Chapter 1

1. Introduction

1.1. Background

The South African Environmental Management Act 107 of 1998 states that everyone has the right to have the environment protected for the benefit of present and future generations, through reasonable legislative and other measures that prevent pollution and ecological degradation (DEAT, 2009). It also promotes conservation and secure ecological sustainable development and use of natural resources while promoting justifiable economic and social development. However, an increase in the industrial development throughout the world leads to high levels of pollution which seriously threatens the natural resources upon which humankind and other living forms depend for their survival.

Chapman (2007) considered contamination as pollution when the presence of pollutants results in adverse biological effects to resident communities. Pollutants can be biodegradable materials that rapidly decompose by natural processes or nondegradable materials that either do not decompose or decompose slowly in the natural environment. Basically, there are three types of pollution, namely: air pollution, land/soil pollution and water pollution. Amongst pollutants, metals can be distinguished from all other pollutants, since they are non-degradable and can accumulate in living tissues, causing various diseases and disorders (Malik, 2004; Gode and Pehlivan, 2005; Pehlivan, 2006). In South Africa, the Minister of Environmental Affairs and Tourism commented that South Africa is highly industrialized; hence at times it carries the burden of industrial pollution including metals and synthetic organic pollutants (DEAT, 2000). These pollutants are not easily removed by conventional treatment technologies (chemical precipitation, reverse osmosis, etc.) which are presently used by the different wastewater treatment plants; hence the concern is not only for the potable use of water, but

also for the aquatic life and the organisms indirectly dependent on aquatic life (DEAT, 2000).

The effective mechanisms to deal with this unavoidable waste are necessary and much greater attention must be directed to the introduction of preventive strategies aimed at waste minimization and pollution prevention. Various countries have set standards in legislation dealing with the concentration levels of heavy metals that are believed to be low enough to protect public health. World Health Organization (WHO) and other environmental agencies have also specified safe limits of heavy metals in drinking water as well as water used for other purposes (WHO, 1997). Although South Africa has extensive environmental pollution and waste management legislation, the responsibility for its implementation is spread over a number of departments and institutions (DEAT, 2000).

Environmental pollution by toxic metals also occurs globally through military, industrial and agricultural processes and waste disposal. For example, in previous years, it was estimated that fuel and power industries annually generate 2.4 million tons of arsenic, cadmium, chromium, copper, mercury, lead, vanadium and zinc (Brower *et al*., 1997; Pagnanelli *et al*., 2000; Veglio *et al*., 2003). Metal manufacturing industries, agricultural practices, and waste disposal also add several million tons per year of the same metals to the environment (Pagnanelli *et al*., 2000). In addition, mineral processing and extractive-metallurgical operations generate toxic liquid wastes (Ahluwalia and Goyal, 2007). All these are activities that human life depends on and are practiced on daily basis and contribute to further pollution of the environment with heavy metals.

Although pollution generated by human activities presents a very serious problem, natural processes have been reported to immobilize heavy metals. For example, through natural erosion processes like weathering and abrasion of rocks, soils and sediments by wind and water, a small but significant fraction of

natural metals are continuously being mobilized and transported in the environment (Raab and Fieldman, 2003). Volcanic eruptions, forest fires and aerosol formation above the sea also contribute to the natural transport of metals. These processes cause cycling of metals in the environment, resulting in natural background levels in the air, surface waters and soil (Mighal *et al.*, 2002; Raab and Fieldman, 2003).

The present study focuses on contamination of water with two toxic heavy metals, cadmium and copper. Water, the most valuable natural resource on our planet and the main source of life, is critical and it is the most limiting natural resource in South Africa (DWAF, 1986; Roux, 1990). The quality of water is affected by the presence of metals, and this compels different municipalities to monitor the levels of these pollutants in water.

Different conventional technologies are routinely used for the treatment of heavy metal polluted wastewater but the application of such processes is often limited because of technical and economic challenges. Chemical precipitation, ion-exchange, chemical oxidation and reduction, filtration, electrochemical treatment, membrane technologies, etc. are commonly applied to the treatment of industrial wastewater containing heavy metals (Veglio *et al*., 2003; Potgieter *et al*., 2006; Ahluwalia and Goyal, 2007). These processes may be ineffective or extremely expensive when metals in solution are present in the range of 1-100 mg L^{-1}, and they often require a high skill for operation and use of hazardous chemicals (Liu *et al*., 2004; Zouboulis *et al*., 2004; Ahluwalia and Goyal, 2007). Another disadvantage of the conventional treatment technologies is the production of toxic sludge as a result of the chemicals used (Gupta *et al*., 2000), which is often difficult to dewater and also requires extreme caution in its disposal (Kapoor *et al*., 1999). Unpredictable metal ion removal and high reagent requirements are also disadvantageous (Barros, *et al*., 2003).

Amongst chemical adsorbents, ion exchange resins are considered as the option for remediation with least ecological problems (Gupta *et al*., 2000). The treated water/effluent is often sufficiently pure that it can be recycled and reused, and the sorbed metals can be recovered and purified in efficient regeneration processes (McKay, 1995). However, the chemical resins are expensive and increasing demand for eco-friendly technologies has led to the search for low-cost alternatives (Gupta *et al*., 2000). Environmentally friendly processes need to be developed to clean up the metal contaminated environment without creating harmful waste products and to reduce metal content in wastewater or discharge to acceptable levels at affordable cost. The need for effective and economically viable technologies is driven by strict regulations with regard to the metal discharges which are enforced particularly in industrialized countries. Toxicological studies also confirm the dangerous impacts of toxic heavy metals in living systems (Rana, 2008; Zhou *et al*., 2008).

Therefore, there is a challenging task to develop appropriate low cost technologies for treatment of wastewater in order to provide clean water. The search for alternative and innovative treatment techniques has focused attention on the use of biological materials such as algae, fungi, yeast and bacteria for the removal and recovery technologies and this has gained importance during recent years because of the better performance and lower-cost of these biological materials (Kratochvil and Volesky, 1998; Volesky, 2001; Iqbal and Edyvean, 2004). Besides flexibility to handle the range of physico-chemical parameters in effluents, selectivity to remove only the desired metals and the cost-effectiveness are some of the added advantages of biological clean-up techniques (Malik, 2004). Many microorganisms have been intensively examined for their abilities to be applied in bioremediation of toxic heavy metals since microbial biomass can passively bind large amounts of metals during biosorption and provide a cost-effective solution for wastewater management (Malik, 2004).

1.2. Literature review

1.2.1. South African water quality guidelines

Regulatory constrains on the concentration of an individual contaminant vary according to the intended water use and specific contaminant. Four broad categories of water uses are recognized in the South African Water Act, namely: domestic, agricultural, industrial and aquatic purposes (DWAF, 1991). The quality of water can affect the intended use of water. Firstly, if water is intended for consumption by living organisms, the health of the organisms will be negatively affected if the quality of water is poor. Secondly, the productivity or yield of a crop being irrigated and also the biodiversity of aquatic ecosystems can be affected by the poor quality of water (DWAF, 1991). As suggested by the World Health Organization (2004), it is essential to monitor the quality of water. The main purposes of water quality guidelines are to protect public health and to support the development and implementation of risk management strategies that will ensure water safety through the control of hazardous constituents of water. These strategies may include regional or national standards developed from the scientific basis provided in the guidelines (WHO, 2004). The nature and form of water quality guidelines may vary among countries and regions because of the different approaches, therefore, it is essential that each country reviews its needs and capacities in developing regulatory frameworks (WHO, 2004). It is also important that the recommended water quality guideline values are both practical and feasible to implement as well as being protective. The guideline values are normally set as concentrations lower than the detection limits achievable under routine laboratory operating conditions and are established taking into account available technologies for controlling, removing or reducing the concentration of the contaminants to the desired levels (WHO, 2004).

The main challenges which disturb improving water quality are the sophistication of the technologies required to treat water to adequate quality and also the cost

of treating water before it can be used. For different water supplies, the Department of Water and Environmental Affairs formerly called the Department of Water Affairs and Forestry, as a South African custodian has established Maximum Contaminant levels (MCL) as indicated in Table 1.1, for different water uses. This guideline takes into account the important developments in risk assessment and its linkages to risk management.

Table 1.1

South African Water Quality guidelines (in mg L^{-1}) for different water uses where no health effects are expected (Adapted from DWAF, 1996).

Water quality constituents	Aquatic water use	Domestic water use	Agricultural water use		
			Livestock	Irrigation	Aquaculture
Cadmium	0.00015	0.005	1	0.1	0.05
Chromium	0.007	0.05	0.01	0.01	0.0002
Copper	0.0003	1	1	0.1	0.002
Lead	0.0002	0.1	0.5	0.2	0.01
Mercury	0.00004	0.001	0.001	-	0.000001
Nickel	-	-	1	0.2	-
Zinc	0.002	3	20	1	0.03

- No guideline is given

Table 1.2

International target values (in mg L^{-1}) for natural seawater environment (Adapted from DWAF, 1996).

Water quality constituents	US-EPA	EEC	Australia	South Africa
Cadmium	0.0093	0.0025	0.002	0. 0015
Chromium	0.050	0.015	0.002	0.007
Copper	0.0029	0.005	0.005	0.000 3
Lead	0.0056	0.025	0.005	0.000 2
Mercury	0.000025	0.0003	0.0001	0. 000004
Nickel	0.140	0.030	0.015	-
Zinc	0.170	0.040	0.050	0.002

- No guideline is given

There is a vast difference between the South African water quality standards and international standards with respect to other contaminants, especially the heavy metals, as shown in Table 1.2. The South African standard levels of heavy

metals in natural seawater are lower than those stipulated for other international seawater environments (DWAF, 1996).

1.2.2. **Heavy metal pollution in South Africa**

South Africa is rich in mineral resources and is one of the leading mineral raw and processed material exporters in the world. The main mineral raw materials are gold, diamonds, platinum, chromium, vanadium, manganese, uranium, iron ore and coal (USGS, 2009). Experts believe that there is still considerable potential for the discovery of other world-class mineral deposits in some areas in South Africa that are yet to be fully exploited. Mining has been the main driving force behind the history and development of Africa's most advanced and richest economy (USGS, 2005). Large scale and profitable mining started with the discovery of diamond on the banks of Orange River in 1867 by Erasmus Jacobs and the subsequent discovery and exploration of the Kimberley pipes a few years later. Gold rushes to Pilgrim's Rest and Barberton were precursors to the biggest discovery of all, the Main Reef Leader on Gerhardus Oosthuizen's farm Langlaagte, Portion C, in 1886, the Witwatersrand Gold Rush and the subsequent rapid development of the gold field. Presently, South Africa experiences negative environmental impact from mining activities, including heavy metal pollution. Pollution from mining activities is probably the most direct cause of groundwater contamination in the country.

Despite prolific mining activities, there is still little information available in South Africa on pollution and the fate of trace metals in surface waters or sediments. This has been attributed to relatively few studies that have been undertaken on levels of heavy metals (Roux *et al*., 1994; Okonkwo and Mothiba, 2005) and primarily because of lack of enforcement of stringent regulatory guidelines in the past and instrumental limitations (Alakendra and Starke, 2006).

The dewatering processes of the mines and disposal of wastes contribute to environmental heavy metal pollution. Alakendra and Starke (2006) described one of the mines which was famous for its prolific gold, coal and uranium deposits and that mining activity had been going on since the late 1800s. One of the activities in the mine was that mine water was pumped out from the shaft on recurrent basis and disposed in the surrounding environment. Also, as mining operations were closed, leaving behind a myriad of shafts underground, water flooded the underground openings and decanted to other deeper mines which were interlinked. Mines which were still active pumped large volumes of mine water to the surface environment (Alakendra and Starke, 2006). This had a negative impact on the environment since it became polluted with toxic heavy metals.

One of the potential hazards of mining operations is that as water flows through settlements around mining operations, heavy metals are dispersed in the environment. Some parts of the country (Gauteng Province), where there are more of these mining operations, experience poor water quality which is likely to impact the fresh water resources on long term basis. For example, a study by Alakendra and Starke (2006) on impact assessment of dewatering of mine waters in the East Rand Blesbokspruit, reported traces of metal concentration higher than the world average concentrations measured in rivers.

Trace metals have little degradation potential and they tend to accumulate in sediments which form metal-rich deposits and with continued accumulation of metals in the sediment, the environmental threshold is often exceeded causing toxicity (Schulin *et al*., 1995). Furthermore, a change in oxidation state can easily remobilize sequestered trace metals making the metal-rich deposits a potential long term source of metal pollution. The metals can then bioaccumulate moving up the food chain causing genotoxicity among living organisms (Patra *et al*., 2004).

Fatoki and Mathabatha (2001) found pollution from both point, as well as, diffuse sources such as urban runoff in and around the East London and Port Elizabeth harbors though the concentrations of the heavy metals were low. Fatoki and Muyima (2003) also published a pollution study on the Umtata River which showed cadmium levels ranging from 0.01 mg L^{-1} to 0.08 mg L^{-1} (the 1996 DWAF guideline value was 0.005 mg L^{-1}). They speculated that this was due to the chemical waste discharge from a wood processing factory situated near the source of the river as well as effluent discharge from Umtata Sewage Treatment Plant. Urban run-off, nickel-cadmium based batteries from the rural communities that had been disposed in the waste disposal sites and run-off from agricultural soils in the catchment also contributed to pollution in the river (Fatoki and Muyima, 2003). As indicated by Fatoki *et al*. (2004), water in the Umtata River was used for various purposes by a large population of the Transkei for agricultural, recreational and mostly for domestic purposes. Hence, heavy metal pollution had a great impact on the society around and the aquatic life at large. Because of this problem, it was further recommended that there should be monitoring and removal of wastes close to the river banks, management of agricultural practices and a valid permit obtained by the Umtata Sewage Treatment Plant to comply with Department of Water Affairs and Forestry effluent regulations according to the National Water Act, 1998. In addition, the development and implementation of routine education programmes to the rural community in order to protect the river was recommended (Fatoki *et al*., 2004). In a follow-up study, the cadmium levels had dropped to acceptable levels indicating the success of the intervention program (Fatoki *et al*., 2004).

Okonkwo and Mothiba (2005) found cadmium and lead contamination in the three rivers (Dzindi, Madanzhe and Mvudi) of Thohoyandou, Limpopo Province of South Africa although Thohoyandou was not known for its large-scale industrial activity. On the other hand, the Nyl River and its flooded plain which fall within the Waterberg catchment area were subjected to various potential impacts via anthropogenic activities, such as mining and farming, as well as the

associated problems caused by formal and informal settlements. However, it has been reported by Greenfield *et al*. (2007) in a study targeting sediment quality that the metals did not appear to pose any environmental problem.

Awofolu *et al*. (2005) studied the levels of trace metals in water and sediment from Tyume River in the Eastern Cape of South Africa and its effect on an irrigated farmland. They found high levels of cadmium (0.038 mg L^{-1}) and lead (0.044 mg L^{-1}) in the river, which could be directly detrimental to aquatic ecosystems and indirectly to man since the river water was used to irrigate a nearby farmland. Thawley *et al*. (2004) studied the accumulation of Zn and Cd in *Potamonautes warreni* (freshwater crab population) from a number of sites in the North-West Province of South Africa that had been heavily mined for gold, diamonds and platinum and therefore vulnerable to metal pollution from the industry. Analysis of water samples indicated the presence of Zn and Cd (in the range 0.00009-0.163 µg mL^{-1}) and accumulation of these metals in a crab population.

1.2.3. **Heavy metal pollution management strategies**

It can be expected that large-scale industrial development will take place in South Africa as a developing country. Unfortunately, the developments may lead to pollution unless certain precautions are taken. The development of towns and industries in the country and the associated accumulation of wastes in built-up areas during the past years induced problems concerning environmental pollution (DWAF, 1991). Precautions can, however, be costly and are therefore not always enforced (Lazewski, 2000).

Industrial management must comply with stipulated standard figures for metal content in discharge wastes before disposal in wastewater streams which are eventually discharged into waterways containing aquatic life. The standard figures are designed to simulate typical aquatic life and to monitor toxicity of the

effluent to such a life. In most developed and developing countries, strict environmental regulations with regard to contaminants discharged from industrial operations, have been introduced (Atkinson *et al*., 1998). Suggestions have been made to develop on-site or in-plant facilities for effluent treatment in order to minimize the contaminant concentrations to acceptable limits prior to their discharge (Banat *et al*., 1996; Vijayaraghavan and Yun, 2008[b]). The impact of industry on water sources is immense and it is only through promotion of good pollution prevention practices that contamination and deterioration of these waters will decrease (Atkinson *et al*., 1998). It is, therefore, the responsibility of various water authorities to inform industry of the methods available to them and to encourage implementation of such practices to safeguard the water environment. In a survey conducted by Umgeni Water (KwaZulu-Natal, South Africa) it was found that regionally, industrial discharges are responsible for 56 % of the contamination present in water sources. However, tremendous effort has been made by this regulatory body to educate senior management within the companies concerned about their legal, social and environmental obligations and ways to improve operational practices (Umgeni Water, 1997).

Therefore, this calls for a continuous assessment of water quality that will assist in making informed management decisions. The application of the methods used for remediation purposes, specifically heavy metal remediation, has to be improved and the cost of remediation should be minimal because of the burden created by pollution from industrialization. The most eco-friendly and cost-effective method for heavy metal remediation is the application of naturally occurring biosorbents, e.g. microbes (Kratochvil and Volesky, 1998; Volesky, 2001; Iqbal and Edyvean, 2004).

1.2.4. **Biosorption and its application in heavy metal polluted wastewater**

Many procedures have been applied for the treatment of heavy metal polluted water. Among the most commonly used techniques are chemical precipitation,

reverse osmosis, electro-dialysis, ion exchange, chemical oxidation or reduction, solvent extraction and filtration (Xia and Liyuan, 2002; Ozdes *et al*., 2009). However, these methods have several disadvantages (Volesky, 2001; Hanif *et al*., 2007). Precipitation is the most common method for removing heavy metals from water (Ahluwaliya and Goyal, 2007). Nevertheless, its efficiency is affected by low pH and it requires addition of other chemicals which finally leads to generation of large volumes of toxic sludge (Gupta *et al*., 2000). The disposal of toxic sludge is cost intensive (Gray, 1999; Kapoor *et al.*, 1999). Ion exchange is used successfully for the removal of heavy metals from effluents although it is relatively expensive as compared to other methods (Gupta *et al*., 2000). In addition, the ion exchange resins are prone to fouling by precipitates and organics (Ahluwaliya and Goyal, 2007). Reverse osmosis and electro-dialysis involve the use of expensive semi-permeable membranes at high pressures for the recovery of metals from dilute wastewater (Volesky, 2001).

Disadvantages of the conventional technologies for the treatment of heavy metal polluted wastewater have been summarized and are shown in Table 1.3. This has led to the search for efficient, eco-friendly and cost-effective technologies for treatment of heavy metal polluted wastewater. Alternative metal removal and/or recovery methods being considered are based on metal-sequestering properties of certain natural materials of biological origin similar to chemical oxidation/ion exchange (Kuyucak and Volesky, 1989). Certain types of microbial biomass can retain relatively high quantities of metal ions by biosorption and/or bioaccumulation, thus providing a cost-effective solution for wastewater management (Terry and Stone, 2001; Akhtar *et al*., 2004; Öztürk *et al*., 2004).

