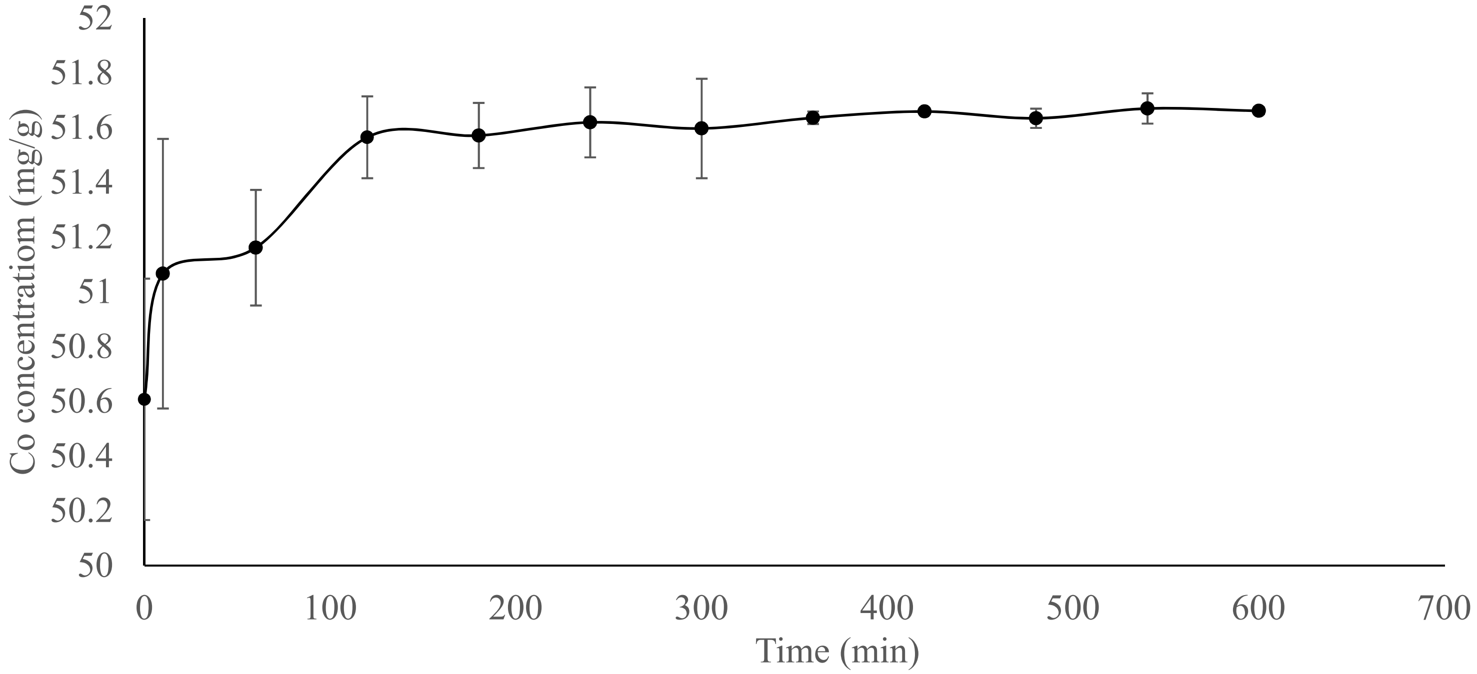

Figure 2.2 Effect of contact time on Co adsorption by soil

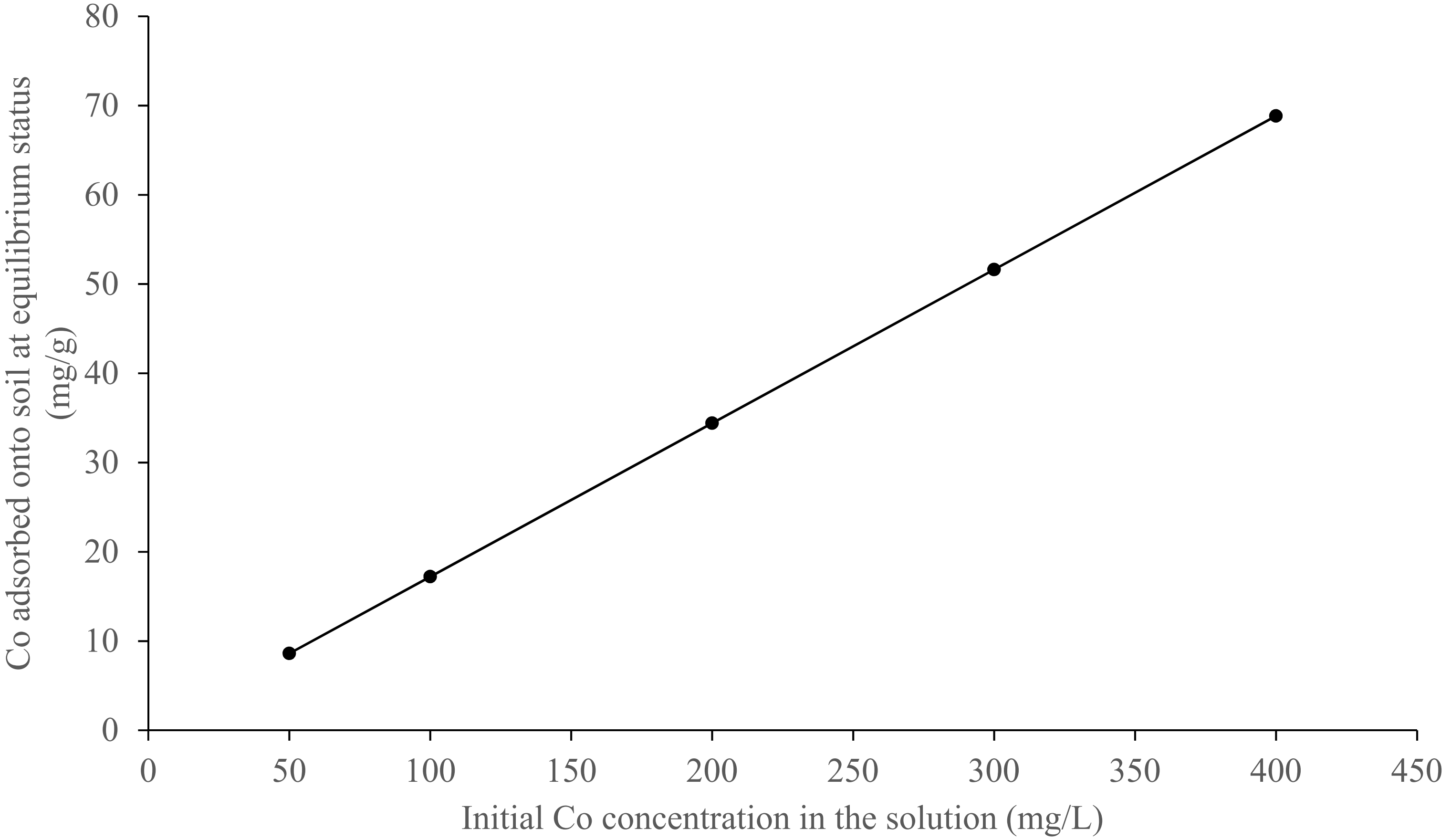

Figure 2.3 Effect of initial concentration on Co adsorption

2.3.2 Biosurfactant Adsorption onto Soil without Existence of Co

Once biosurfactant molecules are adsorbed to the soil surface, the associated capacity for forming complexes with Co decreases. To evaluate the adsorption capacity of each type of biosurfactant during its application, experiments without the existence of Co were conducted. One and two CMCs of each type of biosurfactant were adopted as initial concentrations before adsorption. After each adsorption run, the surface tension of each solution was tested, with results presented in Table 2.7. Under both experimental settings (i.e., 1 CMC and 2 CMC), the resulting surface tension values with the addition of Surfactin were lower than for those runs with Rhamnolipid. The use of Trehalose lipids led to much higher surface tension values after adsorption compared with the other two types of biosurfactant.

The surface tension values of the 1CMC solutions of three Surfactin, Trehalose lipids and Rhamnolipid are 32 mN/m, 33 mN/m and 40 mN/m, respectively. For Surfactin, after adsorption treatment, the surface tension was nearly unchanged, which indicated that Surfactin has an extremely limited adsorption capacity in a soil-water system. The finding delivered the information that Surfactin might be a good candidate for soil washing treatment due to its low adsorption capacity. However, the surface tension of Trehalose lipids was sharply increased after adsorption, showing that the molecules could easily adsorb to the soil surface. The high sorption capacity of a biosurfactant would lead to its low availability for metal complexation (Aşçı et al., 2007).

Table 2.7 Measured ST of solution after biosurfactant adsorption

Initial biosurfactant concentration	Surfactin	Trehalos lipid	Rhamnolipid
	ST	ST	ST
0 CMC (Blank)	72	72	72
1CMC	32.7	70	63.4
2CMC	32.5	69.5	57.6

In addition, when biosurfactants such as trehalose lipids with 1 CMC or 2 CMC concentrations were applied as soil washing agents, their high adsorption capacity led to high biosurfactant residue in soil and potential secondary soil pollution.

2.3.3 Co Adsorption onto Soil with Biosurfactant Addition

The presence of surfactant in soil may affect Co adsorption. By adding various concentrations of each biosurfactant to soil, the pattern of Co adsorption changes. The blank experiments were conducted by adsorbing Co onto soil with different Co initial concentrations (i.e., 50 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm) without the addition of any biosurfactant. The results of blank experiments are shown in Fig. 2.4.

Fig. 2.5 indicates the Co adsorption onto soil with the addition of 1 CMC and 2 CMC of the biosurfactant produced by B. subtilis N3-1P. At lower initial concentrations of Co (i.e., 50 ppm and 100 ppm), the Co equilibrium concentration in the solution is higher in each system with 2 CMC of the biosurfactant addition than that with 1 CMC of the biosurfactant. Once the initial concentration of Co increased to 200 ppm or even 400 ppm, both the Co equilibrium concentration in the solution and the associated Co adsorbed onto soil remained the same when comparing a system with 2 CMC of the biosurfactant's addition to that with 1 CMC of the biosurfactant. Co can be adsorbed onto the surface of soil particles and also react with the biosurfactant to form a complex. The complex can be adsorbed by the soil particles as well. Results illustrate that a higher biosurfactant concentration (i.e., 2 CMC) with lower initial Co concentrations (i.e., 50 ppm, 100 ppm) led to a bit less Co being adsorbed.

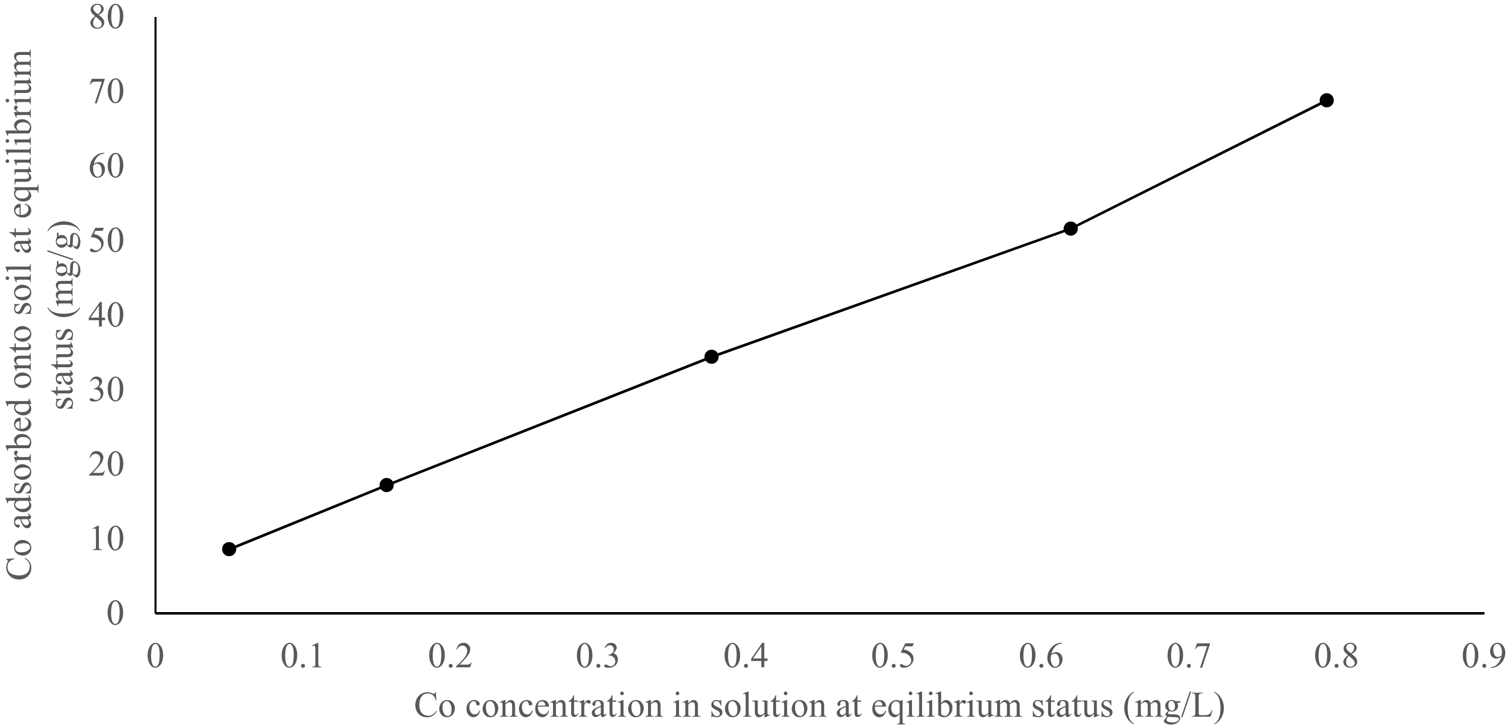

Figure 2.4 Co adsorption by soil without the addition of any biosurfactant

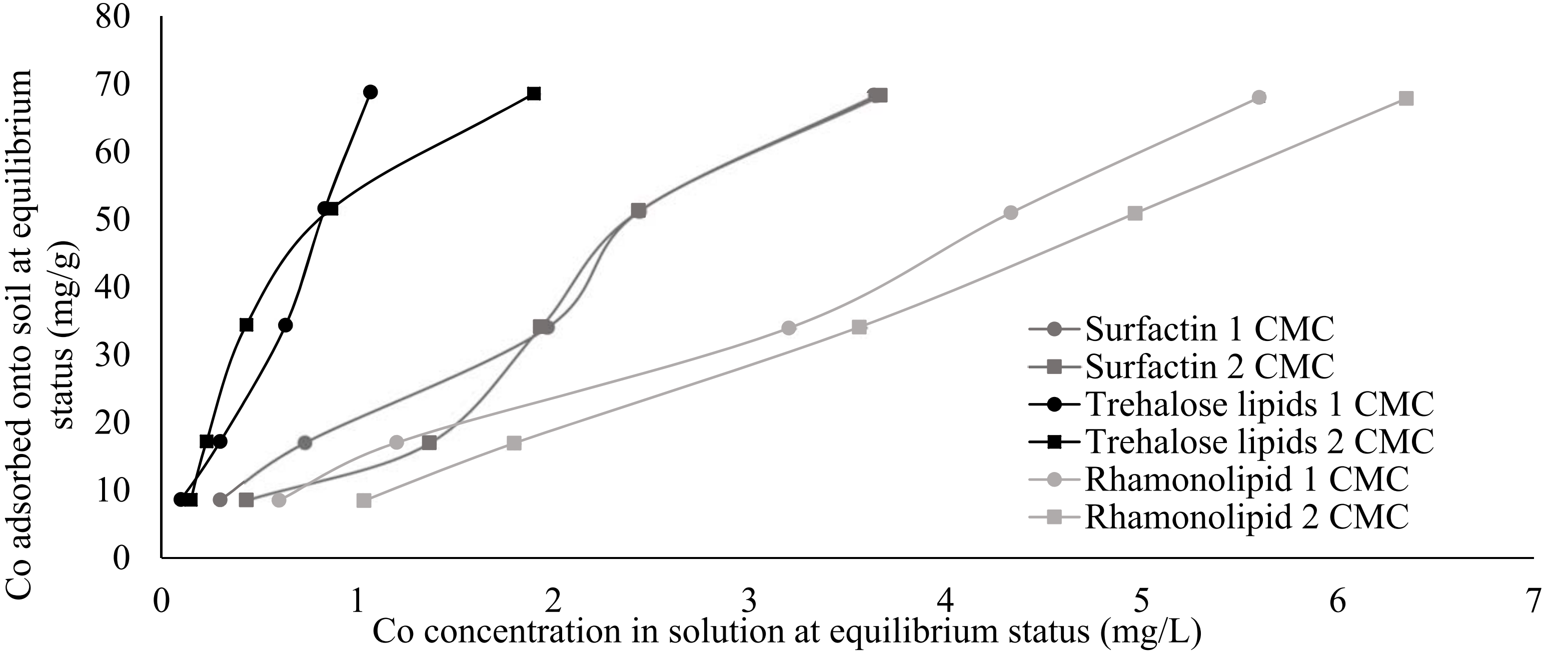

Figure 2.5 Co adsorption by soil by applying 1 CMC and 2 CMC of biosurfactant

Fig. 2.6 presents the change of surface tension in the solution after adsorption versus the initial concentration of Co. At a low initial concentration (i.e., 50 ppm and 100 ppm), the surface tensions in the 1 CMC system are slightly higher than those in the 2 CMC system. In addition, the surface tension values in both systems (i.e., 1 CMC and 2 CMC) are relatively low, which means there are still many free biosurfactant molecules after forming complexes with Co in the solution after adsorption. Once the initial Co concentration was increased to 300 ppm or even 400 ppm, the ratio of the number of Co ions to the number of biosurfactant molecules decreased in both experimental settings. The surface tension values in the solutions for both settings (i.e., 1 CMC and 2 CMC) are quite similar (reaching the surface tension of water) because all free biosurfactant molecules have either been used to form a complex with Co or are attached to the soil surface.

The examination of Co adsorption onto soil with the addition of the second type of biosurfactant, trehalose lipids produced by *Rhodococcus* M36, was conducted with all experimental settings the same as the previous ones with surfactin. Both 1CMC and 2 CMC of the trehalose lipids were applied and the results are presented in Figs. 2.5 and 2.6. Fig. 2.5 indicates that the trehalose lipids' addition resulted in lower remaining Co concentrations in the solutions than those in the matrix with surfactin. Table 2.7 indicates that the trehalose lipids biosurfactant has a higher capacity to be adsorbed by the soil. The surface tensions of those solutions after adsorption are also much higher (Fig. 2.6) due to the sorption of biosurfactant in the soil which causes lower soil surface availability for Co ions. Additionally, Co could adsorb to the soil particles and also make a complex with biosurfactants. Consequently, the surface tension declines when the trehalose lipids biosurfactant transfers from its original form to the complex form. The amount of remaining Co in solution with trehalose lipids falls sharply compared with surfactin. With a high biosurfactant concentration (2 CMC), the remaining Co concentrations are slightly different than those with a low biosurfactant concentration (1 CMC), except for the last data, which almost doubled (rising from 1.06 mg/L in the 1CMC biosurfactant system to 1.9 mg/L in the 2 CMC biosurfactant one).

Rhamnolipid at 1 CMC and 2 CMC concentrations was also applied. In both systems, the increase of initial Co concentration resulted in the increase of Co concentration in the solution at the equilibrium status (Fig. 2.5). The addition of rhamnolipid at 2 CMC led to a higher equilibrium Co concentration compared to the results of the 1 CMC rhamnolipid system. In addition, the addition of rhamnolipid resulted in higher equilibrium Co concentrations in general when compared to the addition of the other two types of biosurfactants.

Fig. 2.6 indicates the surface tension in each solution after adsorption versus the Co initial concentration. The surface tensions at 1 CMC biosurfactant concentration are slightly higher than those at 2 CMC biosurfactant concentration. The surface tension varies between 60-70 (mN/m). The rhamnolipid shows a high tendency of being adsorbed to the soil (Table 2.7).

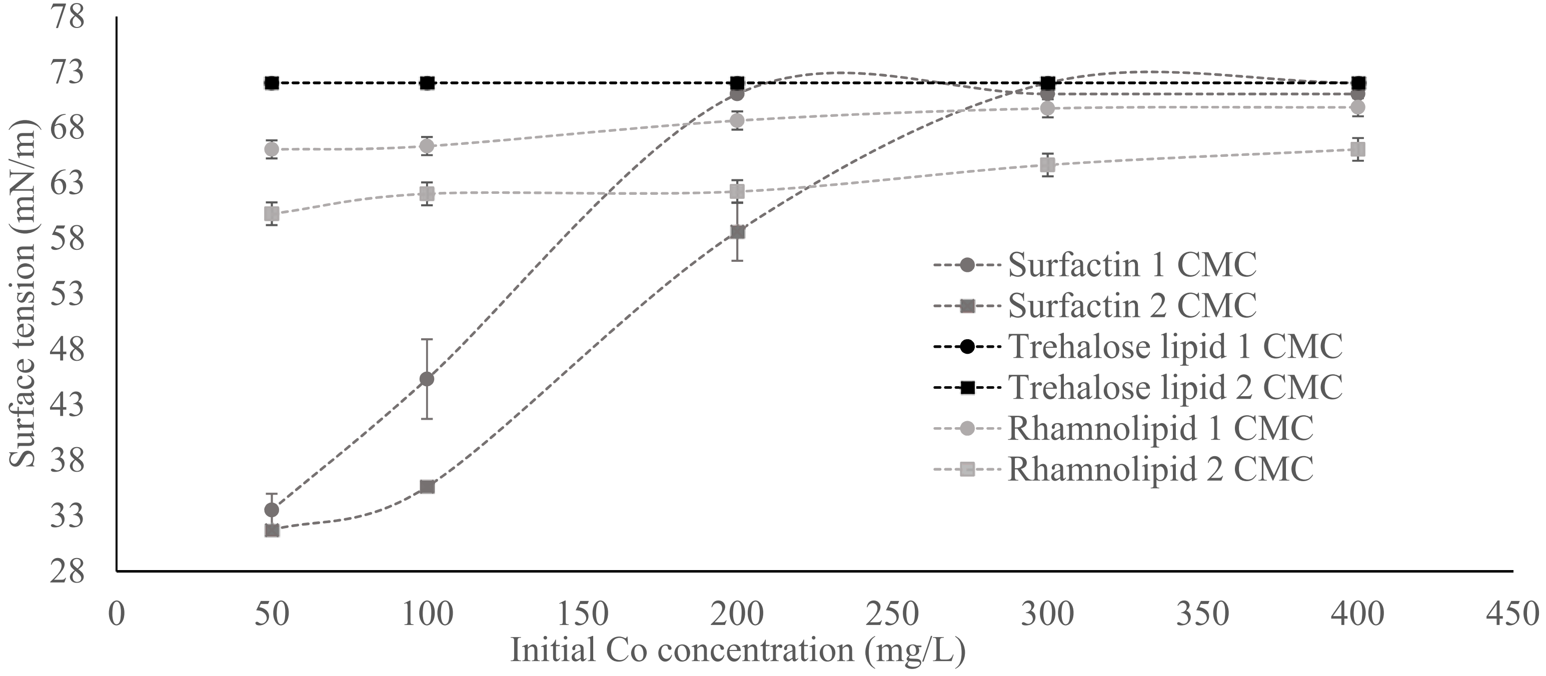

Figure 2.6 Surface tension measured at 1CMC and 2CMC of biosurfactant after adsorption

2.3.4 Kinetic Studies of Co Adsorption to Soil without Existence of Biosurfactants

Adsorption kinetics describe the uptake rate of Co onto the soil particles. Fig. 2.7 shows how the pseudo-first-order kinetic fits the experimental data. The values of $k_1$and q_e are determined by using the intercept and slope of the linear plot of ln (q_e−q_t) versus t. The obtained slope of the model presents the adsorption efficiency of Co onto the soil particles. The determined R^2 and k_1 values are 0.8427 and 0.0078, respectively.