Table 1.3

Conventional technologies for metal removal from wastewaters (Adapted from Volesky, 2001)

Method	Disadvantages	Advantages
Chemical precipitation	Difficult separation Disposal of resulting toxic sludge Not very effective	Simple Relatively cheap
Chemical oxidation or reduction	Chemicals required (not universal) Climate sensitive For high metal concentrations	Mineralization
Electrochemical treatment	Applicable for high metal concentrations Sensitive to specific conditions such as the presence of certain interfering compounds	Metal recovery
Reverse osmosis	Application of high pressures Membrane scaling/fouling Expensive	Pure effluent/permeate (available for recycling)
Ion exchange	Sensitive to the presence of particles Expensive resins Resins are prone to fouling leading to decreased capacity Disposal of spent resins is expensive (incineration)	Effective Possible metal recovery
Adsorption (e.g. activated carbon)	Not very effective for certain metals	Conventional sorbents
Evaporation	Energy intensive Expensive Resulting sludge	Pure effluent (for recycle)

1.2.4.1. **Biosorption**

Biosorption is defined as the ability of biological materials (living or dead) to form complexes with metal ions using functional groups on their outer surfaces. Biosorption does not consume cellular energy. Heavy metal ions can be adsorbed and/or complexed to either living or dead biomass (Lovley and Coates, 1997). Biosorption is influenced by a number of physico-chemical mechanisms, depending on a number of external environmental factors as well as on the type of metal, its ionic state and the type of active binding site responsible for sequestering the metal. An important feature of biosorption is that it can be

responsible for binding metal ions even when the cell is no longer metabolically active (non-viable). The remaining cell debris such as cell walls can still represent a potent biosorbent. Biosorption is a metabolism-independent process which is a function of chemical makeup of the cell's outer protective envelope, the cell wall. This passive physico-chemical uptake of heavy metals takes place in many different ways and is dependent on the degree of affinity resulting in different types of binding between metallic species or its ionic forms and an active site of a molecular structure of the cell wall. Positively charged metal ions are sequestered, primarily through the adsorption of metals onto the negative ionic groups (SH^-, COO^-, OH^-, etc.) on cell surfaces or on extracellular polysaccharides (Lovley and Coates, 1997; Malik, 2004). However, on prolonged contact with the metal-bearing solution, the living biomass may also be able to sequester the metal intracellularly by an active process known as bioaccumulation (Gupta *et al*., 2000).

1.2.4.2. **Bioaccumulation**

Bioaccumulation is the accumulation of materials which are not critical components of an organism by that organism. Usually, it refers to the accumulation of metals (Bains, 1993). Heavy metal ions accumulate in microbial cells through membrane transport proteins by active transport (Nies, 1999). Various microbial species including bacteria, fungi, algae and actinomycetes have been shown to be efficient in bioaccumulation of heavy metal ions from polluted effluents (Yakubo and Dudeney, 1986; Premuzic *et al*., 1991; Wong *et al*., 1993). In comparison with biosorption, bioaccumulation is a growth-dependent process (Gupta *et al*., 2000).Transport of the metal across the cell membrane, which is dependent on the cell's metabolism, yields intracellular accumulation, thus this transportation process must take place only in viable cells. The transport process may be mediated by the same metabolism used to convey metabolically important ions such as potassium, magnesium and sodium (Nies, 1999).

1.2.4.3. **Comparison of biosorption and bioaccumulation**

Several studies on the application of growing microbial cells for metal removal (bioaccumulation process) have shown that they are better than non-viable cells due to the microbe's ability of self-replenishment. In addition, bioaccumulation allows a continuous metabolic uptake of metals after physical adsorption (Malik, 2004). Although the use of living microbes may allow development of a single-stage process for removal of most pollutants from the industrial effluents, there are significant practical limitations to the uptake by living cell systems. The limitations include sensitivity of the systems to extreme pH, high metal/salt concentration and the requirement of external metabolic energy (Dönmez and Aksu, 2001).

The biosorption process possesses certain inherent advantages over bioaccumulation process which have been extensively discussed (Vijayaraghavan and Yun, 2008[a]). The cost of biosorption is usually low, and most biosorbents used are industrial, agricultural and other type of waste biomass whereas in bioaccumulation, the process involves living cells and cell maintenance is costly. Biosorption is usually more rapid than bioaccumulation since the viable cells require time to grow and the intracellular accumulation of metals is also time-consuming (Vijayaraghavan and Yun, 2008[b]). Some studies reveal that the solution pH is the most important parameter affecting the biosorption process (Vasudevan *et al*., 2002; Nuhoglu and Oguz, 2003). Other factors affecting the biosorption process include initial concentration of the metal salt, temperature, contact time, agitation speed as well as the concentration of the biosorbent (microbes applied for bioremediation) in solution (Nuhoglu and Oguz, 2003; Akhtar *et al.*, 2004; Iqbal and Edyvean, 2004).

1.2.5. **Recycling/regeneration of used biomass**

Metals can be desorbed readily and then recovered and purified in regeneration processes if the value and amount of metals recovered are significant (McKay, 1995). The separation and recovery of metal can be done in a form which it can be processed further to the desired final product (either single metal or metal compound). This can be accomplished by following the desorption process when subjecting the heavy metal-loaded biomass to a small volume of desorbing agent (e.g. sodium acetate, sodium chloride, calcium, mercaptoethanol, hydrochloric acid, etc.) that will elute the metal ions from a biosorbent (Deng *et al*., 2008). The possibility of regeneration of spent biomass is important to keeping the cost of remediation down. The efficiency of microbial biosorbents can be evaluated by following adsorption-desorption processes for a number of cycles to see if the microbial biosorbents can still be effective in metal adsorption from wastewater. The recovery process should not cause biosorbent physico-chemical damage. Biosorbents can be regenerated for multiple reuse, offering the metal recovery possibility from concentrated wash solutions whereas metal biosorption in wastewater can meet the environmental discharge criteria. If biomass is plentiful, it can be incinerated, hence eliminating further treatment (Ahluwalia and Goyal, 2007).

1.2.6. **Biosorption mechanisms**

Utilization of biomass in general (Veglio *et al*., 1997; Kratochvil and Volesky, 1998; McKay *et al*., 1999) is considered to be the best alternative for water purification (Sag *et al*., 1998; Savvaidis, 1998; Tobin and Roux, 1998). The passive removal of heavy metals such as Cd, Cu, Zn, Co, Ni, Pb and Hg by inexpensive biomaterials, termed biosorption, requires that the substrate displays high metal uptake and selectivity, as well as suitable mechanical properties for applied bioremediation scenarios.

Microbial walls are composed of macromolecules (chitin, chitosan, glucan, lipids, proteins), which contain carboxyl groups (COO^-), amino groups (NH_3^+), phosphates (PO_4^{2-}), sulphates (SO_3^-) and hydroxides (OH^-) (Fogarty and Tobin, 1996; Kapoor and Viraraghavan, 1998[a, b]; Kapoor *et al*., 1999). These functional groups (Fig. 1.1) are metal sorption sites (Mashitah *et al*., 1999; Tereshina *et al*., 1999; Zhou, 1999).

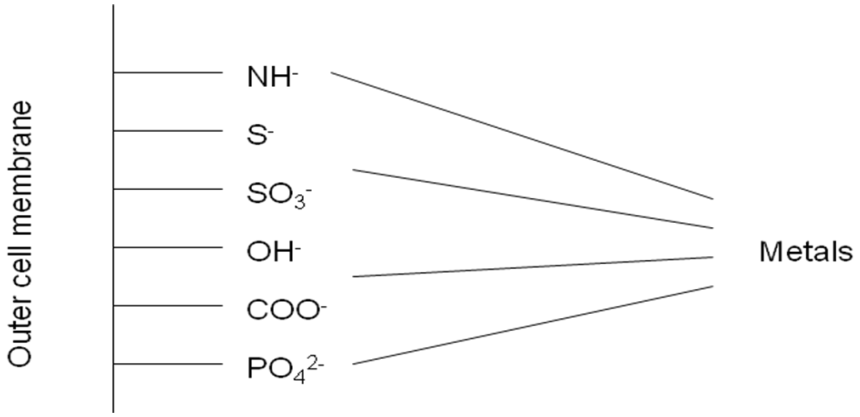

Fig. 1.1. Adsorption sites for the toxic heavy metals (Adapted from Gazsó, 2001).

The metal adsorption capacity of the microbial biomass is directly related to the presence of these various functional groups on microbial membranes (Mullen *et al*., 1989; Volesky, 1990; Fourest and Volesky, 1996). The role of carboxylic groups in the adsorption process has been clearly demonstrated by reduction in cadmium and lead uptake by microbial biomass following partial or complete esterification of the carboxylic sites (Davis *et al.,* 2003). Various metal-binding mechanisms have also been postulated to be active in biosorption. These mechanisms include chemisorption by ion exchange, complexation and chelation, physical adsorption, microprecipitation and oxidation/reduction (Volesky, 2001).

Due to the complexity of the biomaterials used, it is possible that at least some of these mechanisms act simultaneously to varying degrees, depending on the biosorbent and the solution environment. Through these various metal-binding

mechanisms, microbes are able to remove metals from contaminated water (Huang and Huang, 1996; Kapoor and Viraraghavan, 1997; Sarret *et al*., 1998). There are possible oxidation-reduction reactions taking place in the biosorbent (Coulibaly *et al*., 2003). When metals are removed by ionic exchange, they generally replace K^+, Mg^{2+}, Ca^{2+} and H^+ contained in biomasses (Gomes *et al*., 1999; Mashitah *et al*., 1999; Zhou, 1999). Metal sequestrations by microbes are influenced by the mineral and organic composition of the medium in which biomasses are produced. Biomass physiological states (living or dead), co-ions, metal concentration and physical parameters (temperature, pH, ionic strength, presence of others metals) also influence metal removal from polluted waters (Yu and Kaewsarn, 1999; Zhou, 1999; Coulibaly *et al*., 2003). Metals are sometimes completely removed by microbes from various raw effluents (gold mining effluent, tanning effluent, swine water and polluted lake waters); however, the outputs depend on the metal and microbes involved (Coulibaly *et al*., 2003). There is a wide variety of microorganisms, including bacteria (e.g. *Bacillus* spp), fungi (e.g. *Aspergilus* sp.), yeast (e.g. *Saccharomyces cereviciae*), and algae (e.g. *Chlorella* sp.) that can interact with metals through several mechanisms to transform them (Poole and Gadd, 1989; Gupta *et al*., 2000).

Some biomasses undergo physicochemical treatments (e.g. soda or acidic treatments, insertion of functional groups, heat treatment, etc.) to increase metal sorption capacities of the biomasses (Coulibaly *et al*., 2003). Biosorbents can also be treated with different ionic forms such as protons (H^+) or saturated with Na^+, Ca^{2+}, Mg^{2+}, etc. or treating with mineral acids, bases and/or salts (Gupta *et al*., 2000). The treatment varies with the biomass type and the metal species to be biosorbed. A number of microbial biomasses in general, have been treated and their performances in metal biosorption have improved (Kramer and Meisch, 1999; Yin *et al*., 1999; Yan and Viraraghavan, 2000). The biosorption capacity of *Streptomyces rimosus*, fungal biomass, for the removal of zinc was increased from 30 to 80 mg g^{-1} following chemical treatment with sodium hydroxide (Mameri *et al*., 1999). *Cystoseria indica*, algal biomass, was pretreated with hydrochloric

acid to improve the biosorption capacity for the removal of uranium (Khani *et al*., 2008). Another algal biomass of *Oedogonium* sp. was pretreated with hydrochloric acid, nitric oxide, sodium hydroxide, ammonium acetate methanol, acetone and hot water to increase its biosorption capacity for the removal of Cd(II) (Gupta and Rastogi, 2008).

Finally, biotechnological exploitation of biosorption technology for removal of heavy metals depends on the efficiency of the regeneration of biosorbent after metal desorption (Gupta *et al*., 2000). Microbial biomasses that have sequestered metals can be regenerated following washing with a desorbing agent, e.g. nitric acid, 0.05 M HNO_3, and/or with 0.1 M Ca^{2+}, Mg^{2+} and K^{+} (Akthar *et al*., 1996; Kapoor *et al*., 1999; Coulibaly *et al*., 2003). The regenerated biomass can be used again in metal removal process (Iqbal and Edyvean, 2004). Desorption of metals from loaded biomass can be achieved by the basic understanding of the mechanism involved, in particular, metal sequestration. As a physico-chemical phenomenon, metal ions which show a marked pH dependence in binding to the microbial cells can be desorbed by pH adjustment as reported in the case of *Chlorella vulgaris* for the desorption of Cu(II), Cd(II) and Cr(II), by lowering pH (Beveridge and Fyfe, 1985). It is also important that the desorption process does not effect the cell architecture as this can result in reduction of biosorption capacity of the biomass (Kuyucak and Volesky, 1989).

1.2.6.1. **Physical adsorption**

In physical adsorption, metal ions which are positively charged are sequestered through adsorption to the anionic groups on cell surfaces or extracellular polysaccharides. For example, electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and algae *Chlorella vulgaris* (Aksu *et al*., 1992).

1.2.6.2. **Ion exchange**

Microbial cell walls contain polysaccharides and during biosorption, heavy metal ions exchange with the ions of the polysaccharides. For example, the alignates of marine algae occur as salts of K^+, Na^+, Ca^{2+} and Mg^{2+}. These ions can exchange with counter ions such as Co^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky, 1989). The biosorption of some fungal species also occur by ion-exchange mechanism (Muraleedharan and Venkobachar, 1990).

1.2.6.3. **Complexation**

Metal removal from contaminated solutions may also take place by complex formation on the cell surface after interaction between the metal and the active groups. Metal ions form complexes with negatively charged groups (COO^-, PO_4^{2-}, OH^-, SO_3^-) of lipopolysaccharides and glycoproteins on microbial cells (Gupta *et al*., 2000). Complexation was found to be the only mechanism responsible for metal accumulation in a bacterial species, *Pseudomonas syringae*, (Hall *et al*., 2001). Microbes may also produce organic acids (citric, oxalic, gluconic, fumaric, lactic and malic acids), which may chelate toxic metals and result in the formation of metallo-organic molecules. Some metals may be sorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers (Crist *et al.*, 1992).

1.2.6.4. **Precipitation**

Precipitation may be either metabolism-dependent or metabolism-independent. In metabolism-dependent process, the removal of metal from solution is often associated with active defense systems of the microbe in response to metal exposure. Some microbes produce compounds which favor the precipitation of heavy metals (Nies, 1999). Precipitation mechanism was reported in *Citrobacter*

sp. which involved a phosphate-mediated cleavage of glycerol-2-phosphate to release phosphate ion (HPO_4^{2-}) which precipitated Cd(II) on the cell surface as insoluble metal phosphate, $CdHPO_4$ (Ahuja *et al*., 1999). Metabolism independent process may be a consequence of the chemical interaction between the metal and the cell surface (Gadd, 1993).

1.2.7. **Biosorbents**

Biosorbents used may have the following physiological characteristics suitable for metal biosorption: hardness, porosity, particle size, density and resistance to a broad spectrum of variable solution parameters, such as temperature, pH and solvent content, etc. (Kim, 2004). There are a number of natural biosorbents used for biosorption of heavy metals. Microbes extensively studied for biosorption include the fungi (Cho and Kim, 2003; Prasanjit and Sumathi, 2005), yeast (Yavuz *et al*., 2006), algae (Cordeo *et al*., 2004; Mohapatra and Gupta, 2005; Padilha *et al.*, 2005) and bacteria (Ozdemir *et al*., 2003; Salehizadeh and Shojaosadati, 2003; Melo and D'Souza, 2004). Plant derived biomasses have also been used for biosorption of metals (Verma and Shukla, 2000; Inbaraj and Sulochana, 2004; Ho, 2005). The potential of any biosorbent for bioremediation of metals is dependent on the chemical makeup of the biosorbent for reasons already discussed above.

It is also more beneficial to look for biosorbents that are readily available in large quantities to support potential demand. While choosing a biomaterial for metal sorption, its origin is a major factor to be considered. A biosorbent may be obtained as a by-product of fermentation industry, or organisms naturally available in large quantities in nature or organisms cultivated for biosorption purposes using inexpensive media.

1.2.8. **Bacterial morphology and its role in heavy metal ion removal**

The identification of the microbial morphology is also crucial as it gives information about the cell wall for metal biosorption. Bacteria can be divided into two major groups, namely; Gram-positive and Gram-negative cells. They differ markedly in the appearance and composition of their cell walls (Cabeen and Jacobs-Wagner, 2005). Summary diagrams of Gram-positive and Gram-negative bacterial cell walls are shown in Fig. 1.2. The Gram-positive cell wall is composed of a thick, multilayered peptidoglycan sheath outside of the cytoplasmic membrane. Teichoic acids are linked to and embedded in the peptidoglycan, and lipoteichoic acids extend into the cytoplasmic membrane. The main structural component of Gram-positive bacteria cell walls is peptidogylcan, a polymer made up of carbohydrate strands cross-linked by a small peptide. The peptide contains a glutamic acid residue, whose carboxylate side chain is probably important in metal binding. The peptidogylcan is linked to a second polymer, teichoic acid or teichuronic acid (Fig. 1.2a). Cell walls of Gram-negative bacteria are made of two membrane bilayers. The outer membrane contains lipopolysaccharide and is usually linked chemically to a peptidoglycan monolayer which lies between the outer and plasma membrane (Fig. 1.2b).

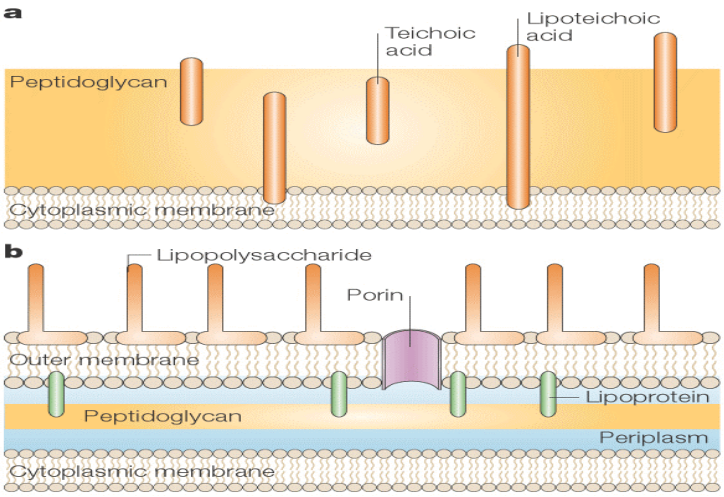

Fig. 1.2. Summary diagram of Gram-positive (a) and Gram-negative (b) bacterial cell walls (Cabeen and Jacobs-Wagner, 2005).

Several types of metals (magnesium, zinc, cadmium, etc.) accumulate, with some being heavy metals that upon accumulation contaminate biomass (Smith *et al*., 1994). Bacterial surfaces are typically anionic and, therefore, interact with metal cations (Beveridge, 1981; Beveridge *et al*., 1982). Gram-positive bacterial walls consist of a variety of hetero- and homopolymers which in combination produce an electronegative charge density throughout the wall fabric at neutral pH (Beveridge, 1981). Evidence suggests that peptidoglycan layer is responsible for most of the metal deposition (Matthews *et al*., 1979; Beveridge and Murray, 1980; Beveridge *et al*., 1982). Interactions between microorganisms and metals can be conveniently divided into three distinct processes (Ford *et al*., 1995), all of which may be important in heavy metal bioremediation: i.e. intracellular interactions, cell-surface interactions, and extracellular interactions. The three processes are not mutually exclusive.