To determine the values of q_e and k_2 in the pseudo-second-order model, the intercept and slope of the linear plot of t/q_t against t were applied (Fig. 2.8). The k_2 value calculated is equal to 0.0677. The coefficient of determination (R^2 value: 1) proves the reliability of the pseudo-second-order to explain the Co adsorption kinetic onto the soil. The high R^2 value obtained and the calculated Co equilibrium concentration ($q_{e\text{-}cal}$ equal to 51.8134 mg/g) show that the pseudo-second-order could be a better model to designate the kinetic of the process. The Co equilibrium concentration obtained from experiments q_e is 51.6591 mg/g. According to R^2 and $q_{e\text{-}cal}$, the pseudo-second-order model suggests that the rate limiting step in divalent metal sorption on the soil involves valence forces through exchange or a sharing of electrons between the sorbates and sorbent (Ho and McKay, 1999). Thus, the significant correlation between experimental data and this kinetic model is helpful for q_e prediction and comparison regarding Co adsorption (Coleman et al., 2006).

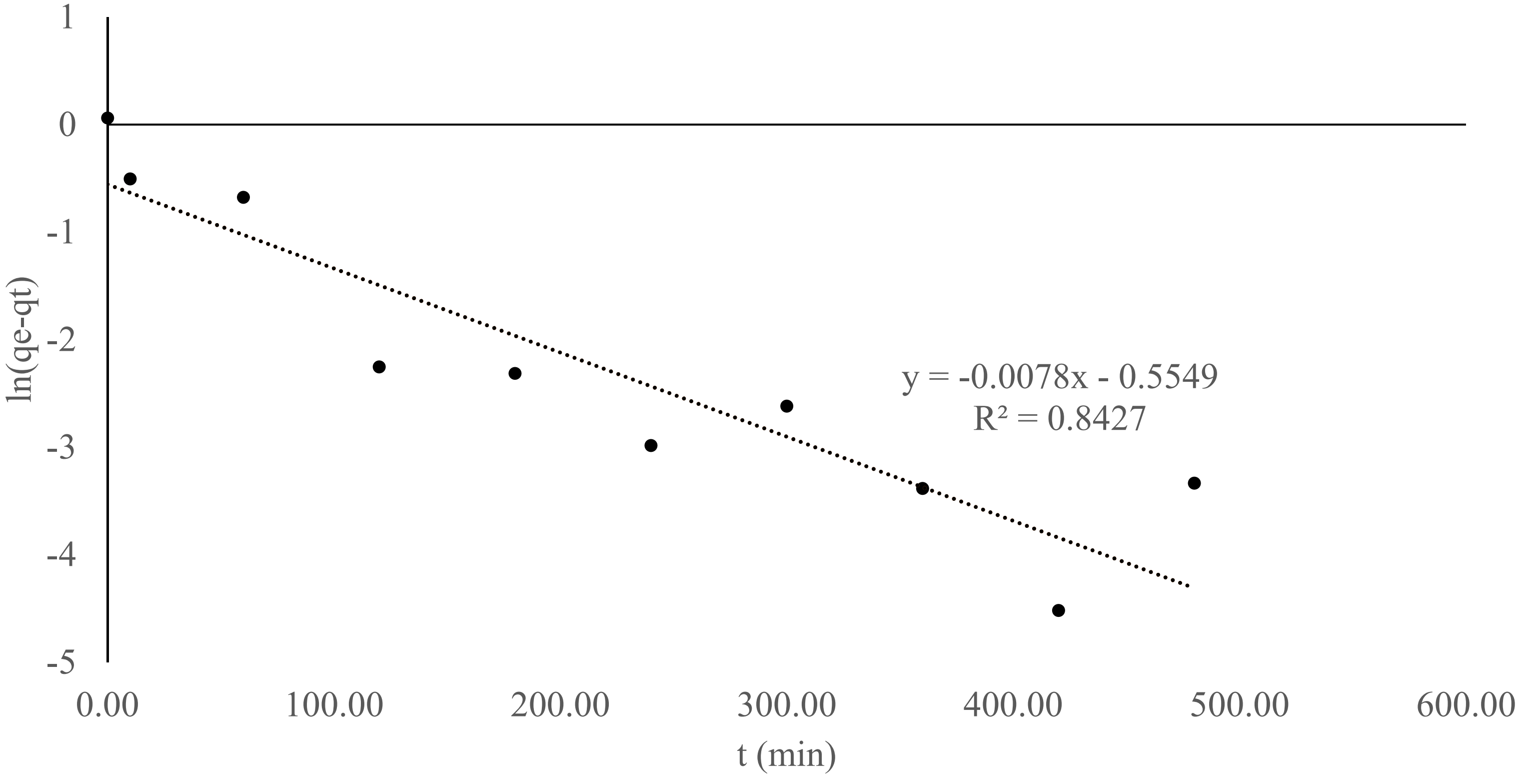

Figure 2.7 Pseudo-first-order adsorption kinetic of Co onto the soil

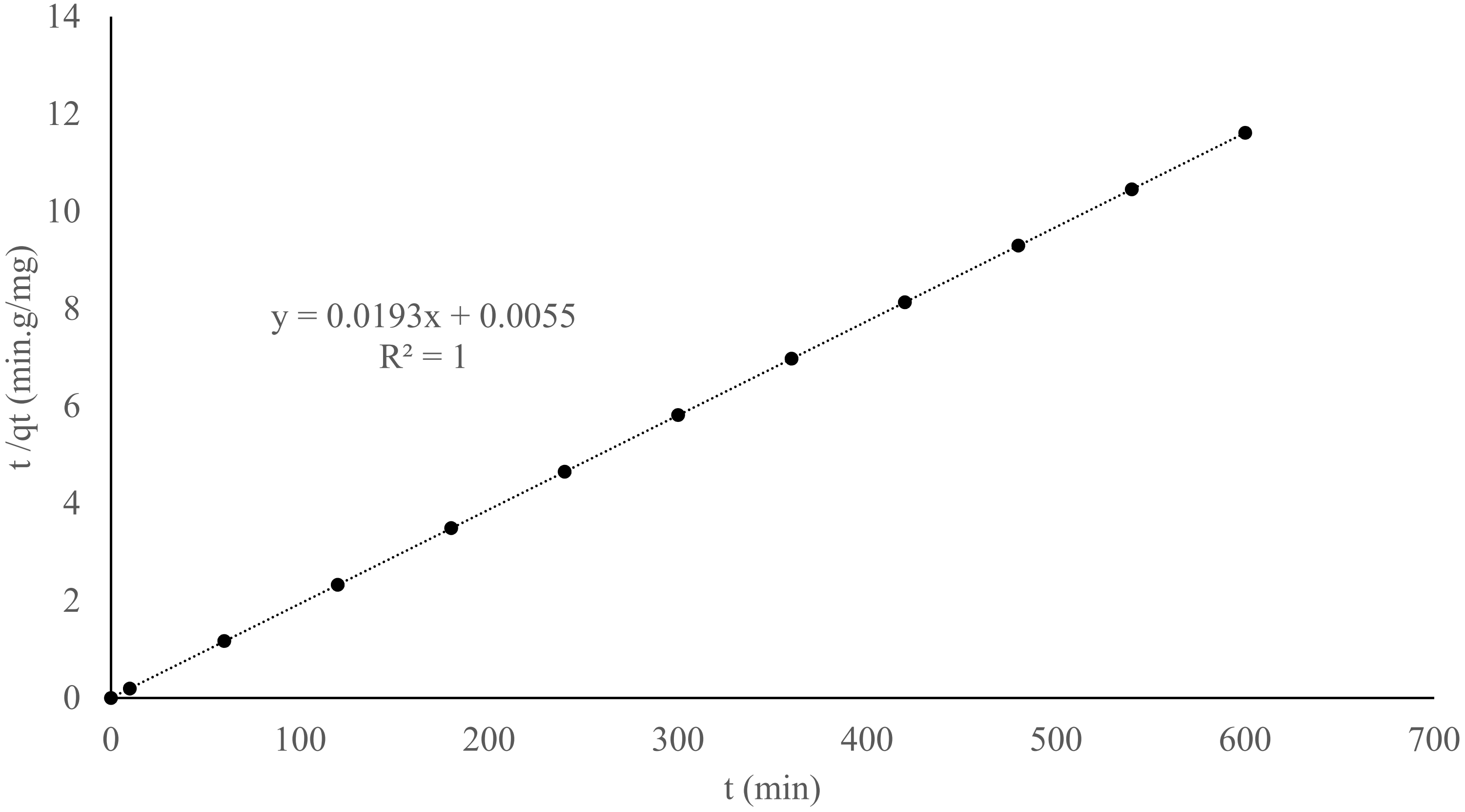

Figure 2.8 Pseudo-second-order adsorption kinetic of Co onto the soil

In Fig. 2.9, the terms 1/ß ln (αß) and 1/ß for Elovich kinetic model are calculated from the intercept and slope of the linear plot of q_t versus ln (t). The value of the correlation coefficient (R^2) of the Elovich model was 0.8741.

In terms of rate-limiting regarding the adsorption, the slowest step of the reaction (rate-limiting step) could be the boundary layer (film) or the intra-particle (pore) diffusion of solute from the bulk of the solution on the solid surface in a batch process. A higher value of intercept (I) means a greater influence of the boundary layer. If the plot of q_t versus $t^{0.5}$ becomes a straight line and passes through the origin, the rate-limiting step can be linked to the intra-particle diffusion (Sheela et al., 2012). In Fig. 2.10, plot deviation from linearity shows the rate limiting step is controlled by the boundary film diffusion. Non-linearity of the plot describes the two or more Co adsorption steps in soil. This means that intra-particle diffusion might not be the only mechanism, the adsorption rate is controlled by a combination of the two processes (Arabloo et al., 2016). Table 2.8 presents the correlation, RMSE and R^2 obtained from applied kinetic models. The coefficient of determination (R^2), k_{id} and intercept (I) calculated from the intra particle kinetic model are 0.7988, 0.0375 and 50.9, respectively.