With intracellular interactions, assimilation of metals (zinc, iron, magnesium, phosphate, etc.) may be important to the microbe in detoxification, enzyme function and physical characteristics of the cell. Cell surfaces contain functional groups (e.g., carboxylic, amino, thiol, hydroxy, and hydroxy-carboxylic groups) that can interact with metal ions (Xue *et al*., 1988). Gram-negative bacteria possess lipopolysaccharides and phospholipids in their cell walls, with phosphoryl groups as the most abundant electronegative sites available for metal binding (Coughlin *et al*., 1983; Ferris, 1989). Gram-positive bacterial cell walls possess teichoic acids and peptidoglycan, providing carboxyl and phosphoryl groups that are potential sites for metal binding (Beveridge *et al*., 1982; Doyle, 1989). Metal binding to cell-surface functional groups is thought to be an important step to intracellular accumulation of trace metals required for enzyme function in Gram-negative and Gram-positive bacteria.

Many microorganisms produce extracellular polysaccharides (EPS), often containing proteins that strongly bind metals (Black *et al*., 1986; Chanmugathas and Bollag, 1988). Extracellular polymeric substances play an important role in

biosorption of heavy metals and their presence increases the efficiency of biosorption (Oliveira-Martins *et al*., 2008). Interactions between EPS and metal ions are generally considered as a direct consequence of negatively charged functional groups on the exopolymer. The EPS comprise a mixture of polysaccharides, mucopolysaccharides and proteins, depending on the microbial strain and culture conditions (Oliveira-Martins *et al*., 2008). They contain ionizable functional groups such as pyruvyl, phosphoryl, hydroxyl, succinyl, and uronyl and amine groups. Ion-exchange, complexation with negatively functional groups, adsorption and precipitation are the mechanisms involved in metal biosorption onto EPS (Zang *et al*., 2006). In addition, siderophores are low molecular weight organic compounds produced by a number of microorganisms to sequester metals and are thought to be highly specific (Davis and Byers, 1971).

1.2.9. **Biosorption experimental procedures**

A biosorption process can be performed via several modes, of which batch or continuous modes of operation are frequently employed to conduct laboratory scale biosorption processes. A schematic diagram of batch biosorption experimental procedure is shown in Fig. 1.3. Although most industrial applications prefer a continuous mode, batch experiments have often been used to evaluate the required fundamental information, such as biosorbent efficiency, optimum experimental conditions, biosorption rate and possibility of biomass regeneration (Vijayaraghavan and Yun, 2008[b]). Batch experiments usually focus on the study of factors affecting biosorption, which are important in the evaluation of the full biosorption potential of any biosorbent. These include the pH of metal polluted solution to be studied, temperature at which the biosorption process would be carried out, ionic strength of a metal ions to be adsorbed, biosorbent dosage and size, initial metal ion concentration, the speed of agitation and time required to reach equilibrium (Iqbal and Edyvean, 2004; Vijayaraghavan and Yun, 2008[b]).

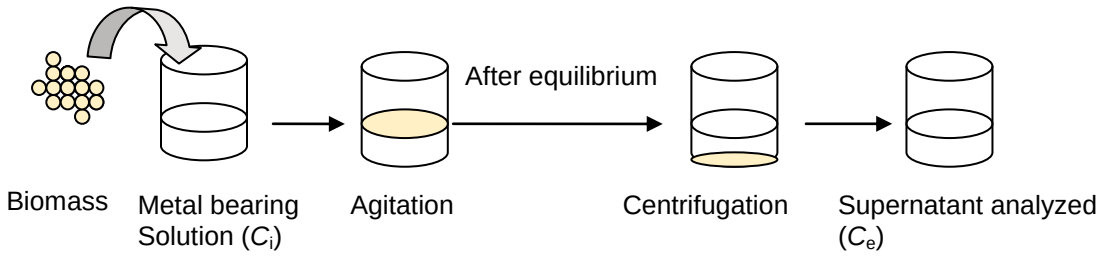

Fig. 1.3. Schematic diagram of batch biosorption experimental procedure. C_i and C_e are the initial and equilibrium metal ion concentrations (mg L^{-1}), respectively.

Earlier studies have shown that the most critical parameter in the treatment of heavy metal with biosorbent material is the initial pH of the sorption medium (Esposito *et al*., 2002; Vijayaraghavan and Yun, 2008[b]). It affects the solution chemistry of the metal ions and the activity of the functional groups of the biosorbent. The pH strongly influences the speciation and biosorption availability of the metal ions. Some of the metal ions precipitate at high pH values, which may complicate the biosorption process (Vijayaraghavan and Yun, 2008[b]). The different biosorbents also exhibit different pH optima for the sorption of the different metals during biosorption which means that a further increase or reduction of pH reduces the metal uptake.

1.2.10. **Equilibrium Modeling**

Biosorption has been studied as a simplified sorption system, usually containing one heavy metal. Equilibrium data at a given temperature, commonly known as adsorption isotherms, are basic requirements for the design of adsorption processes. Biosorption isotherms, a plot of solute uptake (q) versus the equilibrium solute concentration in the solution (C_e), are often used to evaluate the sorption performance (Vijayaraghavan and Yun, 2008[b]). Adsorption isotherms have been commonly used to describe experimental results for the sorption of metal ions by microorganisms and other biosorbents. Although there are a number of adsorption isotherms (Kuyucak and Volesky, 1990), the most

commonly used adsorption isotherms are the Langmuir and Freundlich equilibrium models (Liu *et al*., 2004; Rangsayatorn *et al*., 2004; Febrianto *et al*., 2009). The Langmuir and Freundlich adsorption isotherms are widely used models for single solute system and therefore, were used in this study to fit the equilibrium biosorption data for cadmium and copper. Other equations contain two fitting parameters (Dubinin Radushkevich, Braunauer, Emmer and Teller (BET) models) whereas others can have more than two parameters (Redlich Peterson model). A particular model may not apply in a particular situation, and in some cases more than one model may explain the biosorption mechanism. There is no critical reason to use a more-complex model if a two-parameter model (such as the Langmuir and Freundlich isotherm models) can fit the data reasonably well (Vijayaraghavan *et al*., 2006). Table 1.4 shows the adsorption models which are generally used to describe biosorption data.

Since biosorption is metabolism-independent, it would be expected to be a rapid process. Initial rapid uptake is often observed, and it is believed to be due to binding of metal ions to the cell wall. Many studies have also shown that at low metal ion concentrations, the amount of metal ion accumulated per unit mass of a cell is directly proportional to the concentration of the metal ion in a solution (Omar, 2002).

Metal sorption capacity (q) for the construction of sorption isotherms is determined as follows:

$$q = [V(C_i - C_f)]/M \qquad (1)$$

where C_i and C_f are the initial and final metal ion concentrations (mg L^{-1}), respectively; V is the volume of sample solution (L); and M is the dry weight of added biomass (g).

Table 1.4

Adsorption models used to describe the biosorption data (Adapted from Kuyucak and Volesky, 1990).

Isotherm	Equation	Advantages	Disadvantages
Langmuir	$q_e = (K_L q_{max} C_e)/(1 + K_L C_e)$	Interpretable parameters	Not structured; monolayer sorption
Freundlich	$q_e = K_F C_e^{1/n}$	Simple expression	Not structured
Combination of Langmuir and Freundlich	$q_e = (K_L q_{max} C_e^{1/n})/1 + K_L C_e^{1/n}$	Combination of the above two	Unnecessarily complicated
Radke and Prausnitz	$1/q_e = 1/aC_e + 1/K_L C_e^{B}$	Simple expression	Empirical; requires three parameters
Redlich Peterson	$q_e = (aC_e^{n})/(1 + K_L C_e)$	Approaches Freundlich at higher concentrations	No significant advantages
Braunauer, Emmer and Teller (BET)	$q_e = (\beta C Q^{0})/[(C_s - C)(1 + (B-1)C/C_s]$	Multilayer adsorption	-
Dubinin Radushkevich	$W/W_o = \exp[-K(\varepsilon/p)^2]$	Temperature-independent	Behavior is not limited in the Henry's law regime

- None

1.2.10.1. **Langmuir adsorption isotherm**

The most widely used adsorption isotherm for the description of adsorption equilibrium is the Langmuir isotherm model. The Langmuir equation relates the coverage of molecules on a solid surface to concentration of a medium to solid surface at affixed temperature (Febrianto *et al*., 2009). This isotherm is based on three assumptions, namely, adsorption is limited to monolayer coverage, all surface sites are alike and only can accommodate one adsorbed atom and the ability of a molecule to be adsorbed on a given site is independent of its neighboring site occupancy. The classical Langmuir equation is given as follows:

$$q_e = (q_{max} K_L C_e)/(1 + K_L C_e) \qquad (2)$$

where,

q_e = metal uptake on the biosorbent at equilibrium (mg g^{-1} dry weight); q_{max} = maximum possible amount of metallic ion adsorbed per unit weight of adsorbent;

K_L = Langmuir adsorption constant related to the affinity of the binding sites for metals;

C_e = equilibrium concentration of metal (mg L^{-1}) in the solution.

The Langmuir equation can be linearised as follows:

$$C_e/q_e = 1/K_L q_{max} + C_e/q_{max} \quad (3)$$

The maximum adsorption capacity (q_{max}) and the equilibrium constant (K_L) can be obtained from a linear plot of C_e/q_e versus C_e (Aksu, 2001). The Langmuir constant, q_{max}, is often used to compare the performance of biosorbents whereas the other constant, K_L, characterizes the initial slope of the isotherm. For a good biosorbent, a high q_{max} and a steep initial isotherm slope (i.e. high K_L) are generally desirable (Vijaragavan and Yun, 2008[b]).

As suggested by Hall *et al*. (2001), the equilibrium parameter (K_R) values can be used to predict whether a sorption system (isotherm) is favorable or unfavorable using the essential features of the Langmuir adsorption isotherm. The K_R can be expressed in terms of a dimensionless constant or separation factor or equilibrium parameter, K_R, which is defined by the following relationship:

$$K_R = 1/(1+K_L C_i) \quad (4)$$

where K_R is the equilibrium parameter, K_L is the Langmuir adsorption constant and C_i is the initial concentration of the metal ion in a solution. The parameter indicates the shape of the isotherm accordingly (Table 1.4).

Table 1.5

K_R values for the prediction of the type of isotherm

Values of K_R	Type of isotherm
$K_R > 1$	Unfavorable
$_R = 1$	Linear
$0 < K_R < 1$	Favorable
$K_R = 0$	Irreversible

1.2.10.2. **Freundlich adsorption isotherm.**

The Freundlich adsorption isotherm relates the concentration of an adsorbed metal to the concentration of the metal in the liquid with which it is in contact at equilibrium (Freundlich, 1906).

The linear isotherm model assumes that all sites on the sorbent have equal affinity for the solute (Sawyer *et al.*, 2003). The empirical Freundlich equation based on sorption on a heterogeneous surface is given as follows:

$$q_e = K_F C_e^{1/n} \qquad (5)$$

where,

q_e = metal adsorbed on the sorbent at equilibrium (mg g^{-1} dry weight);

C_e = metal ion concentration at equilibrium (mg L^{-1}) in the solution;

K_F = an empirical constant that provides an indication of the adsorption capacity of biosorbent;

n = an empirical constant that provides an indication of the intensity of adsorption.

The Freundlich equation can be linearized as follows:

$$\ln q_e = \ln K_F + (1/n) \ln C_e \qquad (6)$$

The Freundlich adsorption constants (K_F and n) can be obtained by plotting $\ln q_e$ as a function of $\ln C_e$. High K_F shows easy uptake of the metal and high n values indicate high ability of metal sorption.

1.2.11. **Kinetic modeling**

In order to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes, kinetic models have been used to test experimental data. When the biomass is used as a cell free suspension in a well-agitated batch system, all the cell wall binding sites are made readily available for metal uptake so that the effect of external film diffusion on biosorption rate can be assumed not significant and ignored in any engineering analysis (Wehrheim and Wettern, 1994). Information on the kinetics of metal uptake is required to select the optimum conditions for full-scale batch metal removal processes. A number of kinetic models have been developed to describe the kinetics of heavy metal removal. The kinetic models include pseudo-first order, second order, pseudo-second order and saturation (mixed order) rate equations. Frequently, pseudo-first order and pseudo-second order rate equations have been widely used to describe biosorption data (Liu and Liu, 2008).

The pseudo first-order rate expression based on solid adsorption capacity is generally expressed as follows:

$$dq/dt = k_1 (q_e - q_t) \qquad (7)$$

where q_e and q_t are the amounts of adsorbed metal ions on the biosorbent at equilibrium and the amount of adsorbed metal ions (mg g^{-1}) at time t, respectively, and k_1 is the rate constant of pseudo-first order biosorption (min^{-1}).

The integrated form becomes

$$\ln(q_e - q_t) = \ln q_e - k_1 (t) \quad (8)$$

A straight line of $\ln(q_e - q_t)$ versus t suggests the applicability of this kinetic model (Aksu, 2001). The slope of a plot of $\ln(q_e - q_t)$ versus t can be used to calculate the pseudo-first order rate constant k_1 (Mamisahebei *et al*., 2007).

The pseudo second-order equation is also based on the sorption capacity of the solid phase and has been frequently employed to analyze biosorption data obtained from various experiments using different adsorbates and adsorbents (Ho *et al*., 2000). The pseudo-second order kinetic model is expressed as:

$$t/q_t = 1/k_2 q_e^2 + 1/q_e (t) \quad (9)$$

where q_e is the amount of metal adsorbed at equilibrium (mg g^{-1}) for pseudo second-order biosorption, q_t (mg g^{-1}) is the amount of metal ion adsorbed at time t and k_2 is the equilibrium rate constant of pseudo-second order biosorption (g mg^{-1} min^{-1}). Values of k_2 and q_e are calculated from the plot of t/q_t against t (Aksu, 2001).

The second order adsorption kinetic model is given as

$$1/(q_e - q_t) = 1/q_e + k_3 t \quad (10)$$

where k_3 is the rate constant of second order adsorption (g mg^{-1} min^{-1}). The slope and intercepts of a plot of $1/(q_e - q_t)$ versus t are used determine the second order rate constant k_3.

The saturation (mixed-order) rate equation is given by

$$1/t \ln(C_i - C_t) = -k_o/k_4 - 1/k_4 [(C_i - C_t)/t] \quad (11)$$

where k_0 and k_4 are the mixed order rate constants, C_i and C_t are the metal concentrations. Mixed order rate constants can be determined from a plot of $1/t \ln(C_i/C_t)$ versus $(C_i - C_t)/t]$ (Mamisahebei *et al*., 2007).

1.2.12. **Thermodynamic parameters of biosorption**

The *Gibbs* free energy change is the fundamental criterion of spontaneity of a process and can be determined using the following equilibrium constant equation:

$$\Delta G^o = -RT \ln K_c \quad (12)$$

where K_c is an equilibrium constant ($K_c = C_{ad}/C_e$), C_{ad} is the amount of metal ion adsorbed, C_e is the equilibrium concentration of the metal ion in a solution (mg L^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}) and T is the absolute temperature (K).

The relationship between ΔG^o, ΔH^o and ΔS^o can be expressed by the following equation (Ozdes *et al*., 2009):

$$\Delta G^o = \Delta H^o - T\Delta S^o \quad (13)$$

This equation can be written as:

$$\ln K_c = \Delta S^o/R - \Delta H^o/RT \quad (14)$$

where ΔH^o is the enthalpy (kJ mol^{-1}) of the reaction which can be obtained from the slope and ΔS^o is the entropy (J mol^{-1} K^{-1}) of the reaction which can be obtained from the y-intercept of the plot of $\ln K_c$ versus $1/T$ (Dursun, 2006; Uslu and Tanyol, 2006; Ozdes *et al*., 2009). The *Gibbs* free energy and the entropy are used to determine which process will occur spontaneously whereas the enthalpy refers to the heat of the reaction.

1.3. **Research motivation**

In a previous study, Sekhula *et al*., (2005) described bacterial resistance to different metal ions [Cd(II), Cu(II), Ni(II), Co(II) and Zn(II)]. Of the three bacterial cultures studied (GM 15, GM 16 and GM 17), it was found that GM 16 was resistant in the range of 60-80 % to 5 mM of Cd(II), Cu(II), Ni(II), Co(II) and Zn(II). Therefore, GM 16 was further investigated for its potential in the biosorption of Cd(II) and Cu(II) using viable cells. The initial concentration of each metal ion used for the sorption studies was 100 mg L^{-1} at pH 7. The biosorption capacities of viable GM 16 cells in the treatment of heavy metal contaminated medium were found to be 65 % in the removal of Cu(II) and 48 % in the removal of Cd(II). Therefore, it was decided to study the biosorption of Cd(II) and Cu(II) using the non-viable GM 16 biomass so as to compare with the biosorption of the viable biomass. Since GM 16 was an unidentified isolate from water sample collected from an antimony mine, it was also found necessary to identify it as a test organism used in this study.

1.4. **Aim and objectives**

1.4.1. **Aim of the research**

The aim of this research was to investigate the biosorption of Cd(II) and Cu(II) by non-viable GM 16 cells, study the environmental factors affecting the biosorption of Cd(II) and Cu(II), to determine the kinetic properties of the biosorption and to

identify the bacterial isolate GM 16 isolated from an antimony mine in South Africa.

1.4.2. **Objectives**

The specific objectives are:

- Identification of bacterial morphology into a Gram-positive or Gram-negative.
- 16S rDNA amplification using polymerase chain reaction (PCR) and sequencing.
- Determining the phylogenetic relationship of isolate GM 16 and other bacterial cultures isolated from an antimony mine.
- Determining the biosorption capacities of the non-viable GM 16 cells for Cd(II) and Cu(II).
- Evaluation of the effects of environmental factors on biosorption of Cd(II) and Cu(II) by isolate GM 16 in a batch equilibrium system. The following environmental factors were studied: Effect of
    - contact time
    - optimum pH
    - biomass concentration
    - initial metal ion concentration
    - optimum temperature
    - other metal ion contaminants (Mg^{2+}, Ca^{2+}, K^{+} and Na^{+}) on the biosorption of a desired metal ion [Cd(II) or Cu(II)]
    - agitation speed

- Characterization of biosorption performance by using Langmuir and Freundlich adsorption isotherms.
- Evaluation of biosorption kinetics using pseudo-first order and pseudo-second order reaction models.
- Determination of the thermodynamic parameters of biosorption.

Chapter 2

2. Materials and methods

2.1. Chemicals

Chemicals used were of molecular, analytical or laboratory reagent grade obtained from various commercial suppliers and were used without further purification.

Table 2.1.