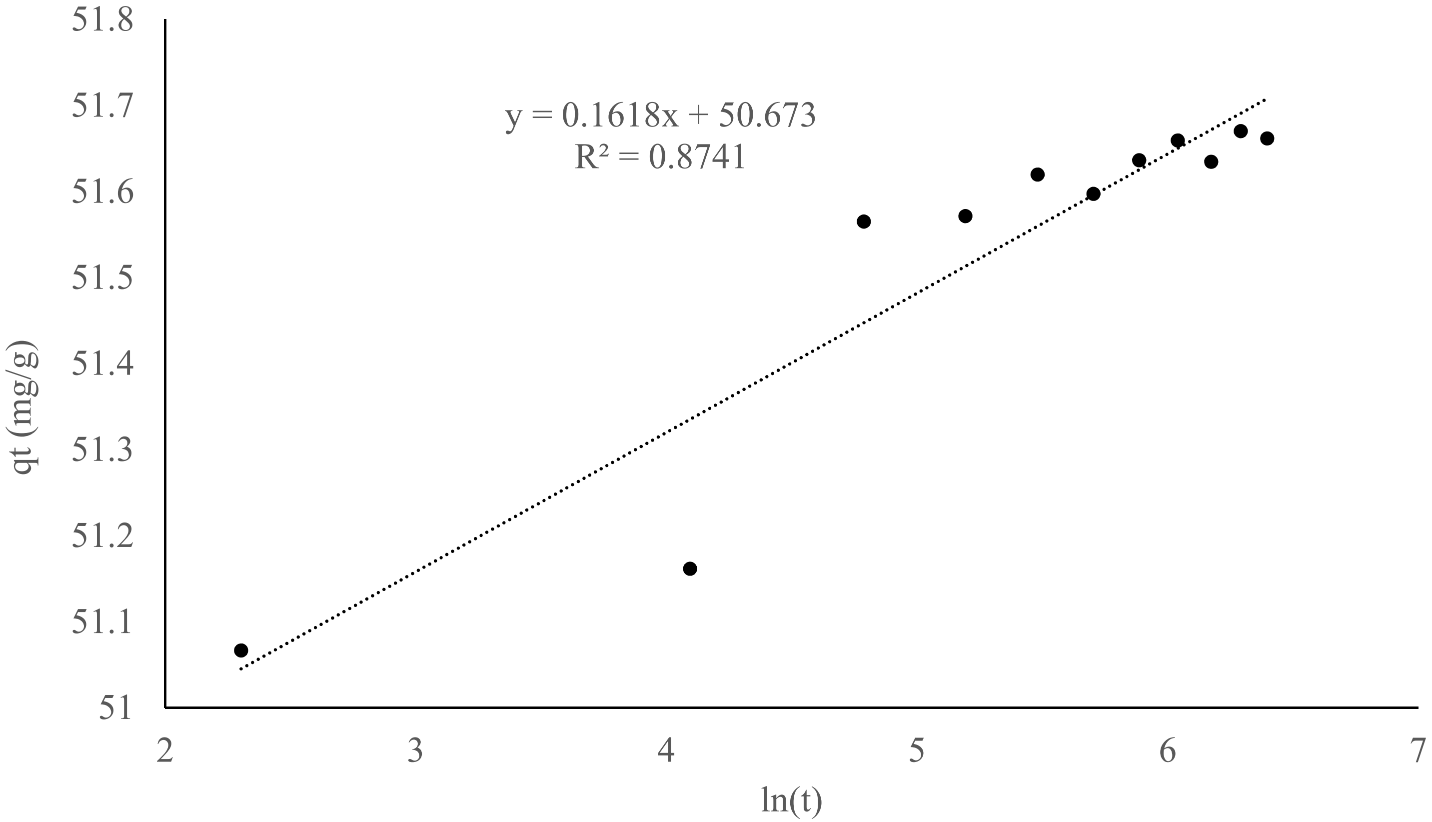

Figure 2.9 Elovich adsorption kinetic of Co onto the soil

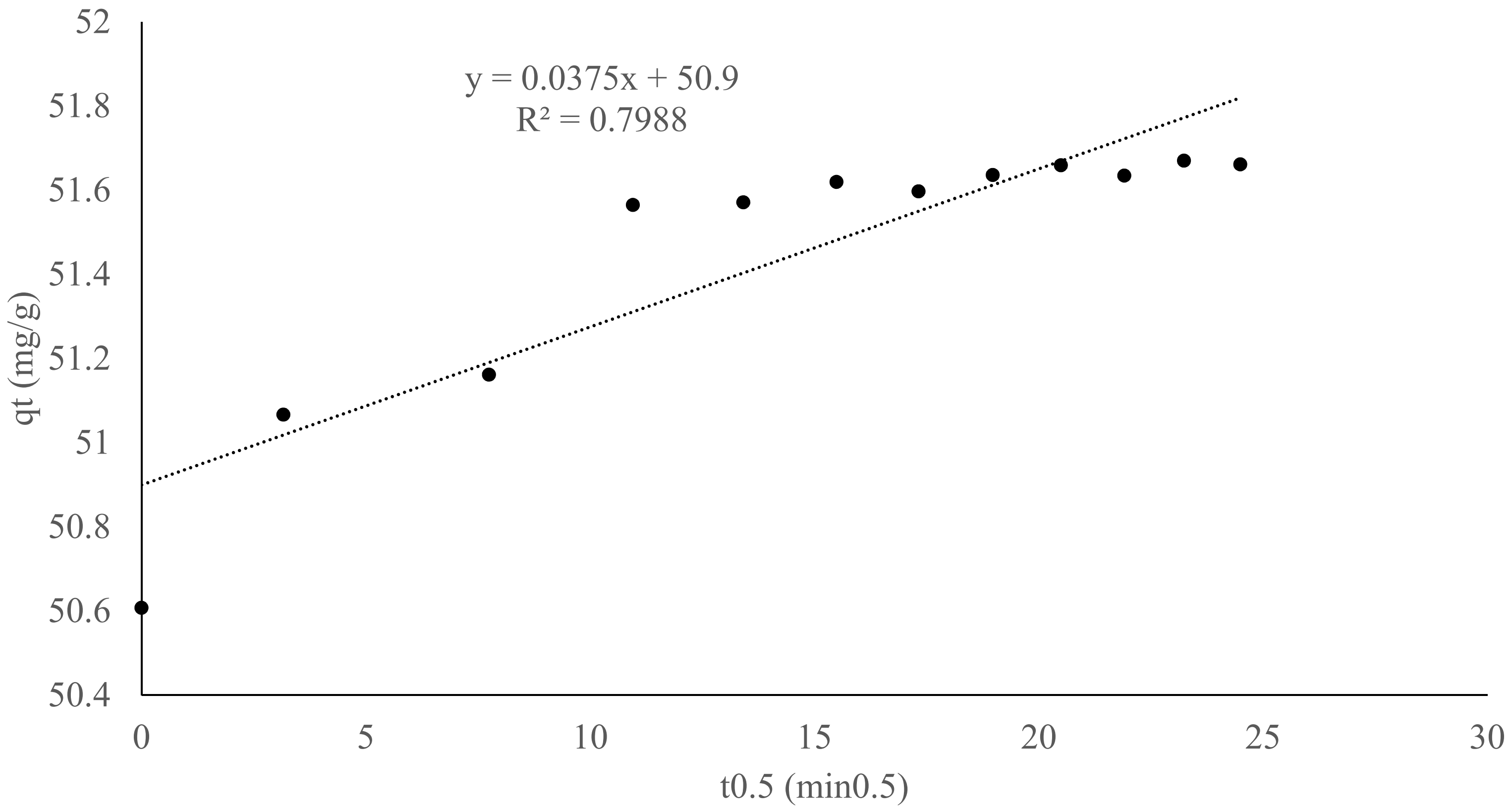

Figure 2.10 Intra-particle diffusion adsorption kinetic of Co onto the soil

Table 2.8 Kinetic models regarding Co adsorption in soil

Kinetic model	**Equation**	**R^2**	**RMSE**
pseudo-first-order	$\ln(q_e-q_t)= -0.5549- 0.0078t$	0.8427	0.6134
pseudo-second-order	$t/q_t= 0.0055 + 0.0193t$	1	0.0035
Elovich	$q_t= 50.673 + 0.1618 \ln(t)$	0.8741	0.0786
Intra-particle diffusion	$q_t= 0.0375 t^{0.5} + 50.9$	0.7988	0.1569

2.3.5 Isotherm Examination of Co Adsorption in Soil with and without Existence of Biosurfactants

The isotherm models were developed using MATLAB software. Four isotherms, including Redlich-Peterson, Langmuir, Freundlich and Temkin, were discussed. The Redlich-Peterson isotherm is the combination of both the Langmuir and Freundlich equations (Rostamian et al., 2011). The Langmuir isotherm supposes monolayer adsorption onto a homogeneous surface of adsorbent with a limited number of identical sites (Farah et al., 2007). The Freundlich isotherm is chosen to evaluate the intensity of adsorption of sorbent in the particles. The Temkin isotherm, similar to the Freundlich model, is one of the early isotherms. Temkin isotherm might be a) due to adsorption sites with an intrinsic distribution of adsorption energies or b) an effect of increasing electrostatic interaction, between the dipoles at the interface, with increasing concentration (Eriksson et al., 1997). The isotherm models are indicated in Figs. 2.11-2.17. Constants and statistical quality measuring of each isotherm model are reported in Tables 2.9 and 2.10, respectively. When comparing the values of $R^{2,}$ R^2_a and RMSE obtained from the sorption models without the addition of any biosurfactant, the Freundlich and Redlich-Peterson models had a better performance of fitting the experimental data. However, the Freundlich model has a higher R^2_a (0.9908). If the magnitude of $n > 1$, then the process of adsorption is favorable (Wang et al., 2007). With no biosurfactant addition, the n value (1.169) from the Freundlich model defined a favorable adsorption process.

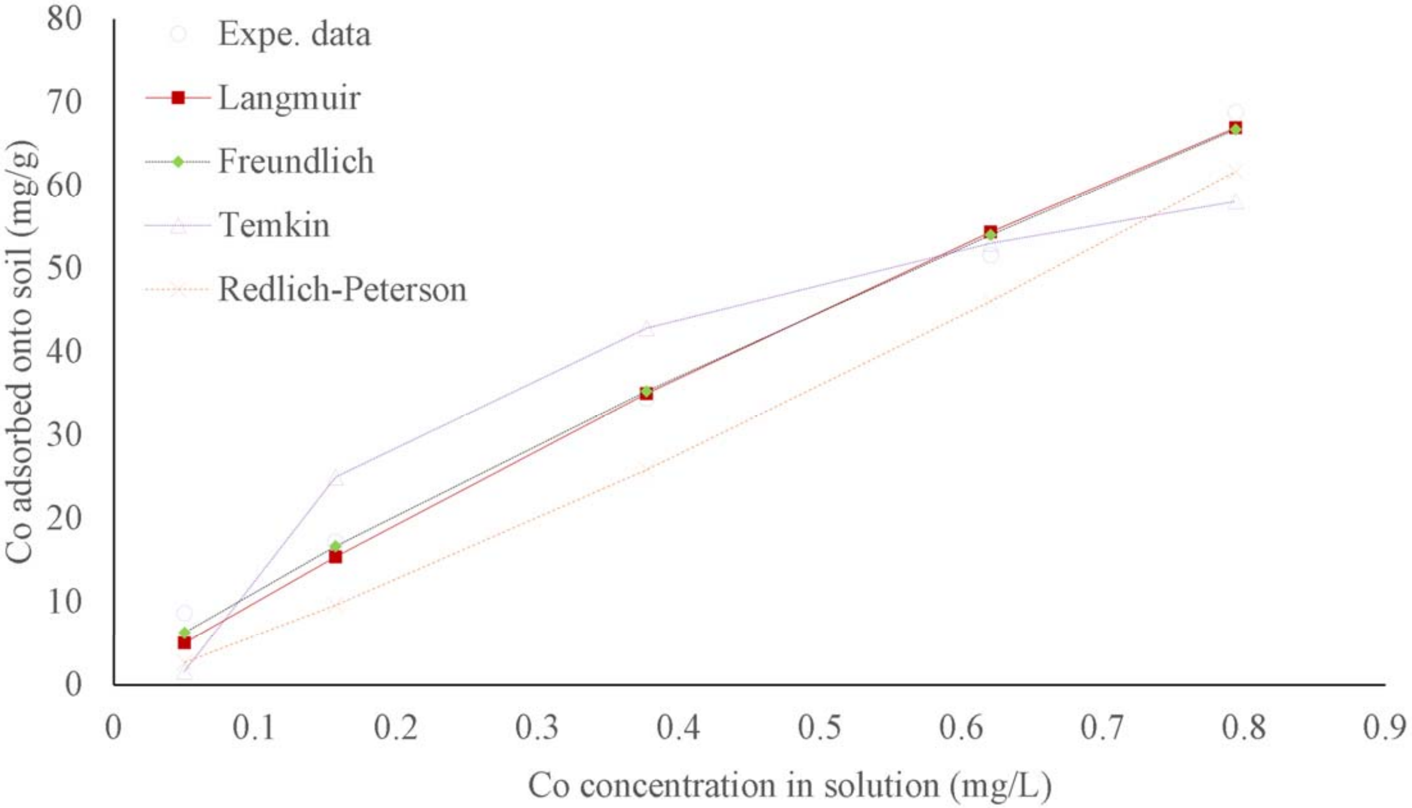

Figure 2.11 Comparison of various sorption isotherms for Co onto soil without biosurfactant

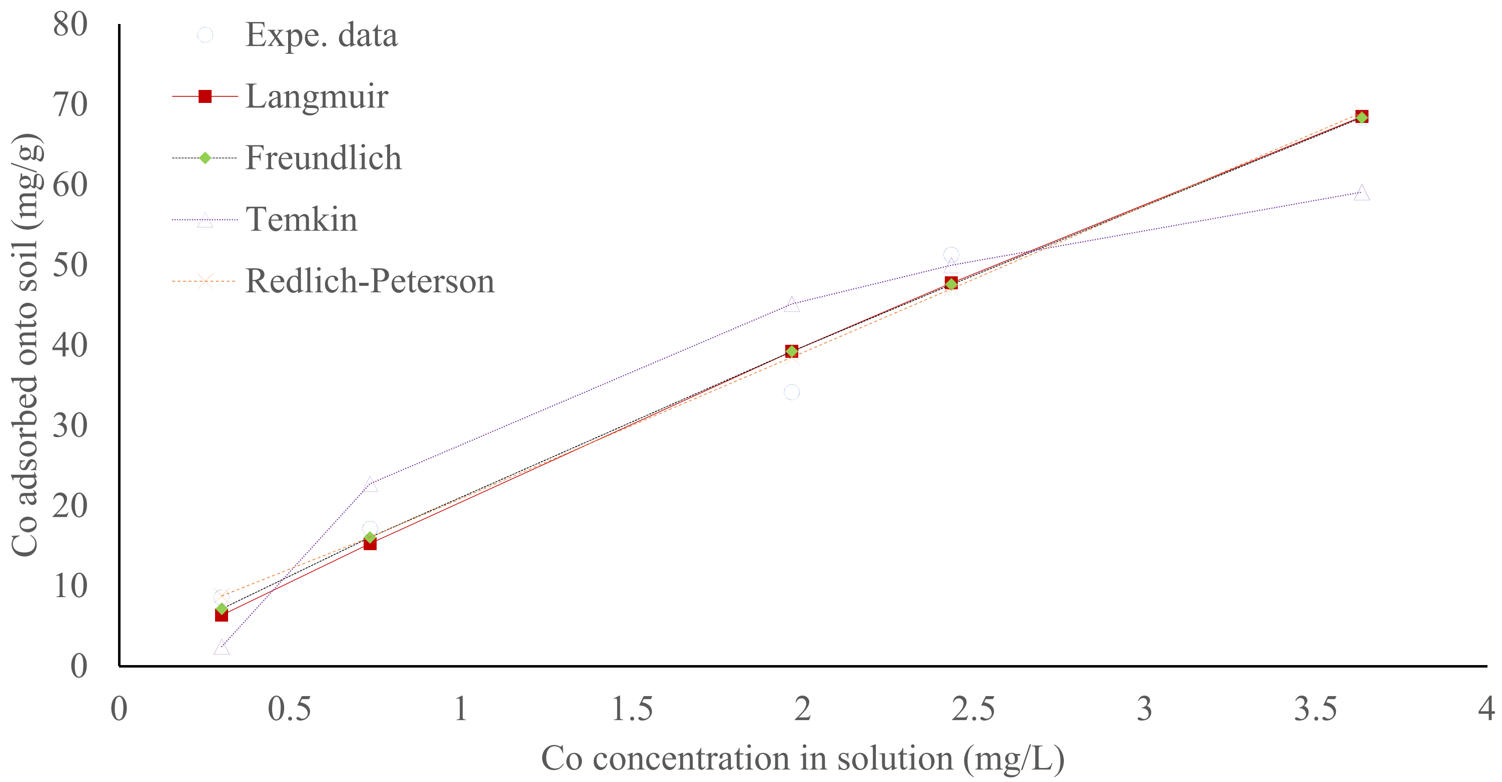

Figure 2.12 Comparison of various sorption isotherms for Co onto soil with 1 CMC surfactin

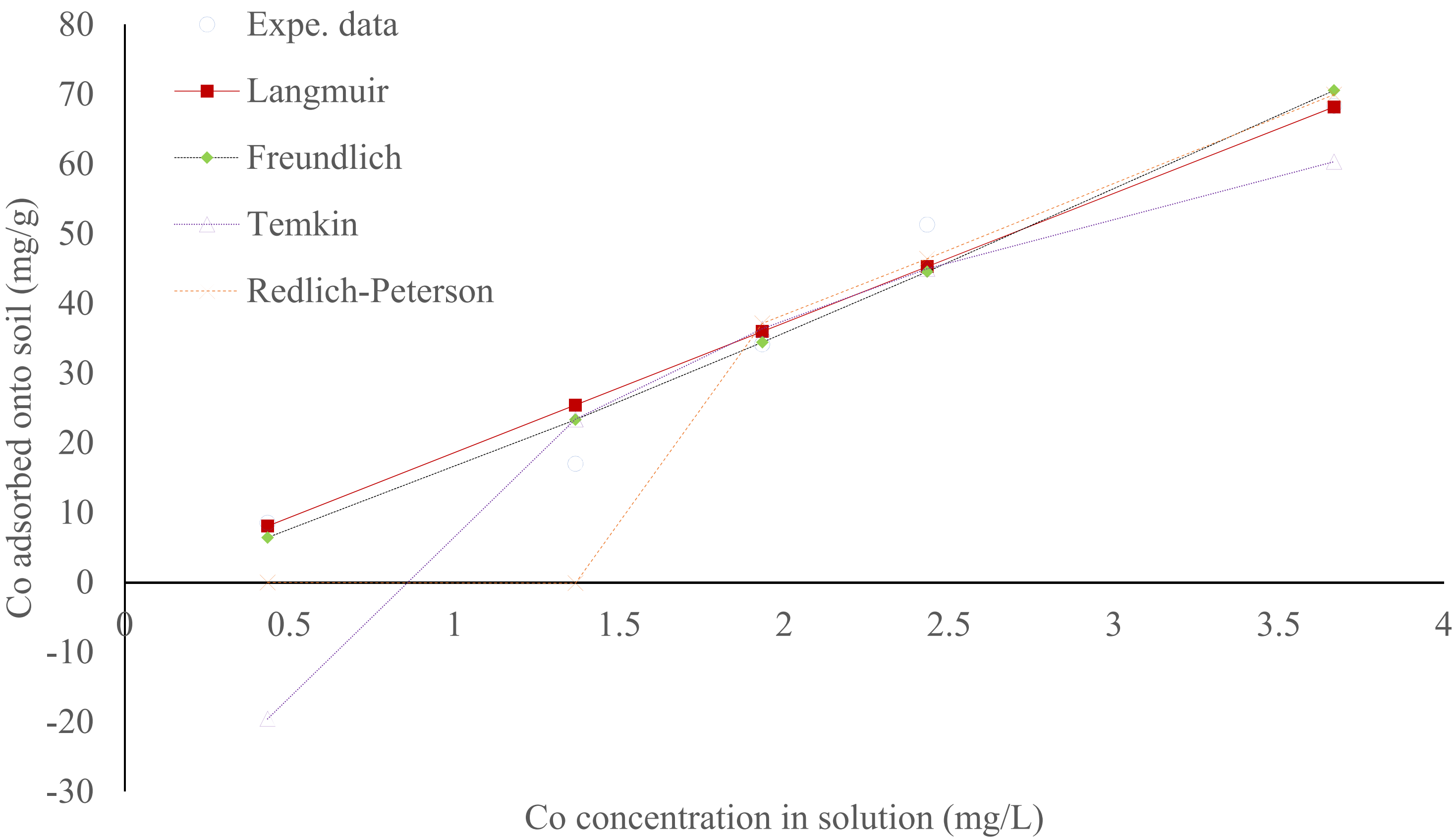

Figure 2.13 Comparison of various sorption isotherms for Co onto soil with 2 CMC surfactin

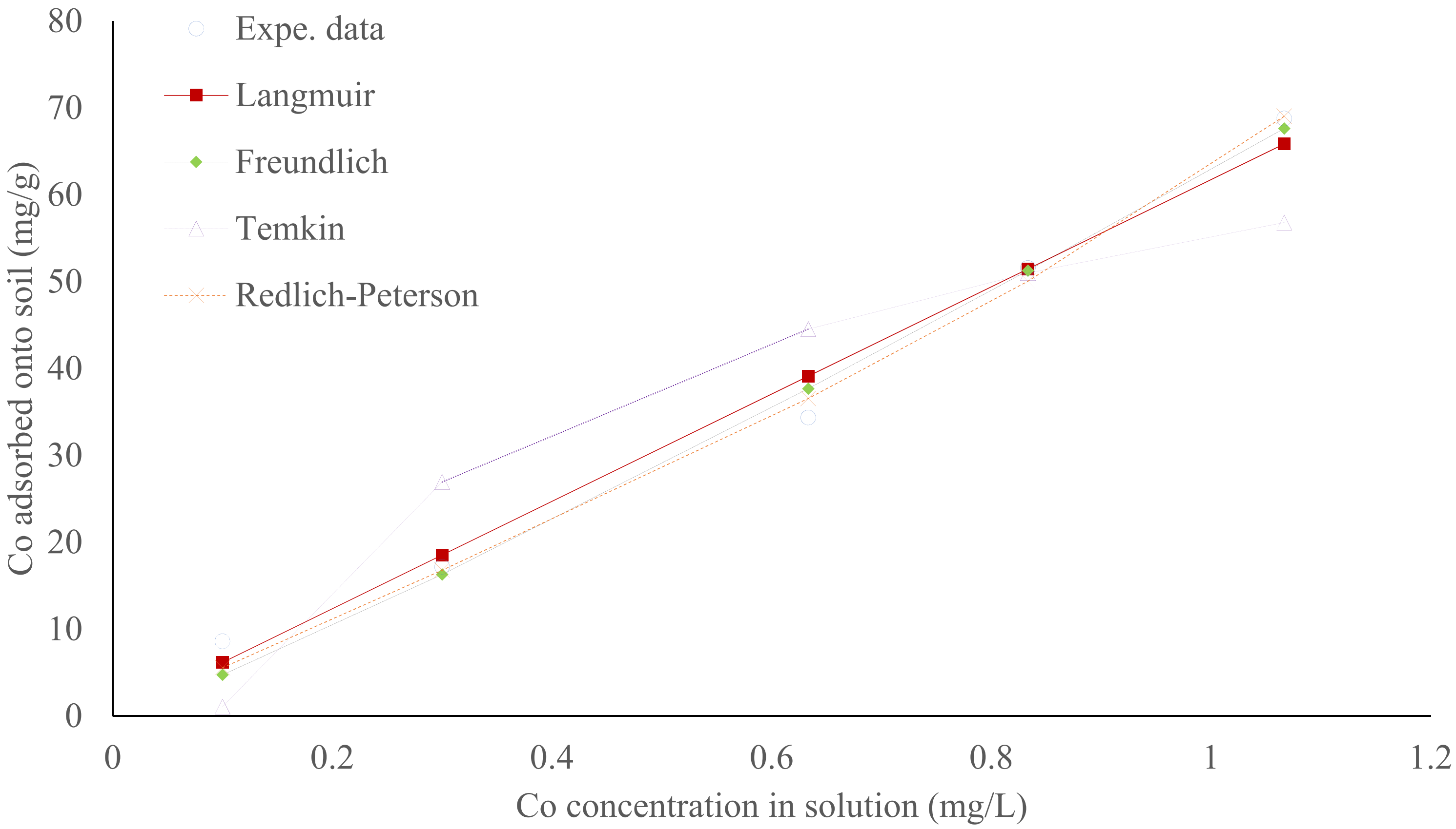

Figure 2.14 Comparison of various sorption isotherms for Co onto soil with 1 CMC trehalose lipids