Chemicals

Reagent	Supplier	Grade
Agar agar powder	Merck, Darmstadt, Germany	Laboratory
Agarose powder	Whitehead Scientific	Molecular Biology
Ammonium acetate	Merck, Darmstadt, Germany	Molecular Biology
Ampicilin	Sigma Chemical Co. St. Louis, MO, USA	Analytical
Arsenite	Sigma Chemical Co. St. Louis, MO, USA	Analytical
Cadmium chloride hemi-pentahydrate	Sigma Chemical Co. St. Louis, MO, USA	Analytical
Calcium chloride dehydrate	Saarchem Pty Ltd, Johannesburg, RSA	Analytical
Chloroform	Saarchem Pty Ltd	Analytical
Crystal violet	Protea Pharmaceuticals, Johannesburg, RSA	Laboratory
Cupric chloride	Sigma Chemical Co. St. Louis, MO, USA	Analytical
D(+) Glucose monohydrate	Saarchem Pty Ltd, Johannesburg, RSA	Laboratory
dNTPs	Fermentas	Molecular Biology
EDTA	Merck, Darmstadt, Germany	Analytical
Ethanol	Merck, Darmstadt, Germany	Analytical
Ethidium bromide	Merck, Darmstadt, Germany	Analytical
Glucose	Merck, Darmstadt, Germany	Laboratory
Glycerol	Merck, Darmstadt, Germany	Laboratory
Hydrochloric acid	Riedel-deHaën AG, Micor, Johannesburg	Analytical
Iodine	Protea Pharmaceuticals, Johannesburg, RSA	Analytical
IPTG (isopropyl β-D-galactoside)	Promega	Molecular Biology
Isopropanol	Merck, Darmstadt, Germany	Analytical
Magnesium chloride	Saarchem Pty Ltd, Johannesburg, RSA	Analytical
MassRulerTM DNA ladder mix #SM0403	Fermentas	Molecular Biology
Mueller-Hinton broth	OXOID LTD, Basingstoke, Hampshire, England	Laboratory
Potassium chloride	NT laboratory supplies, Excom, Johannesburg	Analytical
Primers 27F & 1492R	Whitehead Scientific	Molecular Biology
RNAse	Fermentas	Molecular Biology
Safranin O	Protea Pharmaceuticals, Johannesburg, RSA	Laboratory

Table 2.1 *continues*

SDS (sodium dodecyl sulphate)	Merck, Darmstadt, Germany	Molecular Biology
Sodium arsenite	Sigma Chemical Co. St. Louis, MO, USA	Analytical
Sodium chloride	Riedel-deHaën AG, Micor, Johannesburg	Laboratory
Sodium hydroxide granules	Riedel-deHaën AG, Micor, Johannesburg	Analytical
Taq polymerase	Southern Cross Biotech	Molecular Biology
Thermopol Buffer $MgCl_2$	Biolabs	Molecular Biology
TRIS (trisaminomethane)	ICN Medicals, Inc, Aurora, Ohio	Analytical
Tryptone	Sigma Chemical Co. St. Louis, MO, USA	Molecular Biology
X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside)	Promega	Molecular Biology
Yeast extract	Sigma Chemical Co. St. Louis, MO, USA	Laboratory
Zinc chloride	Sigma Chemical Co. St. Louis, MO, USA	Analytical

2.2. **Bacterial cultures**

Bacterial isolates (GM 10(1), 10(2), 14, 15, 16 and 17) were obtained from the Department of Microbial, Biochemical and Biotechnology, University of the Free State (UFS). The bacterial isolates were isolated from samples collected from Murchison Antimony Mine, Gravellote, Limpopo Province of South Africa. Of particular interest, was the isolate GM 16 isolated from water and silt from draining hole No. 5 in dam 3 of Antimony Mine. Other bacterial samples were isolated from the mine samples collected as follows: GM 10(1) and GM 10(2) (silt from North wall in dam 2), GM 14 (biofilm from North in dam 2), GM 15 (water from dam 3 at Danger sign) and GM 17 (water and sludge from dam 3).

Stock bacterial cultures were grown on tryptone-yeast extract-glucose (TYG) agar plates containing 5 g L^{-1} tryptone, 3 g L^{-1} yeast extract, 1 g L^{-1} glucose and 16 g L^{-1} agar, at pH 6.5. TYG agar medium was sterilized by autoclaving at 121 °C for 30 min and allowed to cool to 50 °C. Agar medium was supplemented with 100 µM of sodium arsenite, mixed and 20 mL was transferred into the Petri dishes to form nutrient agar plates. A loopful of bacterial culture from the stock samples was streaked on nutrient agar plates and incubated in an environmental incubator (New Brunswick, New Jersey) at 37 °C for 24 h. Cryopreservation of bacterial cultures was performed according to the method of Perry (1995). A

single colony from the agar plates was transferred into TYG broth supplemented with 100 µM arsenite and grown at 37 °C for 24 h. The bacterial cultures were diluted in a 1:1 (v/v) ratio of 40 % sterile glycerol and stored at -80 °C until use.

2.3. **Determination of bacterial morphology by Gram-staining**

Gram staining was performed to confirm the gross morphology of the isolated bacteria and to determine whether the culture was pure. Bacterial smears were prepared by placing drops of bacterial broth culture in the middle of clean separate microscopic slides. The bacterial cultures were fixed by passing the microscopic slides few times through a flame. Bacterial smears were then stained with crystal violet, washed off with distilled water after 1 min and flooded with Lugol's iodine for 60 sec. The slides were washed off with 70 % ethanol to remove crystal violet stain until no more dye was removed and counter-stained with Safranin O for 30 sec. Following air drying, the slides were examined with a Zeiss Axioplan microscope. Gram-positive cells appeared violet while Gram-negative cells were stained red (Madigan *et al*., 1997).

2.4. **Identification of bacterial isolates by 16S rDNA amplification and sequencing**

2.4.1. **Isolation of bacterial genomic DNA**

From each plate, a loopful of bacterial culture was inoculated in TYG broth at pH 6.5 supplemented with 100 µM of $NaAsO_2$ and incubated on a rotary shaker incubator until A_{560} value of 0.4 was reached. Bacterial cells were harvested by centrifuging (11000 x g for 4 min) 2 mL of broth culture, and the cells were lysed by vortexing the pellet with 500 µL DNA isolation buffer (Appendix) and 200 µL glass beads for 4 min followed by immediate cooling on ice for 5 min. Ammonium acetate (2.48 M, pH 7) was added, vortex mixed, incubated at 65 °C for 5 min followed by cooling on ice for 5 min. Other cellular components were precipitated

by 500 µL of chloroform, vortex mixed and centrifuged at 8944 x g for 5 min at 4 °C. From the supernatant, DNA was precipitated with an equal volume of isopropanol for 5 min at room temperature and centrifuged at 8944 x g for 5 min. The DNA pellet was washed with 70 % ethanol and centrifuged 8944 x g for 5 min at 4 °C. The DNA pellet was air dried and resuspended in Tris-EDTA buffer (pH 8) containing 0.05 mg L^{-1} RNAse. The concentration of DNA was determined using NanoDrop spectrophotometer (ND-1000V_3.3, Fermentas). Fifty nano grams were loaded on 0.8 % (w/v) agarose gel with a molecular maker (MassRuler™ DNA ladder mix # SM0403, Fermentas), for the determination of size of DNA fragments on agarose gel (containing 0.75 µg L^{-1} ethidium bromide), and electrophoresed at 7.5 $V.cm^{-1}$ for 45 min. The DNA was visualized using Quantity One 1-D Analysis Software analyzer (Bio-Rad). DNA samples of the bacterial isolates were stored at -20 °C until use.

2.4.2. **PCR amplification and cloning of 16S rDNA**

The 16S rDNA genes of bacterial isolates were amplified using the method described by Botes *et al*., (2007). Using bacterial specific primer set 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Lane, 1991), 16S rDNA genes were amplified by PCR. The primer set 27F and 1492R (forward and reverse primer set, respectively) were selected based on the fact that they are bacterial specific, and are used as universal primers for bacterial 16S rDNA. PCR consisted of 1 X Thermopol Reaction Buffer with $MgCl_2$ (Biolabs), 2.5 U Taq Polymerase, 200 µM of each primer, 200 µM of each dNTP and approximately 50 ng template DNA. Amplification was performed after an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, primer annealing at 49 °C for 45 sec and product extension at 72 °C for 1 min. The final extension was performed at 72 °C for 10 min. The amplified products were electrophoresed on a 0.8 % agarose gel (containing 0.75 µg L^{-1} ethidium bromide), excised and purified using Zymoclean™ Gel DNA Recovery kit (Bio-Rad). The purified products were ligated into a pGEM®-T Easy

vector (Promega) and used to transform *Escherichia coli* TOP 10 competent cells. Transformed cells were then cultured in LB- AIX [10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl, 15 g L^{-1} agar supplemented with 50 µg mL^{-1} ampicilin, 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 40 µg mL^{-1} X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) agar plates to select cells with the gene of interest. Following the pGEM®-T Easy protocol, transformed cells were not be able to breakdown the substrate (X-gal) as opposed to non-transformed ones which broke down X-gal and produced a blue-colored product, hence, a blue color appeared in non-transformed cells. The IPTG served to induce β-galactosidase gene expression allowing the selection of *Lac*+ recombinant bacteria in X-gal containing medium. Single colonies were inoculated into LB broth amended with 50 µg mL^{-1} ampicilin and grown for 16 h at 37 °C, and the plasmid DNA was extracted using Biospin Plasmid DNA extraction kit (Bioflux). Inserts of the correct sizes were digested and sequenced according to the method described by Botes (2007). Sequencing was performed using T7 (TAATACGACTCACTATAGGG) and Sp6 (TATTTAGGTGACACTATTAG) promoters with a BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems) on an ABI377 DNA sequencer (PE Biosystems). The sequencing of the deoxynucleotide chain terminators was labeled with a different fluorescent dye, each fluorescing at a different wavelength. The sequences obtained were aligned with closest matches obtained from BLAST searches in GenBank database and a phylogenetic tree was also constructed using Mega 4 software program (Kumar *et al*., 2009).

2.5. **Preparation of the metal salt solutions used for biosorption**

Analytical grade salts of $CuCl_2.2H_2O$, $CdCl_2.H_2O$, $MgCl_2$, $CaCl_2$, NaCl and KCl were used to prepare 1000 mg L^{-1} stock solutions which were used in the experiments whereas 1 M stock solution of $NaAsO_2$ was also prepared. $NaAsO_2$ was sterilized with 0.2 µm pore-size sterile filters (Millipore). Required concentrations of the metals for the biosorption studies were made by dilution of

stock metal solutions with deionized water. Also, the pH of the working solutions was adjusted using 1 M HCl and 1 M NaOH.

2.6. **Preparation of non-viable bacterial biomass**

Cryo-preserved cultures were thawed at 4 °C, pelleted by centrifugation at 3220 x g using GS-15R centrifuge (Beckman) and suspended in 5 mL of TYG broth. Suspended cells were kept for 24 h at 4 °C and 1 mL of bacterial sample was transferred into 100 mL of TYG broth and incubated at 37 °C for 24 h. A drop of bacterial sample was spread on TYG agar (pH 6.5) plates and the cells were grown at 37 °C for 24 h. To generate enough biomass for the biosorption experiments, the GM 16 isolate was grown as previously described (Sekhula *et al*., 2005). A loopful of bacterial culture from a single colony was transferred into 50 mL of 23 g L^{-1} Mueller-Hinton (MH) broth (without any metal) at pH 7.4 in a 250 mL Erlenmeyer shake flask. The cells were incubated at 37 °C on a rotary shaker at 150 rpm for 16 h and 50 mL of bacterial cells (which were grown for 16 h) was transferred into 500 mL of 23 g L^{-1} MH broth in a 1000 mL Erlenmeyer shake flask and incubated for 16 h. Finally, the 500 mL of bacterial culture was transferred into 1000 mL of 23 g L^{-1} MH broth in a 2000 mL Erlenmeyer shake flask and incubated for 16 h. Cells were collected by centrifugation at 3220 x g using J2-21 centrifuge (Beckman) and washed with adequate amount of distilled water. Thereafter the pellets were dried at 80 °C (to render the cells non-viable which was confirmed when no growth was observed when suspended in MH both at 37 °C) for 24 h (Yilmaz and Ensari, 2005) and ground in a mortar to a very fine powder and stored at 4 °C until required.

2.7. **General biosorption procedures**

The biosorption of Cd(II) and Cu(II) by GM 16 biomass was studied in a batch technique. Known concentrations of metal solutions were prepared and adjusted to the required pH using 1 M HCL and 1 M NaOH into a 50 mL final volume. The

initial concentration of metal solution was recorded (determined by atomic absorption spectrophotometer) before biomass was added. In all experiments, 0.2 g of biomass (not in the case of biomass concentration effect) was suspended in 50 mL of metal solutions of known concentrations in Erlenmeyer shake flasks at a fixed temperature of 40 °C (not in the case of temperature effect) in an Environmental shake incubator for 1 h. Samples (1 mL) were collected at 15 min intervals for 1 h. Samples were centrifuged at 8944 x g using GS-15R centrifuge and the supernatants were analyzed for residual metal ion concentrations using atomic absorption spectrophotometer (Varian). The effects of parameters such as contact time, initial pH, speed of agitation, biomass concentration, initial metal ion concentration, temperature, other metal cations and effect of Cd(II) on the biosorption Cu(II) and *vice versa* were studied. The adsorption of metal ions on the walls of Erlenmeyer flasks and pipette tips was determined by running blank experiments and was found negligible. The metal sorption capacity (*q*) was calculated using equation 1 described in Section 12.9.

2.8. **Reproducibility and data analysis**

All biosorption experiments were performed in triplicates and Sigma Plot 4.0 software package was used to evaluate the standard error of the mean values and levels of significance of differences between controls and experimental values. Arithmetical average values were used in calculations.

2.9. **Effect of contact time and pH on metal biosorption**

Dried biomass (4 g L^{-1}) was suspended in 100 mg L^{-1} metal solution of Cd(II) adjusted to pH values varying between 4-10 and Cu(II) adjusted to pH values between 2-6 in 250 mL Erlenmeyer flasks and placed on a rotary shaker (100 rpm) at 40 °C for 160 min. One milliliter samples were collected at 15 min intervals. Supernatants were collected by centrifugation (GS-15R centrifuge, Beckman) at 3220 x g and residual metal concentrations were analyzed using

atomic absorption spectrophotometer (SpetrAA 110 series, Varian). The metal adsorbed was determined using equation 1 described in Section 1.2.9. The kinetic profiles of Cd(II) and Cu(II) biosorption were constructed by plotting the metal uptake (q_t) versus the time (t) at which the samples were collected. The optimum pH for metal adsorption by the biomass was determined from a plot of metal uptake (q) versus pH.

2.10. Selective adsorption of Cd(II) and Cu(II) in a binary system [(Cd(II) and Cu(II)] at pH 6 and 7

Since the optimum pH for the sorption of Cd(II) and Cu(II) were found to be 7 and 6 respectively, it was necessary to investigate the effect of the presence of Cu(II) on the adsorption of Cd(II) and *vice versa* at both pH 6 and 7. Low (50 mg L^{-1}) and high (100 mg L^{-1}) metal ion concentrations were used for this study. The sorption experiments of Cd(II) and Cu(II) were performed under the same conditions (biomass, 4 g L^{-1}, and metal concentrations, 50 or 100 mg L^{-1}, temperature, 40 °C, and the speed of agitation, 100 rpm).

The sorption of Cd(II) or Cu(II) was studied at pH 6 and 7 in a binary [Cd(II) and Cu(II)] metal solutions. The concentrations of the metal solutions were prepared as 100 mg L^{-1} or 50 mg L^{-1} of both Cd(II) and Cu(II) in one 250 mL Erlenmeyer shake flask. The pH of the metal solutions was adjusted to 6 or 7 and 0.2 g of biomass was suspended in 50 mL metal solutions and incubated at 40 °C for 1 h. The supernatants were collected by centrifugation (GS-15R centrifuge, Beckman) at 3220 x g and then analyzed for the residual concentration of Cd(II) or Cu(II) using atomic absorption spectrophotometer (SpectrAA 110 series, Varian). The metal sorption capacity (q) was determined using equation 1 as described in Section 1.2.9.

2.11. **Effect of speed of agitation on the biosorption of Cd(II) or Cu(II)**

Dried GM 16 biomass (4 g L^{-1}) was exposed to 100 mg L^{-1} of Cd(II) or Cu(II) solutions at the respective pH optima [pH 6 for Cu(II) and 7 for Cd(II)], agitated on a rotary shake incubator at 100-200 rpm at 40 °C for 1 h. A non-agitated biosorption system was used as a control. Supernatant solutions were obtained by centrifugation (GS-15R centrifuge, Beckman) at 3220 x g and analyzed for residual metal ion concentrations. The metal sorption capacity (*q*) was determined using equation 1 described in Section 1.2.9.

2.12. **Effect of increasing biomass concentration on the sorption of Cd(II) and Cu(II)**

Preliminary studies on the biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass was done using biomass concentrations of 1, 2 and 4 g L^{-1} and it was found that 4 g L^{-1} (as the highest concentration used in that study) showed high metal adsorption capacity (data not shown). To determine the effect of biomass concentration on the biosorption of Cd(II) and Cu(II), 0.8 to 4.8 g L^{-1} biomass concentration range was used. The effect of biomass concentration (0.8-4.8 g L^{-1}) on the sorption of Cd(II) and Cu(II) was evaluated in 50 mL of 100 mg L^{-1} metal solutions at constant pH (pH 6 for Cu and 7 for Cd) and temperature (40 °C) in 250 mL Erlenmeyer flasks. The flasks were agitated at 100 rpm on a rotary shake incubator and samples were collected at 15 min intervals for 1 h. Supernatants were collected and residual metal ion concentrations were analyzed as previously described.

2.13. **Effect of other metal cations on the biosorption of Cd(II) and Cu(II)**

Heavy metal bearing wastewater often contains other metal ions that may interfere with the uptake of the heavy metals of interest (Hernainz *et al*., 2008; Babarinde *et al*., 2008). Therefore, batch equilibrium experiments were

conducted to evaluate the effect of some common cations (Mg^{2+}, K^{+}, Na^{+} or Ca^{2+}) on the sorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass. The metal cations (Mg^{2+}, K^{+}, Na^{+} and Ca^{2+}) were chosen because they are naturally occurring elements and are most likely to be found in mining sites as well as constituents of polluted wastewater/effluent. Some (Mg^{2+}, K^{+}, Na^{+} and Ca^{2+}) have been used as eluants of metals from biosorbents (Gupta *et al*., 2000). Equimolar concentrations, 0.5 mM (mg L^{-1} concentrations are shown in Table C of Appendix), of the metal ions were used in order to investigate the effect of these metal cations on the sorption of Cd(II) and Cu(II). Dried biomass (0.2 g) was suspended in a final volume of 50 mL of 0.5 mM (mg L^{-1} concentrations in appendix) of Cd(II) or Cu(II) solution in the presence of 0.5 mM Mg^{2+}, K^{+}, Na^{+} or Ca^{2+} in 250 mL Erlenmeyer shake flasks and placed on a rotary shaker (100 rpm) at 40 °C for 1 h. The metal solutions were adjusted to pH 7 for Cd(II) and pH 6 for Cu(II) biosorption. Supernatant solutions were collected and analyzed as described previously. The percentage metal ion adsorbed was calculated using the following equation:

$$\% \text{ Metal adsorbed} = [(C_i - C_e)/C_i] \times 100 \qquad (15)$$

where C_i and C_e are the initial and equilibrium metal ion concentrations (mg L^{-1}), respectively.

2.14. Effect of temperature and the initial metal ion concentrations on the biosorption of Cd(II) and Cu(II)

Batch sorption isotherm experiments were carried out in 50 mL metal solutions of different initial metal ion concentrations (40-120 mg L^{-1}) adjusted to pH 6 for Cu(II) and pH 7 for Cd(II). The concentration range selected was used in order to observe increase in metal sorbed on the biomass until saturation was reached. The metal solutions were incubated with 4 g L^{-1} biomass and agitated at 100 rpm for 1 h. The biosorption experiments were performed at temperatures ranging

from 25 to 40 °C. Supernatant solutions were collected at 15 min intervals, analyzed and metal adsorbed was determined as previously described.

Langmuir and Freundlich adsorption isotherms were used to describe the biosorption data and to elucidate the possible sorption mechanisms. The maximum metal removal capacity q_{max} and Langmuir adsorption isotherm constant K_L for Cd(II) and Cu(II) were calculated from equation 3 described above. The equilibrium parameter (K_R) was also calculated from Equation 3 to predict whether the sorption process was favorable or unfavorable according to Table 1.5 above.

The Freundlich adsorption constants (K_F and n) were obtained by plotting $\ln q_e$ as a function of $\ln C_e$ from equation 6 described above. K_F and n define the biosorption capacity of the biomass and the intensity of biosorption, respectively. A favorable adsorption tends to have Freundlich constant n between 1 and 10 (Febrianto *et al*., 2009). Large values of n imply strong interaction between biosorbent and a metal ion whereas n with a value of 1 indicates a linear adsorption leading to identical adsorption energies for all sites (Febrianto *et al*., 2009).

2.15. **Sorption kinetics**

Kinetic models were used to test experimental data. The kinetic behaviors of Cd(II) and Cu(II) adsorption were analyzed using pseudo-second order and pseudo-first order kinetics. Pseudo-first order rate constant (k_1) and the equilibrium metal sorption capacity (q_e) were calculated from slope and y-intercept, respectively, of a linear plot of Equation 7. Pseudo-second order rate constant (k_2) and equilibrium metal sorption capacity (q_e) were calculated from y-intercept and slope, respectively, of a linear plot of Equation 8.

The initial sorption rate (h) in mg g^{-1} min^{-1} was also calculated using the following equation (Ucun *et al*., 2007):

$$h = k_2 q_e^2 \qquad (16)$$

2.16. **Thermodynamic parameters**

Thermodynamic parameters including Gibbs free energy change (ΔG^o), enthalpy change (ΔH^o) and the entropy change (ΔS^o) were used to predict whether the adsorption process was spontaneous or not. The thermodynamic property ΔG^o in kJ mol^{-1} was determined using Equation 11. The enthalpy, ΔH^o (kJ mol^{-1}), and the entropy changes, ΔS^o (J mol^{-1} K^{-1}), of the reactions were obtained from the slope and the y-intercept, respectively, of the plot of Equation 13.

Chapter 3

3. Results

3.1. Gram-stain

The Gram-stained cells are shown in Fig. 3.1. The stained cells were visualized using a 100 X magnification. The test bacterial isolate (GM 16) was a Gram-positive cell with bacillus-like morphology in Fig. 3.1E. GM 10(1), 10(2) and 15 were also Gram-positive cells and two of the isolates, GM 14 and 17 were Gram-negative cocci.

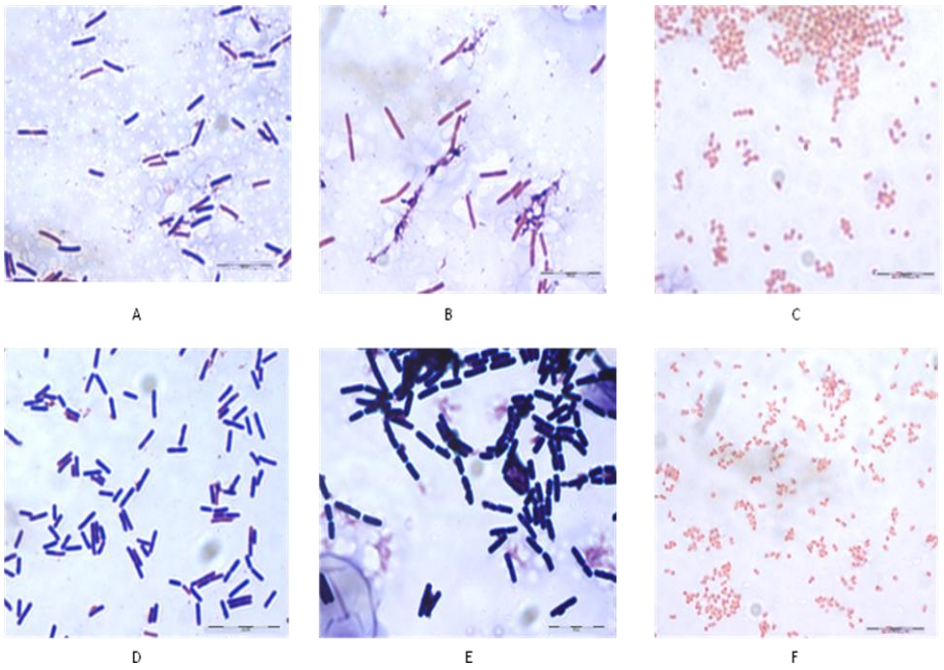

Fig. 3.1. Gram stained cells of GM isolates at a 100 X magnification. The isolates were all incubated for 16 h. A: GM 10(1), B: GM 10(2), C: GM 14, D: GM 15, E: GM 16, F: GM 17.

3.2. PCR products and GM isolates close matches

Fig. 3.2 shows PCR products of the expected size of approximately 1.6 kb of the 16S rDNA genes isolated from the GM isolates. Lanes 2 and 3 show the negative

(DNA template was not added) and positive controls (DNA template was added), respectively, in the reaction mixtures of the PCR. The 16S rDNA sequences of the GM isolates were used to search for their closest matches in NCBI databases (http://www.ncbi.nml.nih.gov/BLAST/) and the results are tabulated in Table 3.1 for all the GM isolates.

A phylogenetic tree of the GM isolates was constructed with 16S rDNA sequences of the closest matches of the bacterial isolates using Mega 4.0.1 software and is shown in Fig. 3.3. A consensus tree was constructed and rooted with the outgroup *Penicillium oxalicum* isolate 68. Generally, the overall identification of GM isolates revealed two groups of genera, members of the *Bacillus* and *Serratia* species. From the phylogenetic tree, isolate GM 16 was found to be a member of the *Bacillus* species, more closely related to *Bacillus thuringiensis* and *Bacillus cereus* strain CSB09 with 99 % and 98 % sequence homologies, respectively.

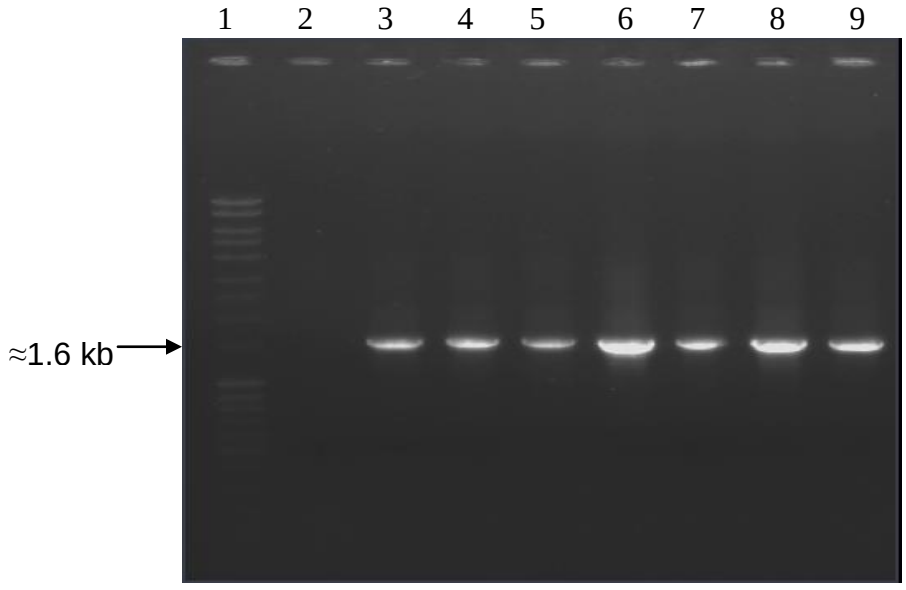

Fig. 3.2. 16S rDNA PCR products (≈ 50 ng) of bacterial isolates from Antimony Mine. Lane 1: MassRuler^{TM} DNA ladder mix (molecular weight marker), Lane 2: negative control (No DNA template was added), Lane 3: positive control (DNA template was added), Lane 4: GM 10(1), Lane 5: GM 10(2), Lane 6: GM 14, Lane 7: GM 15, Lane 8: GM 16, Lane 9: GM 17.

Table 3.1

Close matches for 16S rDNA genes of pure cultures.

Sample ID	Accession No.	Close match	Length (bp)	Max score	Query coverage (%)	E-value	BLAST identity (%)
GM 10(1)	FJ189764.1	*Bacillus subtilis* strain MSB10	1607	645	81	0	72
GM 10(2)	AY1308758	*Paenibacillus favisporus* strain GMP03	1547	1227	94	0	98
GM 14	EU221361.1	*Serratia marcescens* strain J2P3	1539	1694	91	0	95
GM 15	FJ189780.1	*Bacillus* sp. CSB02	1606	544	95	2e-151	93
GM 16	FJ189786.1	*Bacillus cereus* strain CSB09	1619	1386	98	0	98
	AY138287.1	*Bacillus thuringiensis* strain 2000031496	1554	1308	96	0	99
GM 17	AY551938.1	*Serratia marcescens* isolate SA Ant16	1506	457	84	1e-125	98
	AM157437.1	*Serrate proteamaculans*	1552	462	96	3e-127	94

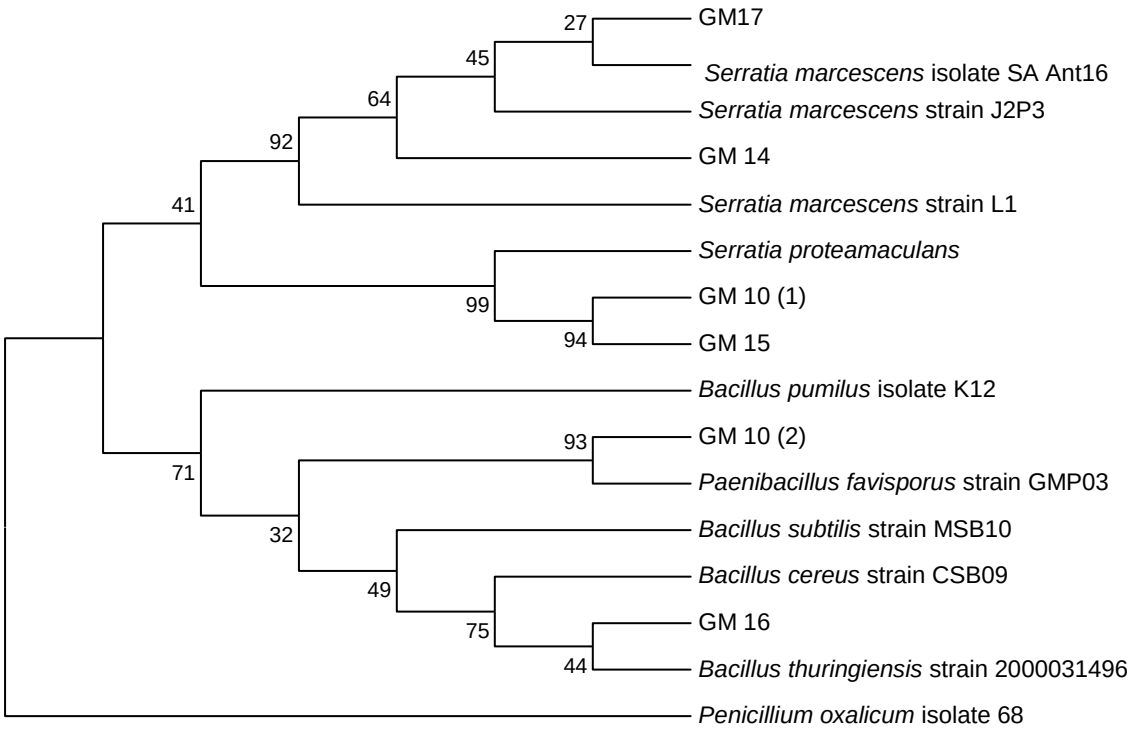

Fig. 3.3. The phylogenetic tree generated from the 16S rDNA sequences of the GM isolates.

3.3. **Effect of contact time and pH on the biosorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass**

The kinetics of Cd(II) and Cu(II) biosorption were studied in a batch equilibrium system on a rotary shaker at 100 rpm at 40 °C at different pH values. The initial and the equilibrium metal ion concentrations (C_i and C_e respectively) were determined and used to calculate the metal sorption capacity (q) at different time intervals. The initial sorption processes of both Cd(II) and Cu(II) were rapid during the first 15 min, followed by a long period of much slower adsorption and equilibria were reached within 1 h (Figs. 3.4A and B). The metal sorption also increased with increase in pH from 4-10 for the biosorption of Cd(II) whereas for the biosorption Cu(II), the metal sorption increased with increase in pH from 2-6 (Figs. 3.4A and B).

The maximum sorption of Cd(II) and Cu(II) was found at pH 7 and 6, respectively. The maximum metal adsorption capacity (q_{max}) was found to be 16.5 mg g^{-1} of dry weight of biomass at pH 7 for Cd(II) and 13.7 mg g^{-1} at pH 6 for Cu(II) (Fig. 3.5). Adsorption of Cu(II) at higher pH values (above pH 6) was not done due to precipitation of the metal in solution. All subsequent experiments were performed for 1 h at pH 7 for Cd(II) and pH 6 for Cu(II).

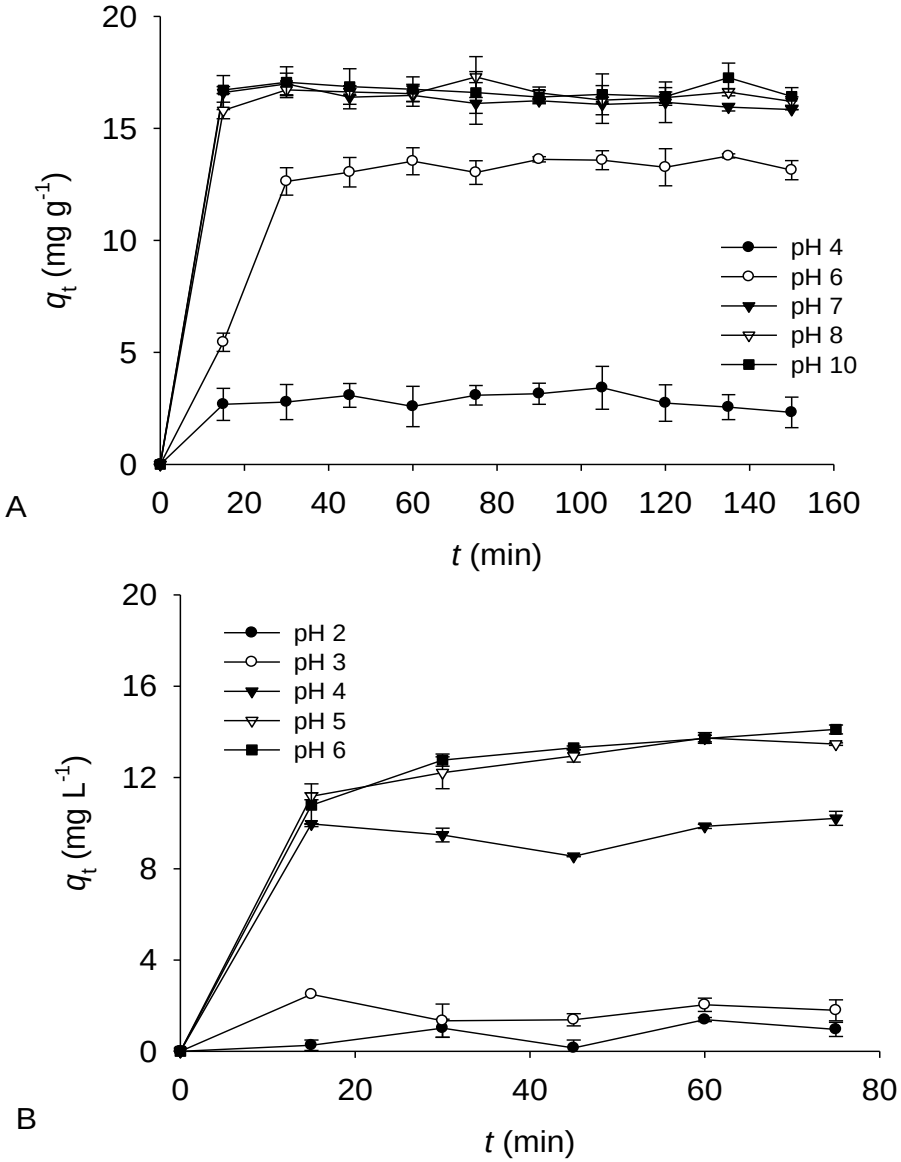

Fig. 3.4. Effect of contact time on the biosorption of Cd(II) (A) and Cu(II) (B) onto non-viable GM 16 biomass at different pH values. Bars indicate the standard errors of the mean values of three separate experiments.

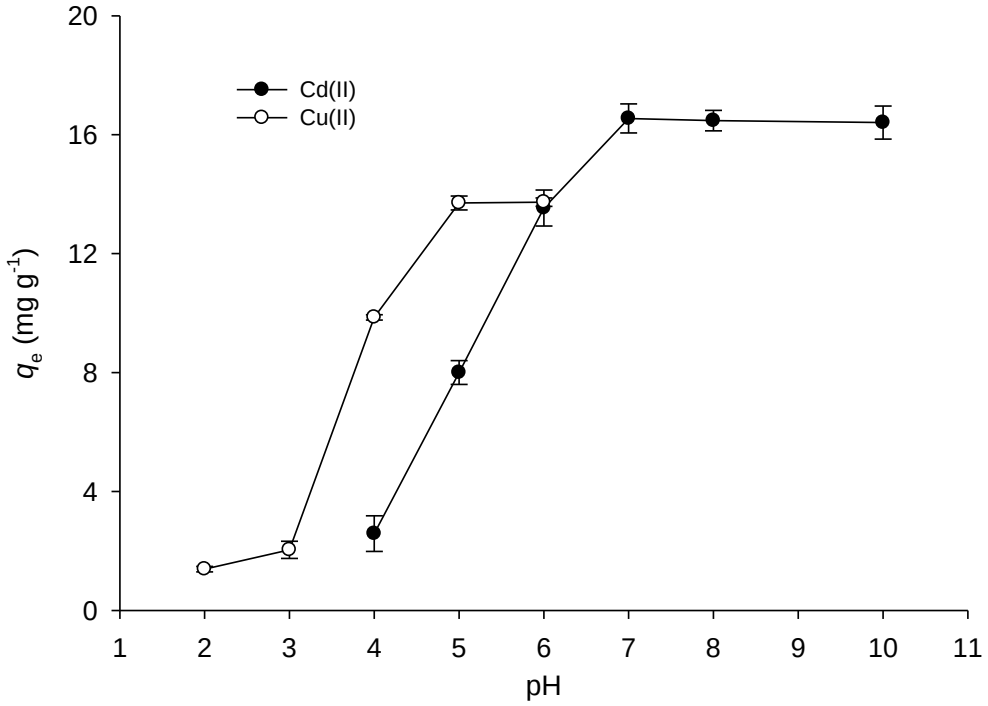

Fig. 3.5. Effect of pH on the biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass at 40 °C. Bars represent the standard errors of the mean values of three separate experiments.