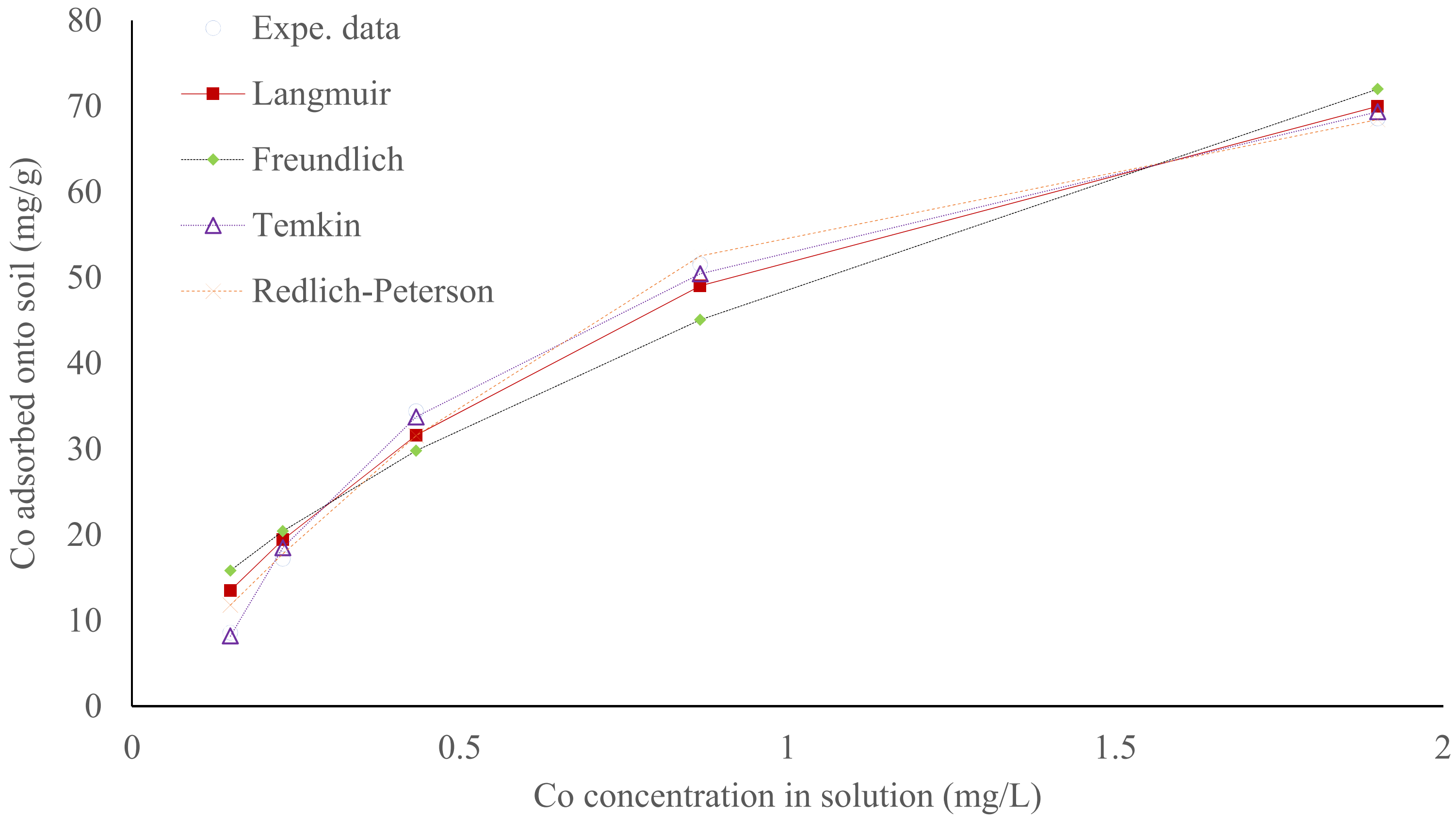

Figure 2.15 Comparison of various sorption isotherms for Co onto soil with 2 CMC trehalose lipids

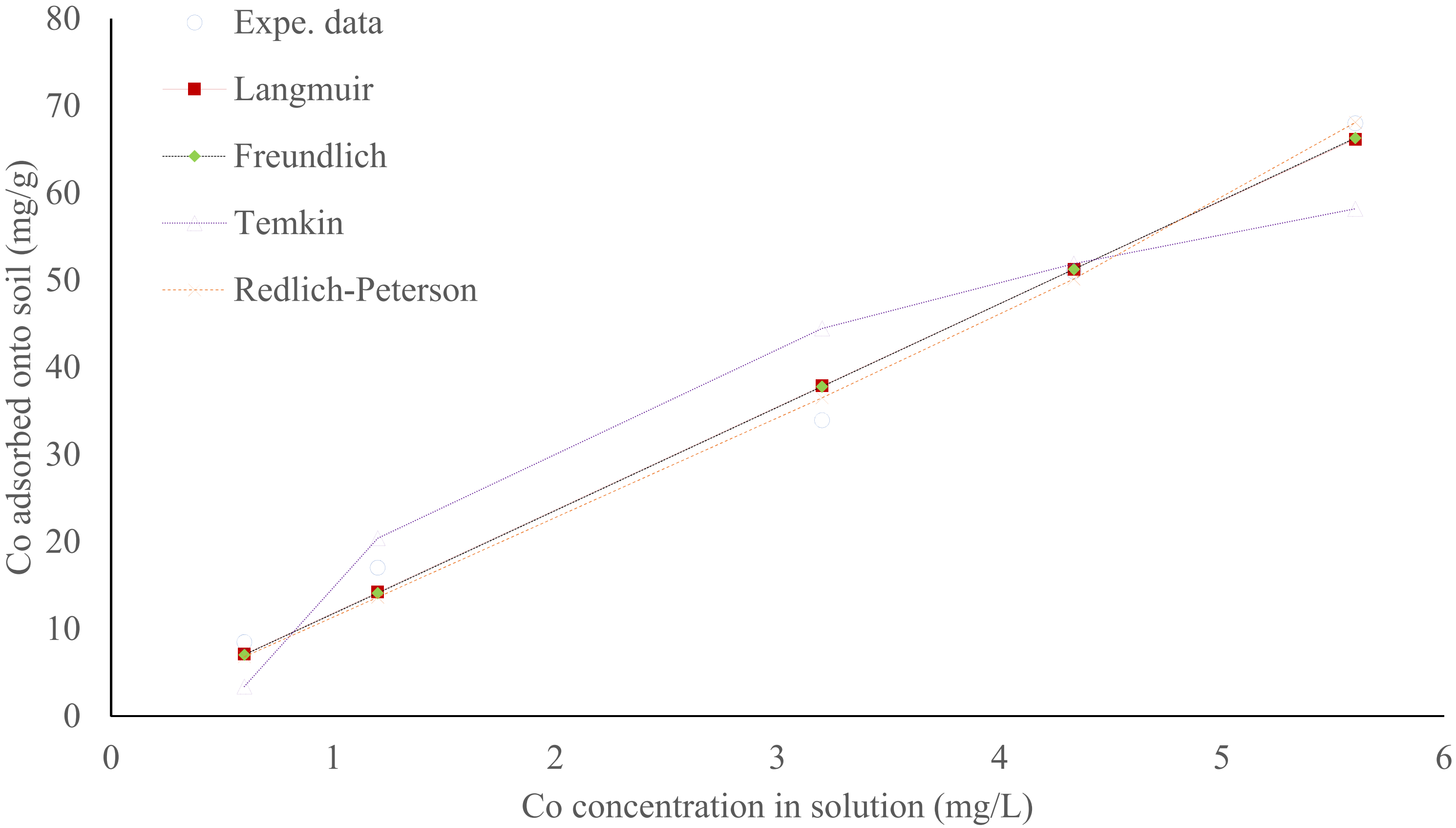

Figure 2.16 Comparison of various sorption isotherms for Co onto soil with 1 CMC rhamnolipid

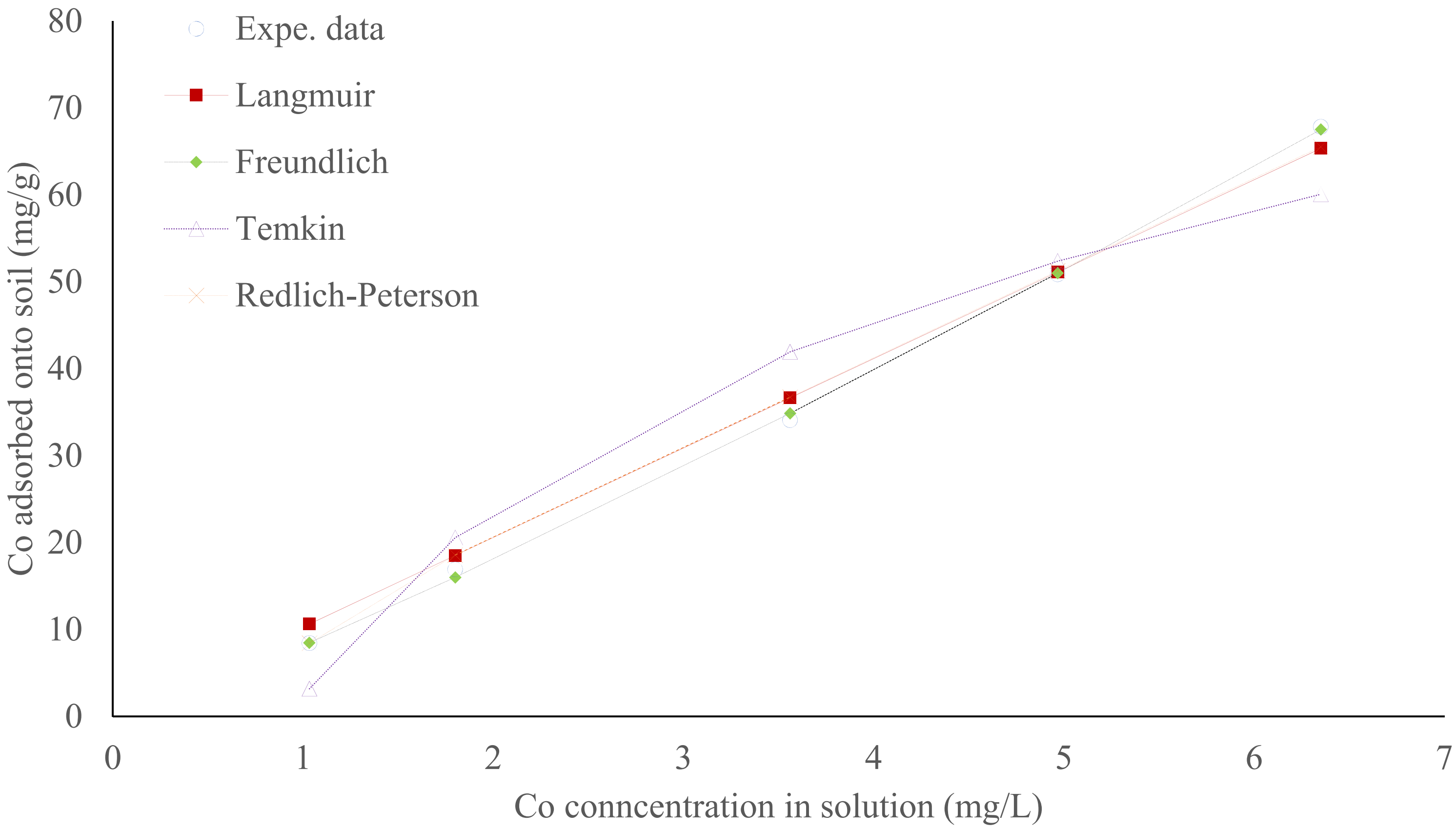

Figure 2.17 Comparison of various sorption isotherms for Co onto soil with 2 CMC rhamnolipid

Table 2.9 Isotherm parameters for various adsorption isotherms for the adsorption of Co onto the soil

Isotherms	Parameters	No Biosurfactant	1 CMC Surfactin	2 CMC Surfactin	1 CMC Trehalose lipids	2 CMC Trehalose lipids	1 CMC Rhamnolipid	2 CMC Rhamnolipid
Langmuir	Q_0 (mg/g)	381.5	572.3	$3.7*10^{4}$	$3.753*10^{4}$	109.1	$1.939*10^{4}$	39150
	K_l (L/mg)	0.2683	0.03741	0.0005037	0.001648	0.9424	0.0006117	0.0002633
Freundlich	K_f (L/g) (mg/g) ^{nf}	81.37	21.27	16.45	62.91	49.11	11.76	8.184
	n_f	1.169	1.105	0.8922	0.8916	1.677	0.996	0.8759
Temkin	b_T	1.406	0.0639	0.01201	0.3852	0.1495	3.488	0.04999
	A_T (L/g)	21.68	3.713	1.368	10.48	9.375	1.915	1.079
Redlich-Peterson	K_R (L/g)	33.75	18.24	19.08	55.74	80	11.37	10.32
	a_R (1/mg)	-0.5852	-0.1262	$-3.101*10^{6}$	-0.117	0.4081	$-9.283*10^{-6}$	1.249
	g	0.1445	-0.906	-30.11	2.668	1.707	5.139	- 47.45