3.4. Selective adsorption of Cd(II) or Cu(II) in binary [Cd(II) + Cu(II)] metal solutions at optimum pH values for Cu(II) (pH 6) and Cd(II) (pH 7)

The selective adsorption of Cd(II) or Cu(II) in a binary [Cd(II) + (Cu(II)] metal solution was investigated at pH 7 for Cd(II) and 6 for Cu(II). The adsorption of Cd(II) was higher at pH 7 than at pH 6 in 50 and 100 mg L^{-1} metal solutions (Fig. 3.6A). The adsorption of Cu(II) was found to be higher at pH 6 than at pH 7 in both 50 and 100 mg L^{-1} (Fig. 3.6B).

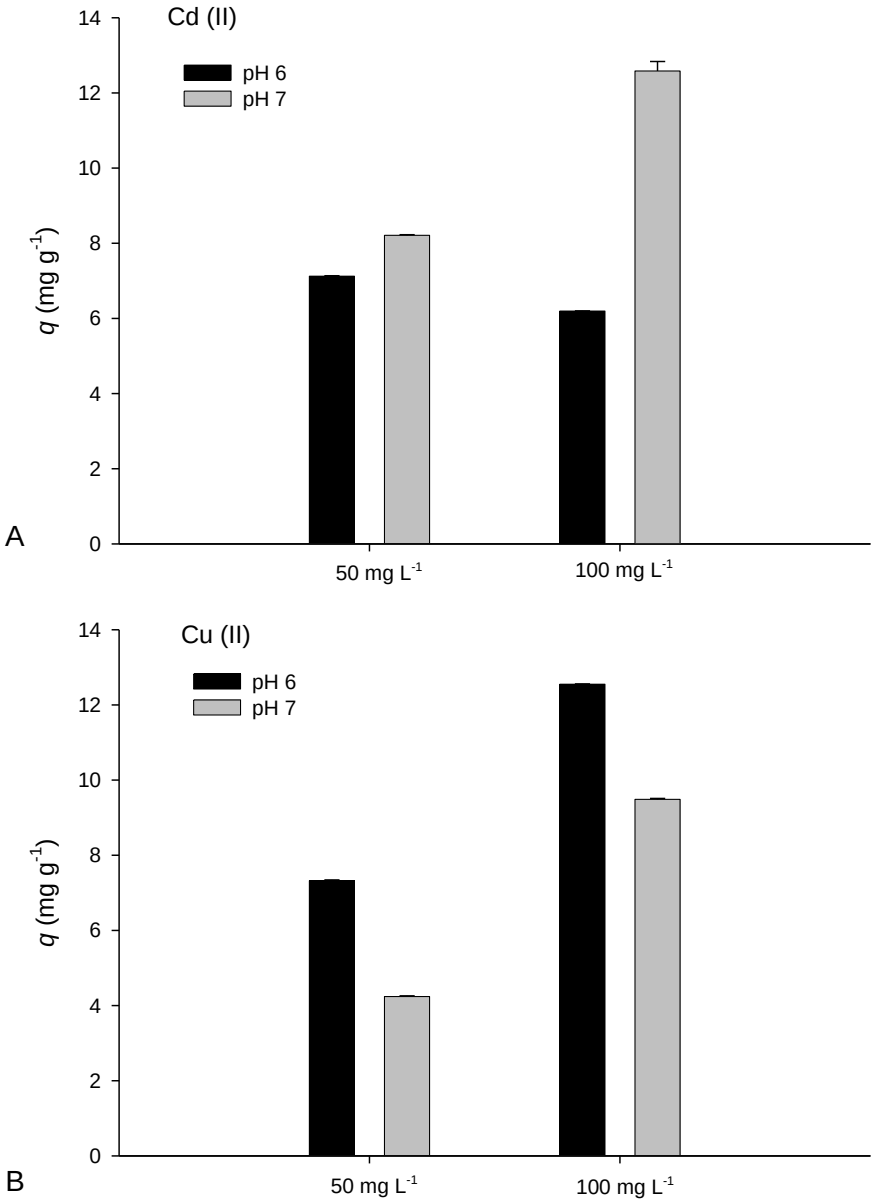

Fig. 3.6. Biosorption of Cd(II) (A) or Cu(II) (B) in mixed metal (Cd + Cu) system by non-viable GM 16 biomass at 40 ^{o}C. Bars represent the standard errors of the mean values of three separate experiments.

3.5. **Effect of speed of agitation on heavy metal ion biosorption**

The influence of the speed of agitation on the sorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass was studied at various speeds of agitation (0 to 200 rpm) (Fig. 3.7). It was found that the adsorption of both metal ions increased with the increase in speed of agitation until the maximum metal uptake, 13.4 mg

g^{-1} for Cd(II) and 14.3 mg g^{-1} for Cu(II), was reached. The speed at which the maximum adsorption of metal occurred was found to be 100 rpm for both Cd(II) and Cu(II). Further increase in the speed of agitation caused a decrease in the adsorption capacity of the biomass.

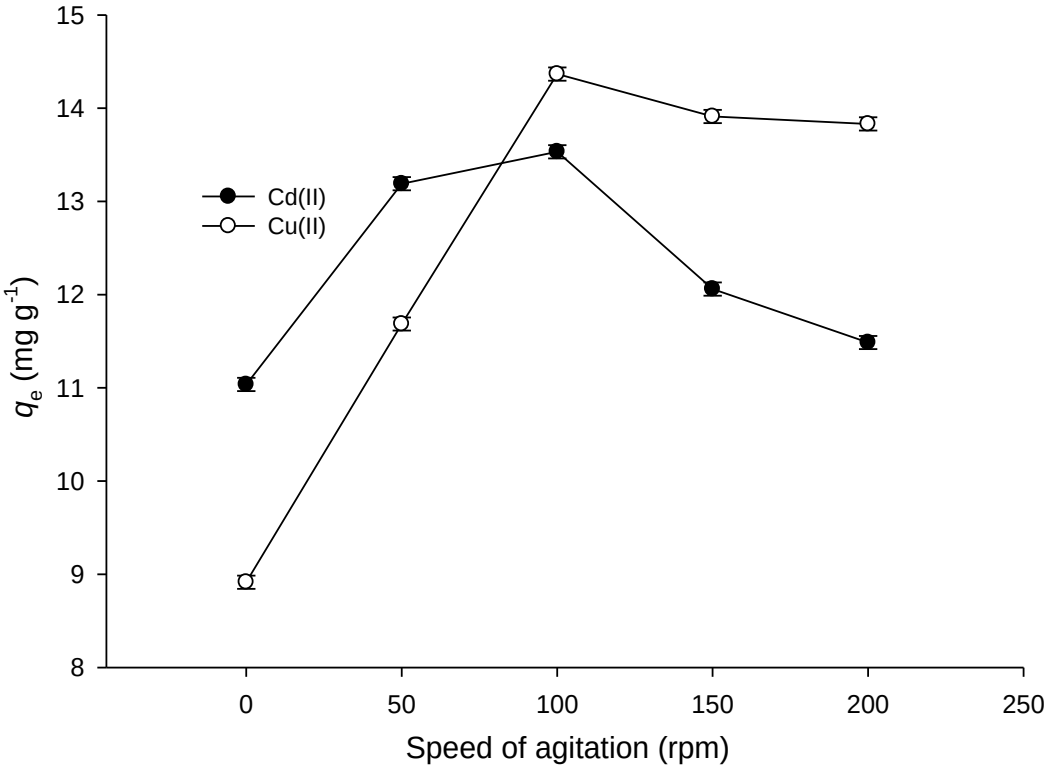

Fig. 3.7. Effect of the speed of agitation on the biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass. Bars represent the standard errors of the mean values of three separate experiments.

3.6. **Effect of increasing biomass concentration on the sorption of Cd(II) and Cu(II)**

The effects of increasing biomass concentration on the biosorption of metal ions were studied in metal solutions of the same initial metal ion concentration (100 mg L^{-1}) at pH 6 for Cu(II) and 7 for Cd(II) (Figs. 3.8A and B). The sorption of Cd(II) and Cu(II) increased rapidly during the first 15 min and equilibrium was reached within 1 h at all the different concentrations of the biomass. The adsorption of Cd(II) increased from 5.5 to 14.5 mg g^{-1} whereas the adsorption of

Cu(II) increased from 2.8 to 14.7 mg g^{-1} when the biomass concentration was increased from 0.8 to 4.8 g L^{-1} for both metals (Fig.3.8A and B).

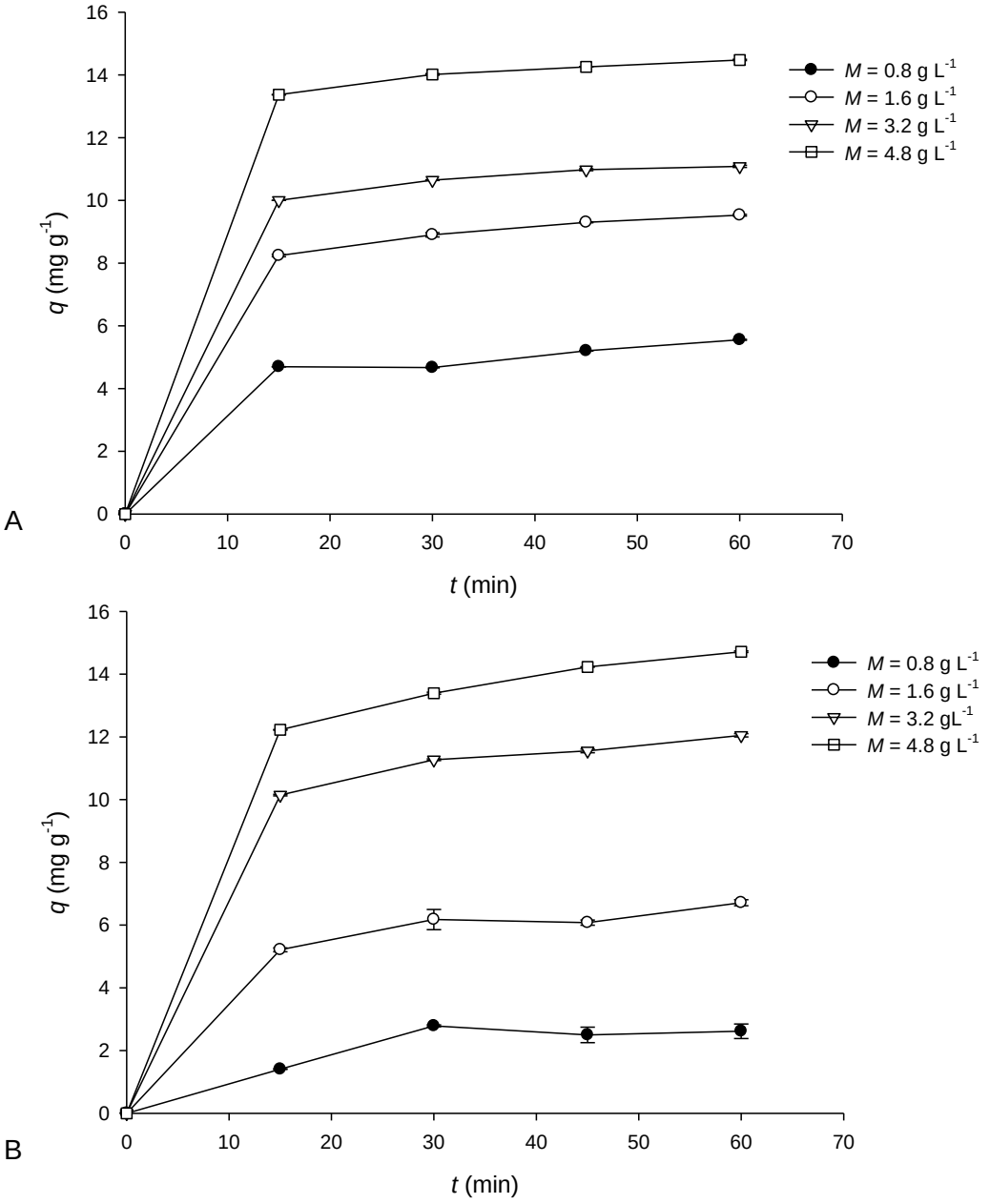

Fig. 3.8. Biosorption profiles of Cd(II) (A) and Cu(II) (B) by GM 16 biomass at different biomass concentrations. Bars represent the standard errors of the mean values of three separate experiments

3.7. **Effect of other metal cations on the biosorption of Cd(II) and Cu(II)**

Since effluent water and biosorbents may contain other metal cations other than Cd(II) and Cu(II), the effects of some other metal cations (K^+, Na^+, Ca^{2+} or Mg^{2+}) on Cd(II) or Cu(II) sorption by GM 16 biomass were investigated. The effect of the metal cations on the biosorption of Cd(II) and Cu(II) were significant ($P \leq 0.05$). The biosorption of Cu(II) on the biomass was inhibited in the presence of the metal cations (Table 3.2). The relative percentage adsorption of Cu(II) on the biomass was lower in the presence of all the metal cations than the control (absence of counter ions). Also, the divalent cations (Ca^{2+} and Mg^{2+}) inhibited Cu(II) adsorption more than monovalent cations (K^+ and Na^+). Ca^{2+} inhibited Cu(II) uptake by 6.4 % whereas Mg^{2+} showed 4.7 % inhibition of the same metal. Only 1.6 % and 1.9 % inhibition of Cu(II) by K^+ and Na^+ was observed.

The presence of calcium, potassium and sodium ions in the metal solution increased the adsorption of Cd(II) on the biomass (Table 3.2). Monovalent cations (K^+ and Na^+) were more potent enhancers of Cd(II) adsorption than the divalent cation (Ca^{2+}). K^+ and Na^+ increased the adsorption of Cd(II) by 12.3 and 8.7 %, respectively, whereas Ca^{2+} enhanced the uptake of Cd(II) by 3.2 %. Mg^{2+} inhibited adsorption of both Cd(II) and Cu(II) by 6.5 % and 4.7 % respectively.

Table 3.2.

Effect of metal cations on percentage Cd(II) and Cu(II) adsorbed at equilibrium by GM 16 biomass (biomass concentration, 4 g L^{-1}; initial metal ion concentration, 5 mM; contact time, 1 h; temperature, 40 °C; speed of agitation, 100 rpm).

Cation	% Cd(II) adsorbed ± SD	Relative % adsorption * ± SD	% Cu(II) adsorbed ± SD	Relative % adsorption * ± SD
Control	57.84 ± 0.029	100 ± 0	66.85 ± 0.24	100 ± 0
K^+	64.93 ± 0.064	112.3 ± 0.110	65.78 ± 0.30	98.4 ± 0.448
Na^+	62.86 ± 0.048	108.7 ± 0.083	65.55 ± 0.24	98.1 ± 0.359
Ca^{2+}	59.67 ± 0.022	103.2 ± 0.038	62.55 ± 0.28	93.6 ± 0.418
Mg^{2+}	54.06 ± 0.064	93.5 ± 0.110	63.69 ± 0.30	95.3 ± 0.448

NB: * Statistically significant (*t*-test): $P \leq 0.05$

Relative % adsorption = (test/control)100 %

3.8. **Isotherm modeling**

3.8.1. **Non-linearized Langmuir adsorption isotherm**

The analysis of the isotherm data is important to develop an equation which accurately represents the results and could be used for the design of the biosorption process. The non-linearized adsorption isotherms (q_e versus C_e), Langmuir adsorption isotherms, of the metal ions adsorbed on GM 16 biomass are shown (Figs. 3.9A and B). The equilibrium sorption capacity (q_e) of the biomass increased when equilibrium Cd(II) and Cu(II) concentrations were increased in the solution. The maximum metal adsorbed for Cd(II) were 16.2, 17.0 and 17.9 mg g^{-1} at 25 °C, 30 °C and 40 °C, respectively. The maximum metal adsorbed for Cu(II) were 16.1, 16.1 and 16.5 mg g^{-1} at 25 °C, 30 °C and 40 °C, respectively. The adsorption data were further fitted to linearized Langmuir and Freundlich adsorption models to find the most suitable model. The results also showed that sorption capacity of the GM 16 biomass was not substantially affected by increase in the temperature range (25-40 °C) studied. The two adsorption models were used to investigate in detail biosorption characteristics and to compare adsorption performance of GM 16 biomass for the sorption of Cd(II) and Cu(II).

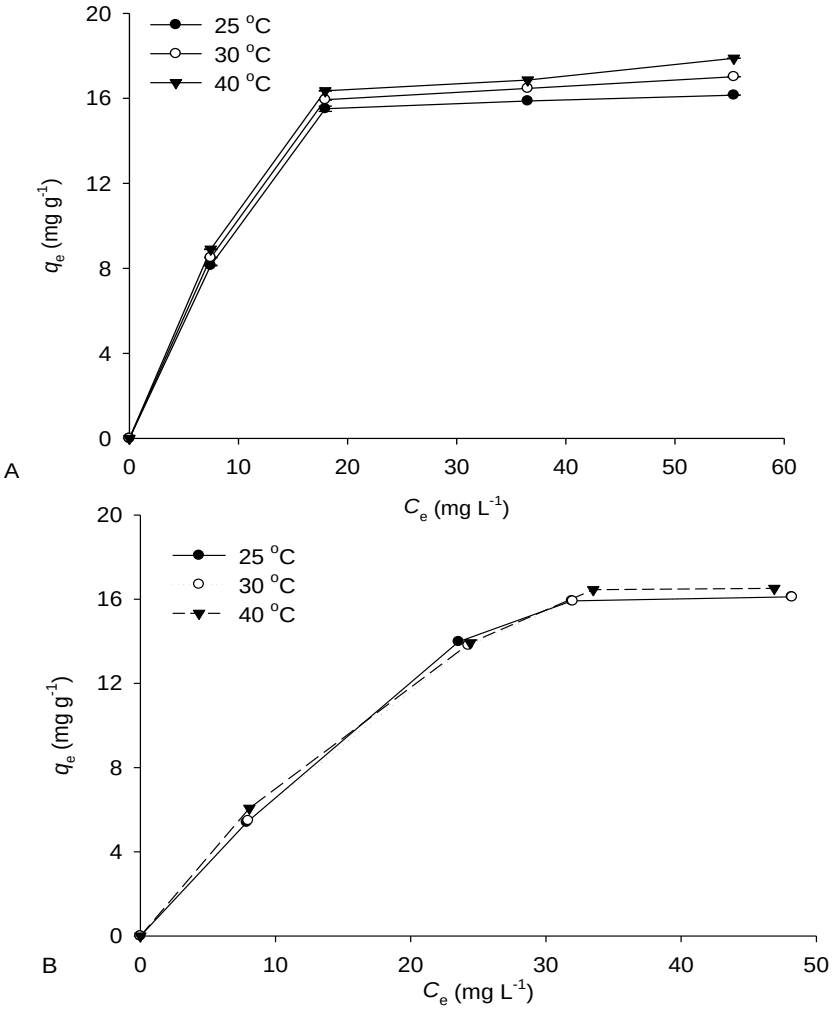

Fig. 3.9. Non-linearized Langmuir adsorption isotherms for the sorption of Cd(II) (A) and Cu(II) (B) onto non-viable GM 16 biomass at different temperatures.