Table 2.10 Isotherm error deviation data related to the adsorption of Co onto the soil

Isotherms	Error functions	No biosurfactant	1 CMC Surfactin	2 CMC Surfactin	1 CMC Trehalose lipids	2 CMC Trehalose lipids	1 CMC Rhamnolipid	2 CMC Rhamnolipid
Langmuir	R^2	0.9886	0.9806	0.9536	0.9839	0.9811	0.9879	0.9914
	Adjusted R^2	0.9848	0.9742	0.9382	0.9785	0.9749	0.9838	0.9885
	RMSE	3.039	3.93	6.086	3.609	3.893	3.096	2.605
Freundlich	R^2	0.9931	0.9822	0.9603	0.9884	0.9427	0.9879	0.9993
	Adjusted R^2	0.9908	0.9763	0.9471	0.9845	0.9236	0.9839	0.9991
	RMSE	2.368	3.767	5.63	3.064	6.786	3.085	0.7281
Temkin	R^2	0.8774	0.8839	0.6079	0.8353	0.9983	0.8964	0.9301
	Adjusted R^2	0.5095	0.5358	-0.5686	0.3414	0.9931	0.5857	0.7202
	RMSE	17.25	16.66	30.66	19.98	2.038	15.66	12.85
Redlich-Peterson	R^2	0.9931	0.984	0.8326	0.9931	0.9918	0.9908	0.9936
	Adjusted R^2	0.9861	0.9679	0.6653	0.9863	0.9835	0.9817	0.9871
	RMSE	2.9	4.379	14.16	2.886	3.149	3.293	2.756

2.4 SUMMARY

In this study, Co adsorption in saline soil with and without the existence of a biosurfactant was examined. The impact of pH, contact time, initial Co concentration, and type/dose of biosurfactant on Co adsorption were evaluated and three biosurfactants including surfactin, trehalose lipids and rhamnolipid products were used. At a low initial concentration of Co, increasing surfactin concentration has a positive impact on the remaining Co in the solution. On the other hand, at a high concentration of Co, the concentration of biosurfactant does not have significant impact on the remaining Co. Surfactin was seen to be weakly sorbed in the soil. Trehalose lipids at any concentration reduced the remaining Co in the solution, compared with surfactin. Studying the adsorption characteristics of Trehalose lipids results in the high capacity of adsorption by the soil. When applying rhamnolipid, by increasing the initial concentration of Co, the remaining Co in the solution increased considerably at any initial concentration of rhamnolipid. The high value range of surface tension of solutions (60-70 mN/m) shows that rhamnnolipid has a high tendency of adsorption to the soil surface.

Adsorption kinetics were also explored. The kinetic parameters were investigated using the pseudo-first-order, pseudo-second-order, as well as the Elovich and intraparticle diffusion rate models. The adsorption kinetic data fit the best in the pseudo-second-order model with the largest regression coefficient (R^2) obtained and the similarity between the Co equilibrium concentration based on model calculation ($q_{e\text{-}cal}$ = 51.8134 mg/g) and on experimental data (q_e = 51.6591 mg/g). The intraparticle diffusion model indicated that boundary layer diffusion affects the adsorption rate.

Langmuir, Freundlich, Temkin and Redlich-Peterson adsorption models were applied for the isotherm investigation. Results indicated that with no biosurfactant addition, the adsorption isotherm was well described by the Freundlich model. In the system with surfactin addition, the Redlich-Peterson and Freundlich models can show the adsorption the best with 1 CMC and 2 CMC, respectively. When using trehalose lipids and rhamnolipid, the best fitting isotherms were the Redlich-Peterson isotherm (1 CMC of trehalose lipids and rhamnolipid), Temkin isotherm (2 CMC of trehalose lipids), and Freundlich isotherm (2 CMC of rhamnolipid), respectively. The highest adsorption of Co (Q_0: 3.753×10^4 mg/g) was achieved when 1 CMC of Trehalose lipids was introduced to the soil.

This study identified that Co has a strong adsorption capacity in low permeable saline soil. The research findings based on the Co adsorption kinetics and isotherms can help to explore the related mechanisms and the impact of biosurfactants on the fate of Co in soil, so as to aid the screening of proper soil remediation technologies.

When 1 CMC of surfactin was added, the greatest value of R^2 (0.984) was achieved using the Redlich-Peterson model to fit the experimental data. When the surfactin concentration increased to 2 CMC, the greatest value of R^2 (0.9603) was obtained using the Freundlich model. The Freundlich model implies that multilayer adsorption is assumed on the heterogeneous surface, and $n < 0.5$ indicates unfavorable adsorption (Dolatkhah and Wilson, 2016). The n value (0.8922) achieved using the Freundlich isotherm indicated a reasonable adsorption.

For trehalose lipids addition at a concentration of 1 CMC and 2 CMC, the Redlich-Peterson isotherm and Temkin isotherm show good performance for fitting the data, respectively. The Temkin model is extracted by simulating that relation between change in the heat of adsorption (ΔH_{ads}) with θ being linear (Rostamian et al., 2011). In terms of the maximum adsorption capacity (Q_0) for the Co adsorption in soil, the Langmuir isotherm (Table 2.9) resulted in the greatest Q_0 (3.753×10^4 mg/g) in the 1 CMC trehalose lipids system. When using rhamnolipid at 1 and 2 CMC concentrations, the Redlich-Peterson fit the data the best in the 1 CMC system and the Freundlich model was the best in the 2 CMC system. The Redlich-Peterson isotherm resulted in the R^2 values ranging between 0.984 – 0.9936 in all systems, except for the one with an addition of 2 CMC of surfactin.

CHAPTER 3 INTERACTIVE PARAMETERS ON THE ADSORPTION BEHAVIOURS OF COBALT ONTO SALINE SOIL WITH DIFFERENT BIOSURFACTANTS

3.1 BACKGROUND

Co is toxic and non-degradable with significant bioaccumulation. The understanding of the effect of interactive parameters on Co adsorption in soil can facilitate the prediction of the mobility of Co and its remediation strategy. The aim of this study is thus to fill the knowledge gaps identified in Chapter 1 by using a statistical design of experiment methodology (i.e., full factorial design) to examine interactive parameters on Co adsorption onto saline soil. The full factorial design is well established to qualified the interactions between main factors.

3.2 MATERIALS AND METHODS

3.2.1 Soil Preparation

To assess adsorption of Co, batch equilibrium experiments were performed using the soil including 50% natural clay and 50% sand. The soil was dried and kept in an oven at 105° C overnight followed by removing gravel and rocks. The soil was firstly sieved to pass through a 2 mm opening size stainless-steel mesh, and then a fraction of the sieved soil was sieved again through a 0.59 mm opening size stainless-steel mesh. NaCl was added to obtain 3% saline soil.

Soil properties including pH, soil bulk density, soil water content, soil cation exchange capacity and major element oxides as well as trace element concentrations were characterized, in chapter 2.

3.2.2 Biosurfactant Production

Two different lab-generated biosurfactants were applied. Both biosurfactants were generated in the NRPOP Laboratory at Memorial University of Newfoundland. The effect of each biosurfactant on Co adsorption in soil was studied. The first one, surfactin, was produced by *Bacillus subtilis* N3-1P isolated from oily seawater (Cai et al., 2014). This product is a lipopeptide biosurfactant which can reduce water surface tension significantly (Zhu et al., 2016). The hyper-production mutant, *Rhodococcus erythropolis* M36, was generated through three runs of UV mutagenesis and productivity screening of a wild strain isolated from produced water samples from offshore Newfoundland, Canada (Cai et al., 2016). The second type of biosurfactant product was generated by this hyper-production mutant and was identified as glycolipids (trehalose lipids).

3.2.3 Co Adsorption onto Soil with Biosurfactant Addition

Co adsorption onto saline soil under various initial Co concentrations, dose/type of biosurfactants and size of soil particles was conducted. The soil was air dried, homogenized, and kept in an oven at 105°C for 1 day. Each 2.9 g sample of 3% saline soil was equilibrated in a polypropylene centrifuge tube with 50 ml of Co solution followed by the addition of a biosurfactant product. Solutions with different Co concentrations (i.e., 100 ppm, 200 ppm, 300 ppm) were prepared. Two different types of biosurfactant products (surfactin and trehalose lipids) were applied at three concentrations (0.5, 1 and 1.5 CMC). Three different sizes of sand, including: 1) 2 mm - 0.59, 2) ≤ 0.59 mm, and 3) 1:1 mixture of 1) and 2), were used in the adsorption process. The clay used was ≤ 0.59 mm.

Applied pH level (9) and reaction time (4 hrs.) for conducting the Co adsorption onto the soil were selected based on previous one-factor-at-time (OFAT) study stated in Chapter 2. Samples before adsorption experiments were adjusted to pH 9 by 1 M HCI or 1 M NaOH solution. During adsorption, the pH level of each run was kept consistent by adding small volumes of 0.1 M HCI or 0.1 M NaOH solution. Co adsorption was performed by shaking each tube in a shaker at 200 rpm at the room temperature for 4 hours. Once reaching the ultimate equilibrium status, the tube was centrifuged at 6,000 rpm for 30 min. The aliquots of supernatant solution were acidified to pH=2 using 2% HNO_3 for flame atomic absorption spectroscopy measurement to determine the Co concentration remaining in the solution after each adsorption treatment. A calibration curve for Co was constructed using standard solutions.

3.2.4 Experiment Design

DESIGN EXPERT 8.0.6 statistical software (Stat-Ease, Inc. Minneapolis, USA) was used to design and analyse the data from the experiment. The 2^4 factorial design was conducted in two levels, high and low. The lower level of factors was coded as '-1' and the higher level was coded as '+1'. The full factorial design results in 16 runs with 6 center points with possible combinations of factors A, B, C and D at the selected levels. The mathematical equation in term of coded factors is expressed as:

$$Y = a_0 + a_1A + a_2B + a_3C + a_4D + a_5AB + a_6AC + a_7AD + a_8BC + a_9BD + a_{10}CD + a_{11}ABC + a_{12}ABD + a_{13}ACD + a_{14}BCD + a_{15}ABCD \tag{3.1}$$

where Y is theoretical yield function (Remaining Co). The a_0, a_{1-4} and a_{5-15} represent global mean, and the regression coefficients corresponding to the main factor effects and interactions, respectively.

The factors and levels include the size of sand particles (≤ 0.59 mm and between 2 mm and 0.59 mm), biosurfactant concentration (0.5 CMC and 1.5 CMC), type of biosurfactant (surfactin and trehalose lipids), initial Co concentration (100 mg/L and 300 mg/L) (Table 3.1). The levels of design were selected based on results from our previous OFAT study. The center points were triplicated for both surfactin and trehalose lipids. The response variable is the concentration of the remaining Co in the solution. Table 3.2 lists the experimental layout of the factorial design.

Table 3.1 Factors and levels selected for factorial design

Factor	Type	Code	Low (-1)	High (+1)
Size of particle (mm)	Numeric	A	0.59	2
Biosurfactant concentration (CMC)	Numeric	B	0.5	1.5
Type of biosurfactant	Categorical	C	Surfactin	Trehalose lipids
Initial Co concentration (mg/L)	Numeric	D	100	300

Table 3.2 Experimental layout of the factorial design

Standard order	Run	Factor 1 Particles size mm	Factor 2 Biosurfactant concentration CMC	Factor 3 Type of biosurfactant	Factor 4 Initial Co concentration mg/L
1	1	-1	-1	Surfactin	-1
5	2	-1	-1	Trehalose lipids	-1
22	3	0	0	Trehalose lipids	0
18	4	0	0	Trehalose lipids	0
3	5	-1	1	Surfactin	-1
19	6	0	0	Surfactin	0
15	7	-1	1	Trehalose lipids	1
20	8	0	0	Trehalose lipids	0
6	9	1	-1	Trehalose lipids	-1
14	10	1	-1	Trehalose lipids	1
17	11	0	0	Surfactin	0
2	12	1	-1	Surfactin	-1
16	13	1	1	Trehalose lipids	1
9	14	-1	-1	Surfactin	1
12	15	1	1	Surfactin	1
21	16	0	0	Surfactin	0
11	17	-1	1	Surfactin	1
10	18	1	-1	Surfactin	1
13	19	-1	-1	Trehalose lipids	1
4	20	1	1	Surfactin	-1
7	21	-1	1	Trehalose lipids	-1
8	22	1	1	Trehalose lipids	-1

3.3 RESULTS AND DISCUSSION

3.3.1 Experimental results and analysis of variance (ANOVA)

The experimental results for each run under various conditions are listed in Table 3.3. The concentrations of the final Co remaining were found in the range of 0.5-1.9. The results of ANOVA for the full factorial model are listed in Table 3.4. The interacting factors affecting the Co adsorption onto the soil were determined by performing the analysis of ANOVA in Table 3.4. ANOVA is generally conducted to survey the reliability of the model (Khataee et al., 2011). ANOVA illustrates whether the variations obtained from a model is significant compared to the variations related to the experimental error (Khataee et al., 2012). A sum of squares (SS) of each factor shows its significance in the process. When the value of the SS increases, the importance of factor in the experiencing process increases. The p-value of model ($0.0345 < 0.05$) and lack of fit ($0.4082 > 0.05$) indicate the model is significant with good fitting. The main effects and interactions of each factor with p-value less than 0.05 are considered as significant. The size of particle-biosurfactant concentration-type of biosurfactant-initial Co concentration interaction (ABCD) has the greatest effect on the residual concentration of Co in the solution, followed by the type of biosurfactant (C). Among the significant terms, size of particle (A) and biosurfactant concentration-type of biosurfactant interaction (BC) have the lowest effect with p-values equal to 0.0429. It should be noted that p-values of AC (0.0788), ABC (0.0580) and BCD (0.0788) are around the α-level (0.05) and can be considered as significant with more than 90% confidence. The interactions and main effects that are not significant were kept in the model to obey the model hierarchical rule as ABCD is significant.

Table 3.3 Factorial design matrix of four factors along with experimental results for remaining Co in the solution after adsorption

Standard Run #	Coded values of independent factors				Remaining Co
	A	B	C	D	Observed
1	- 1	- 1	- 1	- 1	0.5
2	+ 1	- 1	- 1	- 1	1.6
3	- 1	+ 1	- 1	- 1	0.5
4	+ 1	+ 1	- 1	- 1	1.1
5	- 1	- 1	+ 1	- 1	0.9
6	+ 1	- 1	+ 1	- 1	0.7
7	- 1	+ 1	+ 1	- 1	1.4
8	+ 1	+ 1	+ 1	- 1	1.8
9	- 1	- 1	- 1	+ 1	1.2
10	+ 1	- 1	- 1	+ 1	0.8
11	- 1	+ 1	- 1	+ 1	0.6
12	+ 1	+ 1	- 1	+ 1	1.4
13	- 1	- 1	+ 1	+ 1	0.8
14	+ 1	- 1	+ 1	+ 1	1.8
15	- 1	+ 1	+ 1	+ 1	1.9
16	+ 1	+ 1	+ 1	+ 1	0.9
17	0	0	- 1	0	1.5
18	0	0	+ 1	0	1.3
19	0	0	- 1	0	1
20	0	0	+ 1	0	1.5
21	0	0	- 1	0	1
22	0	0	+ 1	0	1.3

Table 3.4 ANOVA for the full factorial model (signifiacance determined with 95% confidence interval)

Source	Sum of Squares	df	Mean Square	F-Value	p-value	
Model	3.49	15	0.23	4.61	0.0345	Significant
A	0.33	1	0.33	6.56	0.0429	Significant
B	0.11	1	0.11	2.09	0.1980	
C	0.44	1	0.44	8.66	0.0258	Significant
D	0.051	1	0.051	1.00	0.3551	
AB	0.031	1	0.031	0.61	0.4655	
AC	0.23	1	0.23	4.47	0.0788	
AD	0.14	1	0.14	2.79	0.146	
BC	0.33	1	0.33	6.56	0.0429	Significant
BD	0.051	1	0.051	1.00	0.3551	
CD	5.63E-03	1	5.625E-003	0.11	0.7498	
ABC	0.28	1	0.28	5.46	0.0580	

ABD	0.051	1	0.051	1.00	0.3551	
ACD	0.076	1	0.076	1.50	0.2667	
BCD	0.23	1	0.23	4.47	0.0788	
ABCD	1.16	1	1.16	22.91	0.0030	Significant
Lack of Fit	0.11	2	0.055	1.13	0.4082	Not significant

3.3.2 Model statistics

An extended statistical analysis of the model is listed in Table 3.5. The mean and standard deviation of the data set were 1.16 and 0.22, respectively. The model R^2 value was at 0.9202, indicating 92.02% of variability in the response was explained by the explanatory variables. The adjusted R^2 is a measure of the amount of variation about the mean explained by the model. Its value of 0.7208 indicated 72.08% of variability in the response could be explained by the variables after the adjustment for the number of parameters in the model relative to the number of points in the design. Adequate precision is a measure of the signal to noise ratio and the value of 7.15 (> the desirable value 4) indicated an adequate signal. Coefficient of Variation (C.V. %) is the error expressed as a percentage of the mean. The predicted residual sum of squares (PRESS) is a form of cross-validation used in regression analysis to provide a summary measure of the fit of a model to a sample of observations that were not themselves used to estimate the model. PRESS is a measure of how well a particular model fits each point in the design. Both C.V.% and PRESS can be used to evaluate the performances of various models. The lower values indicate better structure of the candidate models. However, the predicted R^2 was found to be -5.8477. The negative value of predicted R^2 was resulted as PRESS value is higher than the total sum of squares, which can be caused when the noise is too high. A negative predicted R^2 implies that the overall mean is a better predictor of the responses than the selected model. In such case, it can be concluded that the model described the experimental data well but is not suitable for the prediction.

Table 3.5 Extented statistical results

Standard deviation	0.22	R^2	0.9202
Mean	1.16	Adjusted R^2	0.7208
C.V.%	19.38	Predicted R^2	-5.8477
PRESS	25.97	Adequate Precision	7.15

3.3.3 Recommendations for adjustment

Despite the factorial design, the responses have very little variability. The obtained models were found inadequate to predict the results. In order to improve the quality of the data inputs for the design, it is recommended that the levels of biosurfactant concentration to be adjusted to a wider range. Firstly, an OFAT will be used to determine how much CMC of biosurfactant when added to the system can cause a significantly higher final Co remaining concentration in the solution. Based on such findings, another factorial design can then be used to assist in the analysis of how the main effects and interactions impact the final Co remaining concentration in the solution. Moreover, the established models will help simulate and predict the Co adsorption behavior with the presence of the selected biosurfactants in the range of the tested conditions.

3.4 SUMMARY

Saline soil was applied for the Co adsorption from its aqueous solution in the presence of lab produced biosurfactants, including surfactin and trehalose lipids. The effect of size of soil particles, type/dose of biosurfactant and initial Co concentration on the adsorption were studied with the 22 experiments using the 2^4 full factorial design. The p-value of model (0.0345) and lack of fit (0.4082) show the model is significant with good fitting. Effect of significant terms on remaining Co after adsorption in the solution increases in the order of interaction ABCD > main factor C > main factor A and interaction BC. The coefficient of determination of regression model (R^2) indicates that 92.02% of the variations in response variable would be explained by the model. The adjust R^2 obtained is equal to 72.08% which means that the model is fit enough for present observations.

However, the obtained model was found with a Predicted R^2 value of -5.8477. A negative Predicted R^2 implies that the overall mean is a better predictor of the responses than the selected model. Furthermore, ABCD being the largest effect clearly shows that the model does not make sense. In view of such results, the ranges of the various factors would need to be adjusted to enhance the signal to noise ratio in follow-up experiments before a predictive model can be developed.

CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

This dissertation research has focused on the Co adsorption on saline soil with/without biosurfactants to shed light on the mobility and behaviors of Co in the subsurface environments, so as to provide support on assessing environmental risks of Co contamination and guidance on the selection of soil remediation techniques.

Firstly, Co adsorption onto saline soil was investigated using the one-factor-at-a-time approach and the corresponding kinetics and isotherms were determined. Three biosurfactant products (i.e., Surfactin, Trehalose lipids, Rhamnolipid) each at two concentrations (1 CMC and 2 CMC) were applied during Co adsorption. At a low initial concentration of Co, increasing surfactin concentration has a positive impact on the remaining Co in the solution. On the other hand, at a high concentration of Co, the concentration of biosurfactant does not have significant impact on the remaining Co. Surfactin was seen to be weakly sorbed in the soil. Trehalose lipids at any concentration reduced the remaining Co in the solution, compared with surfactin. Studying the adsorption characteristics of Trehalose lipids results in the high capacity of adsorption by the soil. When applying rhamnolipid, by increasing the initial concentration of Co, the remaining Co in the solution increased considerably at any initial concentration of rhamnolipid. The high value range of surface tension of solutions (60-70 mN/m) shows that rhamnnolipid has a high tendency of adsorption to the soil surface. The kinetic parameters were investigated using the pseudo-first-order, pseudo-second-order, as well as the Elovich and intraparticle diffusion rate models. The adsorption kinetic data fit the best in the pseudo-second-order model with the largest regression coefficient (R^2) obtained and the similarity between the Co equilibrium concentration based on model calculation ($q_{e\text{-}cal}$ = 51.8134 mg/g) and on experimental data (q_e = 51.6591 mg/g). The intraparticle diffusion model indicated that boundary layer diffusion affects the adsorption rate.

Secondly, Interactive parameters on the adsorption behaviors of cobalt onto saline soil with different biosurfactants were examined using a statistically and mathematically supported manner (i.e., full factorial design). A 2^4 factorial design was conducted in two levels, which consists of 22 runs with possible combinations of four factors, i.e. size of soil particles (A), biosurfactant concentration (B), type of biosurfactant (C) and initial Co concentration (D). A linear mathematical model presenting the effect of the factors and their interactions. Analysis of variance (ANOVA) table was applied to study the effects and interaction effects among parameters. The results show interaction of ABCD exerts the most significant influence on the remaining Co. Effect of significant terms on response increase in the order of ABCD > C > A and BC. The R^2 and adjust R^2 obtained are equal to 92.02% and 72.08%, respectively. However, the obtained model was found with a predicted R^2 value of -5.8477. A negative predicted R^2 implies that the overall mean is a better predictor of the responses than the selected model. The ranges of the various factors would need to be adjusted to enhance the signal to noise ratio in follow-up experiments before a predictive model can be developed.

The findings illustrated the adsorption kinetics and isotherms used to estimate mobility of Co in the environment and predict the suitable remediation method for Co contaminated soil.

4.2 RESEARCH ACHIEVEMENTS

It is the first study on Co adsorption on saline soil using three types of biosurfactants produced by three different bacterial strains. It found the impacts of these biosurfactants on the adsorption of Co in soil and their roles in shifting the adsorption kinetics and isotherms. It is also the first study investigating Co adsorption using a statistically and mathematically supported manner, i.e., full factorial design. Although the experiment did not provide an adequate predictive model, it did provide some guidance for follow-up eperiments. The kinetics, isotherms, interactive parameters, and optimization condtions can be of great value to both scientific understanding and the environmental applications involving Co subsurface contamination.

4.3 RECOMMENDATIONS FOR FUTURE RESEARCH

The study can be further extended as follows:

(1) Desorption kinetics and isotherm can be added into the study and correlate with the adsorption studies. A desorption hysteresis (irreversibility) index based on the Freundlich exponent, concentration-dependent metal distribution coefficients, and the irreversibility index based on the metal distribution coefficient can be used to quantify hysteretic behavior observed in the systems.

(2) Based on findings stated in Chapter 3, the formation of assemblies plays an important role in Co adsorption. The use electron Microscopy technique will thus be proposed to study biosurfactant morphology in the presence of Co when considering the

effects of pH and possibly co-contaminated hydrocarbons. The findings may facilitate the understanding of mechanisms behind low Co mobility in the subsurface environment.

(3) Follow-up experiments by increasing the ranges of the factors studied in Chapter 3 so that a statistically valid predictive model of remaining Co can be achieved. The experimental design may also need to be modified in case of non-linearity in the behaviour of the remaining Co.

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