3.7.2. **Linearized adsorption isotherms**

Linearized Langmuir and Freundlich adsorption isotherms were also used to describe the biosorption data (Figs. 3.10A and B). Linearized Langmuir and Freundlich adsorption isotherms were used to calculate the biosorption parameter of Cd(II) and Cu(II). The Langmuir and Freundlich adsorption constants evaluated from the isotherms at different temperatures with their correlation coefficients are also presented in Table 3.3. The isotherms for Cd(II) appeared to follow the Langmuir model more closely than Freundlich model in the concentration range studied with high regression correlation coefficients (r^2 >

0.98). K_L, q_{max}, K_F and n are the adsorption isotherm parameters. K_L is a Langmuir constant related to the affinity of the binding sites, q_{max} is the maximum amount of the metal ions per unit weight of biosorbent to form a complete monolayer on the surface bound at high equilibrium concentration (C_e). K_F represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in comparing the adsorption performance, particularly in cases where the biosorbent does not reach its full saturation capacity in the experiments. K_F and n, Freundlich constants, have been used as relative measures of adsorption capacity and the intensity of adsorption, respectively, (Aksu, 2001). The difference in model parameters estimated for biosorption of Cd(II) and Cu(II) on GM 16 biomass at various temperatures (25-40 °C) were not statistically significant ($P \geq 0.05$).

The Langmuir constants, K_L, and q_{max}, at the different temperatures were calculated from the intercept and the slope, respectively, of linearized Langmuir plots in Fig. 3.10A and the results are also tabulated in Table 3.3. The maximum Cd(II) sorption capacity (q_{max}) values determined from the linearized Langmuir adsorption model only increased slightly whereas the Langmuir constant, K_L, decreased with an increase in temperature. Although the differences were not statistically significant, a higher value of K_L (0.152) at 25 °C than at 40 °C (0.136) implied stronger binding of Cd(II) onto the non-viable GM 16 biomass at 25 °C than at 40 °C (Table 3.3). The sorption of Cd(II) at the various temperatures and initial metal ion concentration ranges did not seem to follow the Freundlich adsorption isotherm model ($r^2 < 0.9$) (Table 3.3).

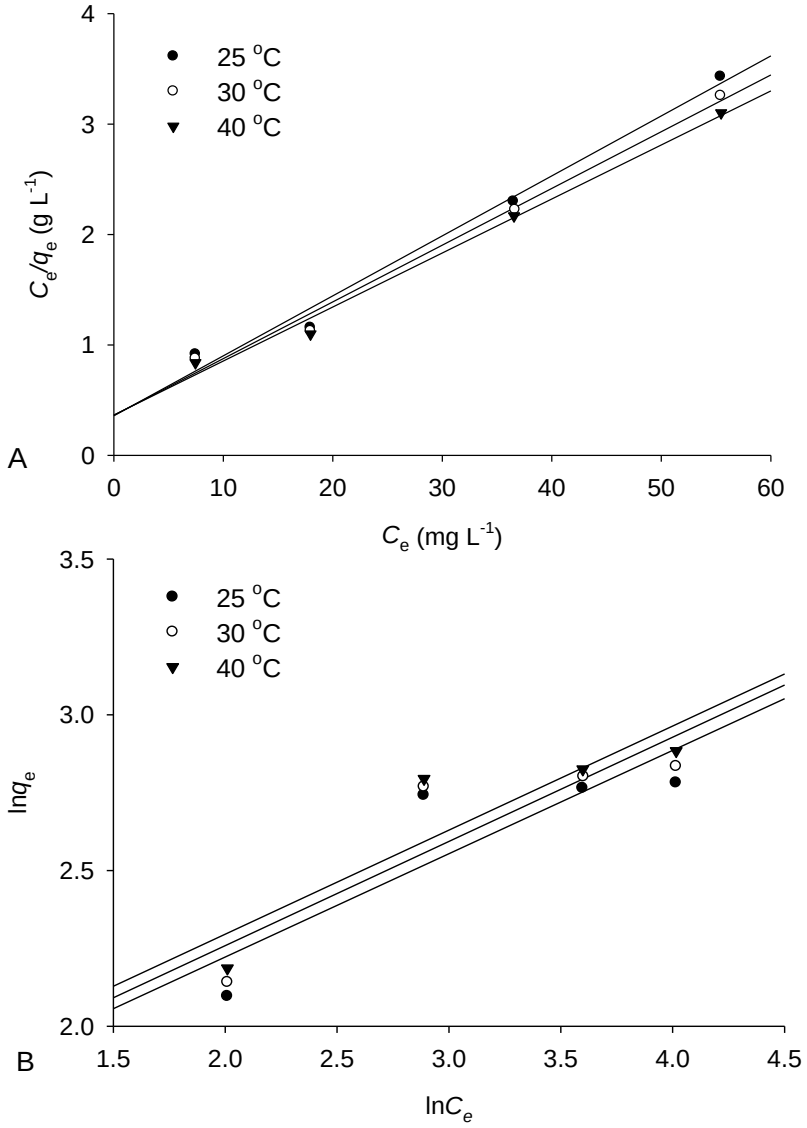

Fig. 3.10. Linearized Langmuir (A) and Freundlich (B) adsorption isotherms for the sorption of Cd(II) onto non-viable GM 16 biomass at different temperatures.

Table 3.3

Isotherm constants for the biosorption of Cd(II) onto non-viable GM 16 biomass.

T (°C)	**Langmuir isotherm** $C_e/q = 1/q_{max}.C_e + 1/q_{max}K_L$			**Freundlich isotherm** $\ln q_e = 1/n \ln C_e + \ln K_F$		
	q_{max} mg g^{-1}	K_L L mg^{-1}	r^2	K_F mg g^{-1}	n	r^2
25	18.4	0.152	0.985	4.768	2.983	0.762
30	19.4	0.142	0.986	4.908	2.973	0.788
40	20.0	0.136	0.989	5.079	2.862	0.809

NB: The differences in model parameters estimated were not statistically significant (*t*-test): $P \geq 0.05$

The linearized Langmuir and Freundlich adsorption isotherms for the biosorption of Cu(II) at the temperatures of 25, 30 and 40 °C are shown in Figs. 3.11A and B. The Langmuir and Freundlich adsorption constants calculated from the isotherms at different temperatures with the correlation coefficients are also presented in Table 3.4. The data fitted well to both Langmuir and Freundlich adsorption models with the regression correlation coefficients greater than 0.90. The correlation coefficients showed that the Langmuir and Freundlich adsorption isotherm models were in fairly good agreement for the biosorption equilibrium of Cu(II) by the non-viable GM 16 biomass in the metal concentration range used. Similar observation was made by Lu *et al*. (2006) when studying the biosorption of Cu by an indigenous isolate *Enterobacter* sp.J1.

The maximum metal sorption capacity (q_{max}) values determined from the Langmuir isotherm only increased marginally with increase in temperature. There were slight decreases in the Freundlich adsorption constant (K_F) and relative adsorption intensity (n) of the biomass with an increase in temperature. A K_F value of 1.54 mg g^{-1} was observed at 25 °C and decreased when the temperature was increased from 25 to 40 °C but the decreases were not significant. The highest value of n was found to be 1.67 at 25 °C (Table 3.4). The values of Freundlich constant, n, were greater than 1, indicating that Cu(II) was adsorbed onto the non-viable GM 16 biomass favorably at all temperatures studied. However, the changes in the Langmuir and Freundlich parameters at the various temperatures were not significantly different ($P \geq 0.05$).

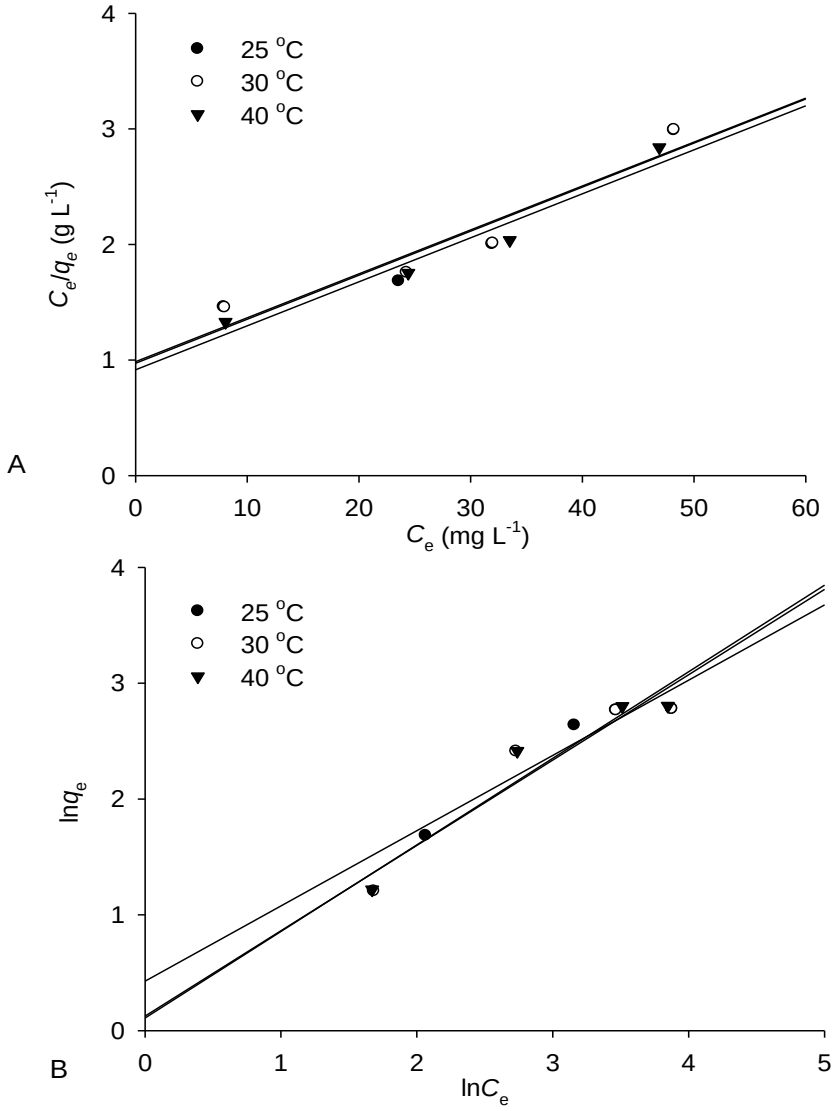

Fig. 3.11. Linearized Langmuir (A) and Freundlich (B) adsorption isotherm for the sorption of Cu(II) onto non-viable GM 16 biomass at different temperatures.

Table 3.4

Isotherm constants for the biosorption of Cu(II) onto non-viable GM 16 biomass.

T (°C)	**Langmuir isotherm** $C_e/q = 1/q_{max}.C_e + 1/q_{max}K_L$			**Freundlich isotherm** $\ln q = (1/n)\ln C_e + \ln K_F$		
	q_{max} mg g^{-1}	K_L L mg^{-1}	r^2	K_F mg g^{-1}	n	r^2
25	26.0	0.039	0.900	1.537	1.67	0.918
30	26.3	0.038	0.912	1.138	1.38	0.909
40	26.4	0.041	0.948	1.127	1.33	0.927

NB: The differences in model parameters estimated were not statistically significant (*t*-test): $P \geq 0.05$

As proposed by Hall *et al*. (2001), the equilibrium parameter (K_R) values can be used to predict whether a sorption system (isotherm) is favorable or unfavorable using the essential feature values of the Langmuir adsorption isotherm. The isotherm parameter values (K_R) for the sorption of Cd(II) and Cu(II) were used to predict whether the sorption systems were favorable or unfavorable at all the different temperatures and initial metal ion concentrations studied. The values of K_R are shown in Table 3.5. For a favorable biosorption process the value of K_R must be greater than zero and less than unity (Ucun *et al.*, 2007; Ozdes *et al*., 2009). The K_R values obtained were found between 0 and 1 indicating that the sorption of Cd(II) and Cu(II) on GM 16 biomass was favorable under the conditions (pH, speed of agitation, biomass concentration, temperature) used in this study. The K_R values also indicated that sorption was more favorable for the higher initial Cd(II) and Cu(II) concentrations than for the lower ones. Furthermore, there was more favorable sorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass at 40 °C as indicated by the K_R values.

Table 3.5

K_R values based on Langmuir adsorption isotherm model.

Initial [metal] mg L^{-1}	**Cd(II)**			**Cu(II)**		
	25 °C	30 °C	40 °C	25 °C	30 °C	40 °C
40	0.153	0.149	0.144	0.472	0.462	0.430
80	0.083	0.081	0.080	0.249	0.244	0.233
100	0.068	0.066	0.065	0.216	0.211	0.197
120	0.057	0.055	0.054	0.189	0.185	0.178

3.9. **Kinetic modeling**

3.9.1. **Effect of temperature on the sorption rates of Cd(II) and Cu(II) onto the non-viable GM 16 biomass and its equilibrium metal sorption capacity**

In order to analyze the biosorption kinetics of Cd(II) and Cu(II) ions onto the non-viable GM 16 biomass at different temperatures, pseudo-first order and pseudo-second order rate kinetic models were applied to the equilibrium adsorption data.

Figs. 3.12 and 3.13 show plots of linearized forms of pseudo-first order and pseudo-second order rate kinetic models for the biosorption of Cd(II) and Cu(II), respectively, at 25 °C, 30 °C and 40 °C. The kinetic parameters of the different kinetic models and the correlation coefficients are also shown in Tables 3.6 and 3.7 for both Cd(II) and Cu(II) respectively.

The comparison of the correlation coefficients (Table 3.6 and 3.7) for the two kinetic models used (pseudo-first order and pseudo-second order rate kinetic models) indicated that the biosorption of Cd(II) and Cu(II) at different temperatures followed pseudo-second order rate kinetic model. The correlation coefficients were found to be high ($r^2 > 0.995$) for pseudo-second order model for the sorption of Cd(II). The pseudo-first order kinetic model showed non-linear relationships with the correlation coefficients less than 0.9 for the sorption of Cd(II). In addition, the theoretical values of q_e for pseudo-first order kinetic model were not in agreement with experimental data for the sorption of both Cd(II) and Cu(II) whereas pseudo-second order values were closer to those of the experimental data (Tables 3.6 and 3.7).

The initial sorption rate (h) increased with increase in temperature for the sorption of both Cd(II) and Cu(II). In addition, the initial sorption rates increased (3.88 to 4.08 mg g^{-1} min^{-1}) when temperature was increased from 25 °C to 30 °C for the sorption of Cd(II) than when the temperature was increased from 30 °C to 40 °C (Table 3.6). There was virtually no change in the initial sorption rate when the temperature was increased from 30 °C to 40 °C, suggesting that a temperature of 30 °C was adequate for the biosorption process. The results also indicated that the equilibrium metal sorption capacities (q_e) increased marginally with increase in temperature for the pseudo-second order kinetic model (Tables 3.6 and 3.7). The equilibrium metal sorption capacities (q_e) obtained from the pseudo-second order kinetic model were in good agreement with the experimental data (from Figs. 3.9A and B). The pseudo-second order rate constants (k_2) decreased marginally for the sorption of Cd(II) but they increased

slightly for the sorption of Cu(II) when temperature was increased from 25 to 40 °C.

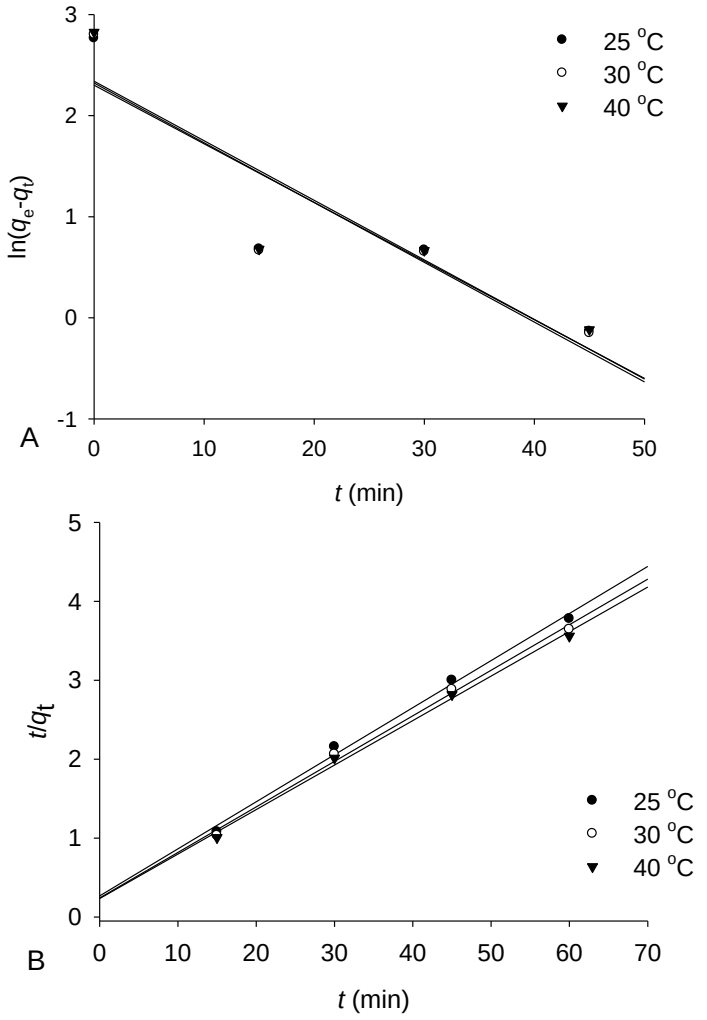

Fig. 3.12. Kinetic plots for the biosorption of Cd(II) onto non-viable GM 16 biomass at different temperatures. The initial concentration of the metal ion was 100 mg L^{-1} at pH 7. Pseudo-first order (A), pseudo-second order (B).

Table 3.6

Kinetic parameters for the effect of temperature on biosorption of Cd(II).

T °C	Pseudo-first order			Pseudo-second order				Experimental
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
25	9.97	0.059	0.821	16.0	0.0151	3.88	0.995	16.2
30	10.22	0.06	0.819	17.36	0.0136	4.08	0.995	17.02
40	10.38	0.06	0.815	17.51	0.0138	4.26	0.995	17.9

NB: The differences in model parameters were not statistically significant (*t*-test): $P \geq 0.05$

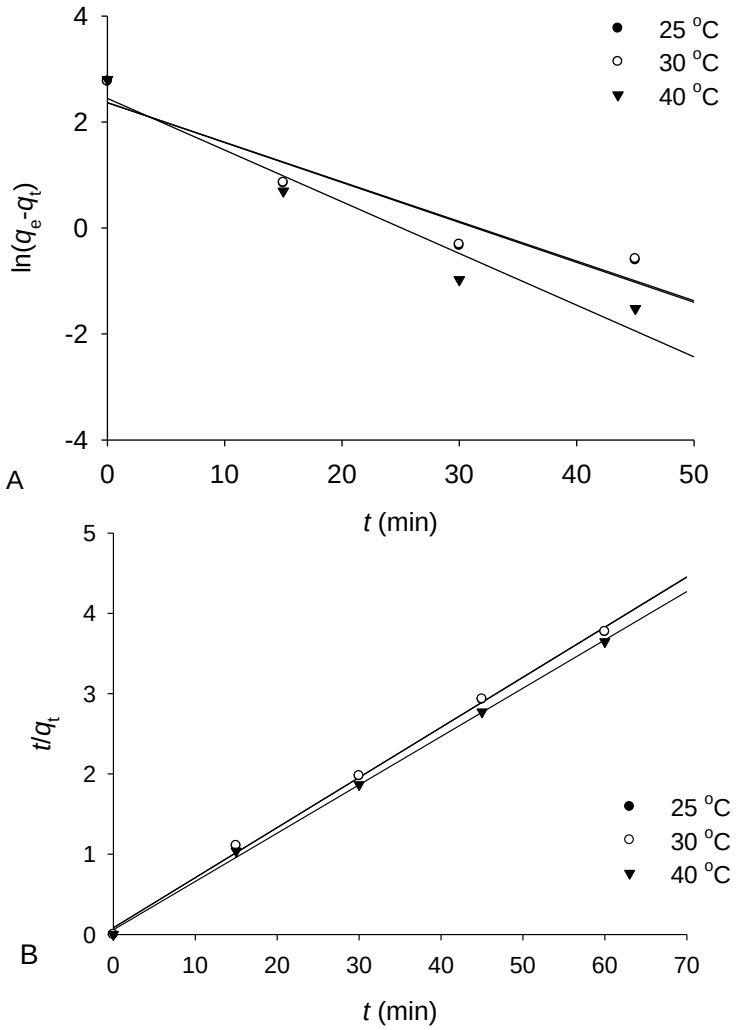

Fig. 3.13. Kinetic plots for the biosorption of Cu(II) onto non-viable GM 16 biomass at different temperatures. The initial concentration of the metal ion was 100 mg L^{-1} at pH 6. Pseudo-first order (A), Pseudo-second order (B).

Table 3.7

Kinetic parameters for the effect of temperature on biosorption of Cu(II).

T °C	Pseudo-first order			Pseudo-second order				Experimental
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
25	10.601	0.0708	0.904	15.92	0.0457	11.583	0.998	16.10
30	10.612	0.0709	0.904	15.98	0.0461	11.772	0.998	16.11
40	11.473	0.10	0.944	16.67	0.0599	16.646	0.998	16.51

NB: The differences in model parameters were not statistically significant (*t*-test): $P \geq 0.05$

3.9.2. **Effect of the initial metal ion concentration on the sorption rates of Cd(II) and Cu(II) onto the non-viable GM 16 biomass and its equilibrium metal sorption capacity**

The effects of initial metal ion concentrations on the sorption rates of Cd(II) and Cu(II) were studied at different concentrations (40-120 mg L^{-1}) of the metal ion at 40 °C (this is the temperature at which high q_{max} values were obtained for the sorption of both Cd(II) and Cu(II), Tables 3.3 and 3.4). Pseudo first-order and pseudo-second-order rate kinetic models were applied to the equilibrium data and the plots are shown in Figs. 3.14 and 3.15 for the sorption of Cd(II) and Cu(II), respectively. The rate constants and the regression correlation coefficients are also shown in Tables 3.8 and 3.9. The correlation coefficients obtained for pseudo-second order model were greater for the sorption of Cd(II) and Cu(II) than for pseudo-first order kinetic model. In addition, higher regression correlation coefficients (r^2 > 0.99) for the pseudo-second order model were obtained (Tables 3.8 and 3.9). For the sorption of Cd(II), the initial sorption rate (h) increased from 2.93 to 7.33 mg g^{-1} min^{-1} when the initial metal ion concentration was increased from 40 to 80 mg L^{-1} but further increase beyond 80 mg L^{-1} initial metal ion concentration resulted in a decrease in the initial sorption rate. Furthermore, increase in initial concentration of Cu(II) increased the initial sorption rates (h) at all initial metal ion concentration range used (Table 3.9). The pseudo-second order rate constant (k_2) also increased with an increase in initial Cu(II) ion concentrations whereas decreases in pseudo-second order rate constants were observed for the sorption of Cd(II) (Tables 3.8 and 3.9).

The equilibrium metal sorption capacity (q_e) of GM 16 increased with increase in initial Cd(II) and Cu(II) concentrations as indicated for the pseudo-second order kinetic model and the values obtained were reasonably in good agreement with the experimental data (from Figs. 3.9A and B), but the model overestimated q_e values. The q_e values obtained for pseudo-first order kinetic models were not in agreement with experimental data for the sorption of both Cd(II) and Cu(II)

(Tables 3.8 and 3.9). The pseudo-first order kinetic model showed non-linear relationships with lower correlation coefficients (r^2 < 0.990) than pseudo-second order kinetic model (r^2 > 0.990). Moreover, the q_e values found for the pseudo-first order kinetic model did not give reasonable values when compared with q_e experimental values at all initial metal ion concentrations.

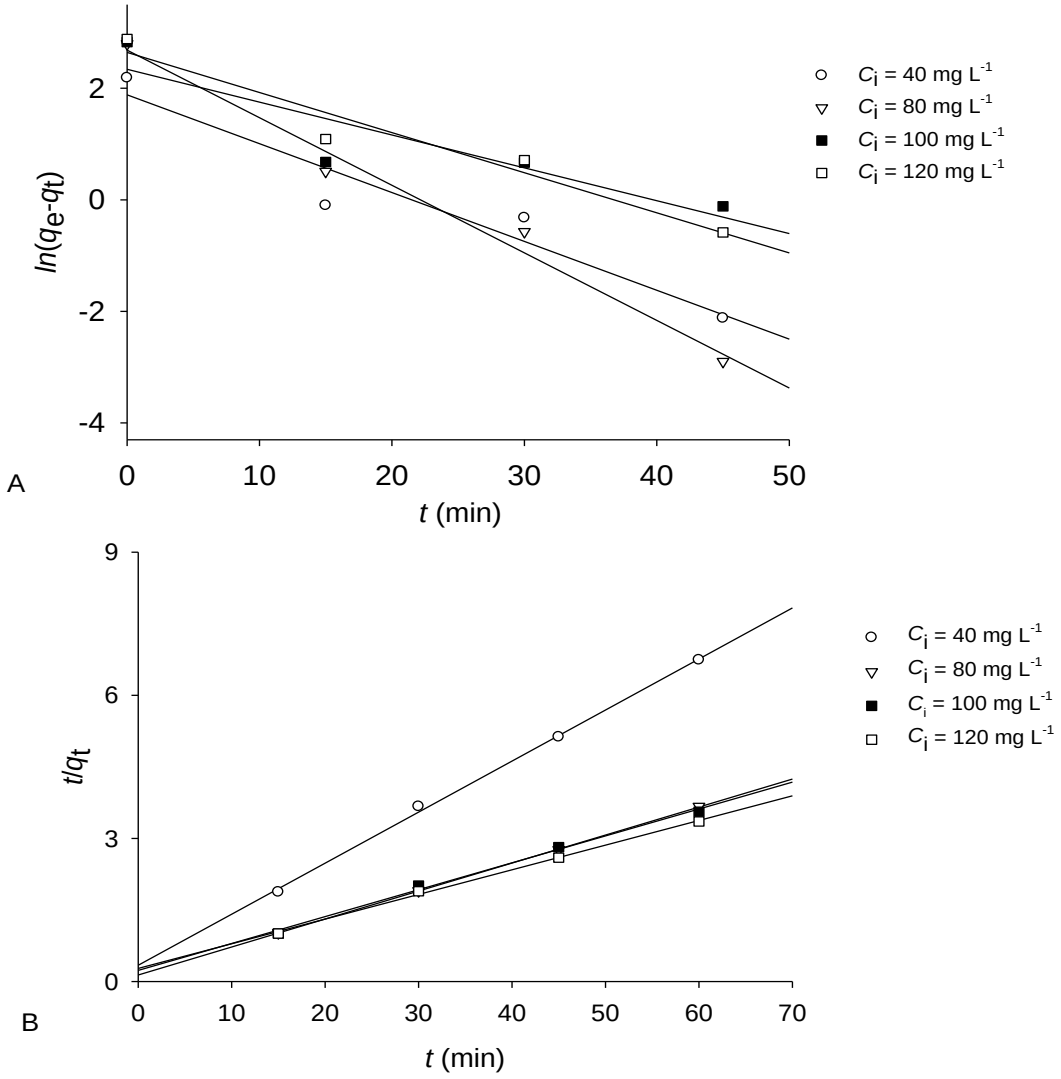

Fig. 3.14. Kinetic plots for the biosorption of Cd(II) onto non-viable GM 16 biomass at different metal concentrations and 40 ^{o}C. Pseudo-first order (A), Pseudo-second order (B).

Table 3.8

Kinetic parameters for the effect of initial metal ion concentration on biosorption of Cd(II).

Initial [Cd] mg L^{-1}	Pseudo-first order			Pseudo-second order				Experimental
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
40	6.39	0.09	0.922	9.4	0.0329	2.93	0.998	8.9
80	14.22	0.12	0.982	16.7	0.0264	7.33	0.999	16.4
100	10.54	0.06	0.815	17.8	0.0134	4.28	0.994	16.9
120	14.24	0.07	0.946	19.2	0.0098	3.64	0.998	17.9

NB: The differences in model parameters were statistically significant (*t*-test): $P \leq 0.05$

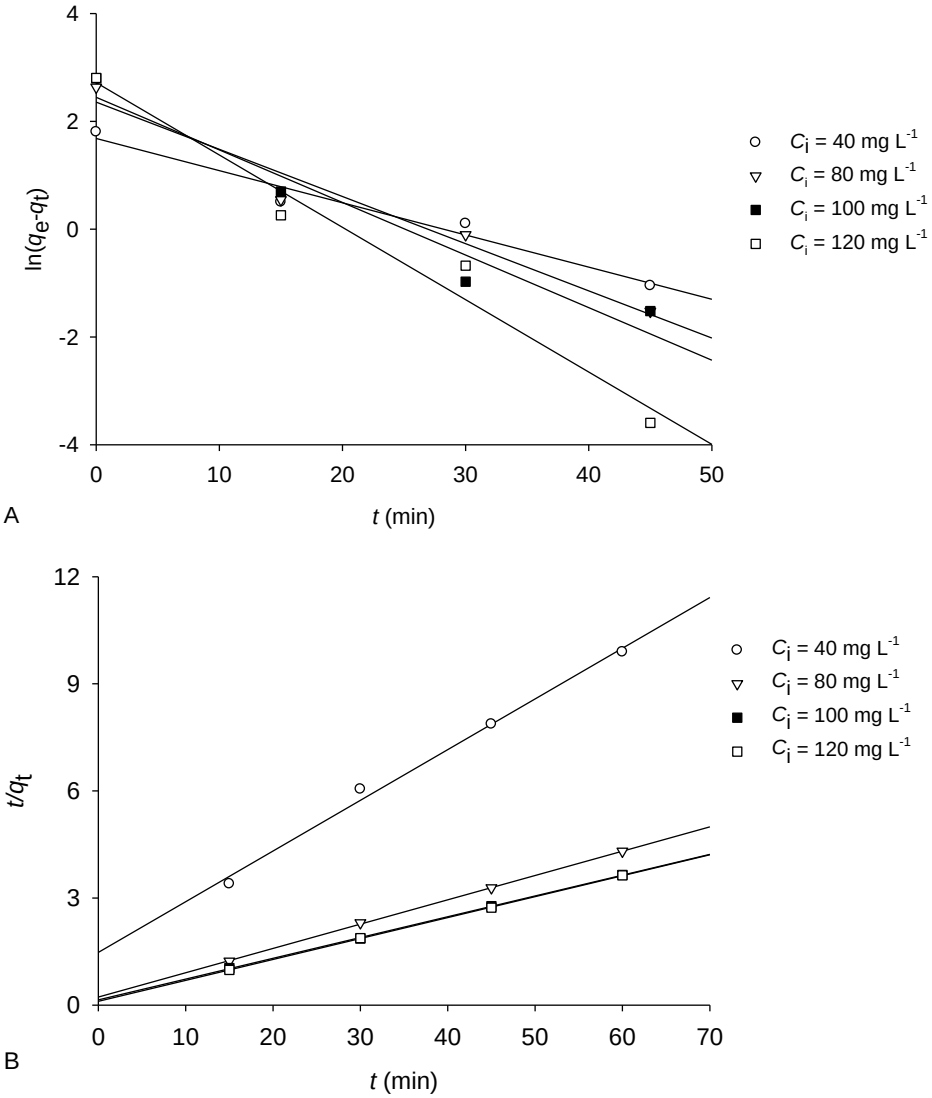

Fig. 3.15. Kinetic plots for the biosorption of Cu(II) by non-viable GM 16 biomass at different metal concentrations and 40 ^{o}C. Pseudo-first order (A), Pseudo-second order (B).

Table 3.9

Kinetic parameters for the effect of initial metal ion concentration on biosorption of Cu(II).

Initial [Cd] mg L^{-1}	Pseudo-first order			Pseudo-second order				Experimental
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
40	5.36	0.063	0.966	7.5	0.0119	0.67	0.993	6.066
80	10.48	0.088	0.960	14.7	0.0186	4.00	0.999	13.93
100	11.58	0.1	0.944	16.7	0.0240	6.67	0.999	16.45
120	15.64	0.138	0.968	17.1	0.0243	7.14	0.999	16.51

NB: The differences in model parameters were statistically significant (*t*-test): $P \leq 0.05$

3.9.3. **Effect of biomass concentration on the sorption rates of Cd(II) and Cu(II) onto the non-viable GM 16 biomass and its equilibrium metal sorption capacity**

The effect of increasing biomass concentration on the sorption rates of Cd(II) and Cu(II) was studied in 100 mg L^{-1} metal solutions at different concentrations (0.8-4.8 g L^{-1}) of biomass and 40 °C. Pseudo-first-order and pseudo-second-order kinetic models were applied to data and the plots are shown in Figs. 3.16 and 3.17 for the sorption of Cd(II) and Cu(II), respectively. The rate constants and the regression correlation coefficients for the two kinetic models were compared (Tables 3.10 and 3.11). Higher correlation coefficients ($r^2 > 0.900$) were obtained for pseudo-second order kinetic model at all the biomass concentrations used than for the pseudo-first order kinetic model. Generally, increase in biomass concentration increased the initial sorption rates of both metal ions on the biomass.

For the pseudo-second order kinetic model, the equilibrium metal sorption capacity (q_e) values increased with increase in biomass concentration. The comparison of the correlation coefficients of the two different kinetic models used (pseudo first-order and pseudo-second-order kinetic models) indicated that the biosorption of Cd(II) and Cu(II) followed pseudo-second order kinetic model. The straight-line plots were constructed for pseudo-second order, but the pseudo-first order model was found not to give good fittings for the biosorption of both metals.

The pseudo-first order kinetic model showed non-linear relationships with low correlation coefficients (r^2 < 0.90) at low biomass concentration (0.8 g L^{-1}) for both Cd(II) and Cu(II). However, the predicted q_e values for pseudo-first order model were in agreement with experimental data when the biomass concentration was increased from 0.8-1.6 g L^{-1}. Although the regression correlation coefficients for the pseudo-second order model were relatively high (r^2 > 0.990) indicating a good fit of the experimental data to this model, the predicted q_e values were overestimated at all biomass concentrations used.

The initial sorption rate (h) increased when the concentration of the biomass was increased from 0.8 to 4.8 g L^{-1} for the sorption of both Cd(II) and Cu(II). For the sorption of Cd(II), pseudo-second order rate constants (k_2) also increased slightly when biomass concentration was increased.

Table 3.10

Kinetic parameters for the effect of biomass concentration on biosorption of Cd(II).

[Biomass] g L^{-1}	**Pseudo-first order**			**Pseudo-second order**				**Experimental**
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
0.8	5.0	0.057	0.846	6.0	0.028	1.0	0.994	5.54
1.6	9.5	0.080	0.954	9.7	0.029	2.7	0.999	9.50
3.2	12.2	0.100	0.964	12.5	0.032	5.0	0.999	11.05
4.8	12.8	0.033	0.914	15.0	0.036	8.0	0.999	14.46

NB: The differences in model parameters were statistically significant (t-test): $P \leq 0.05$

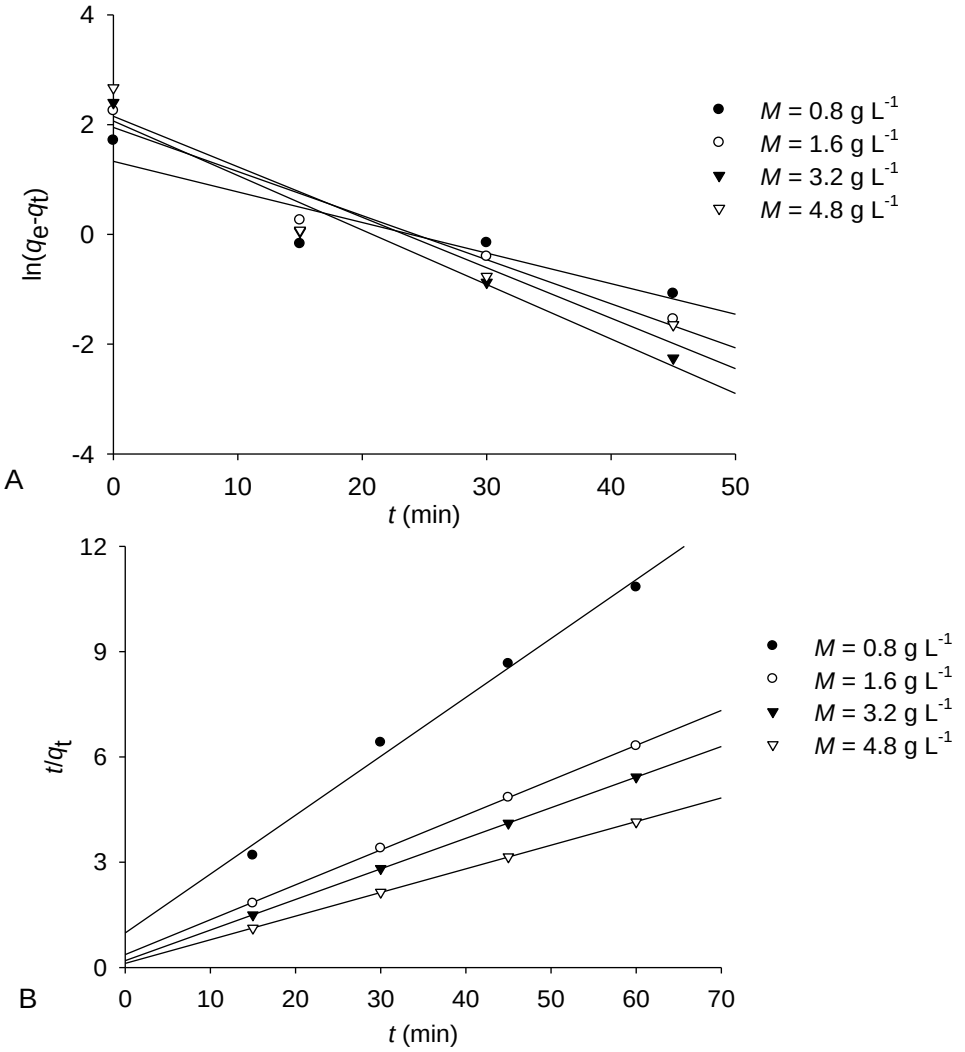

Fig. 3.16. Kinetic plots for the biosorption of Cd(II) onto non-viable GM 16 biomass at different concentrations of biomass and 40 ^{o}C. Pseudo-first order (A), Pseudo-second order (B).

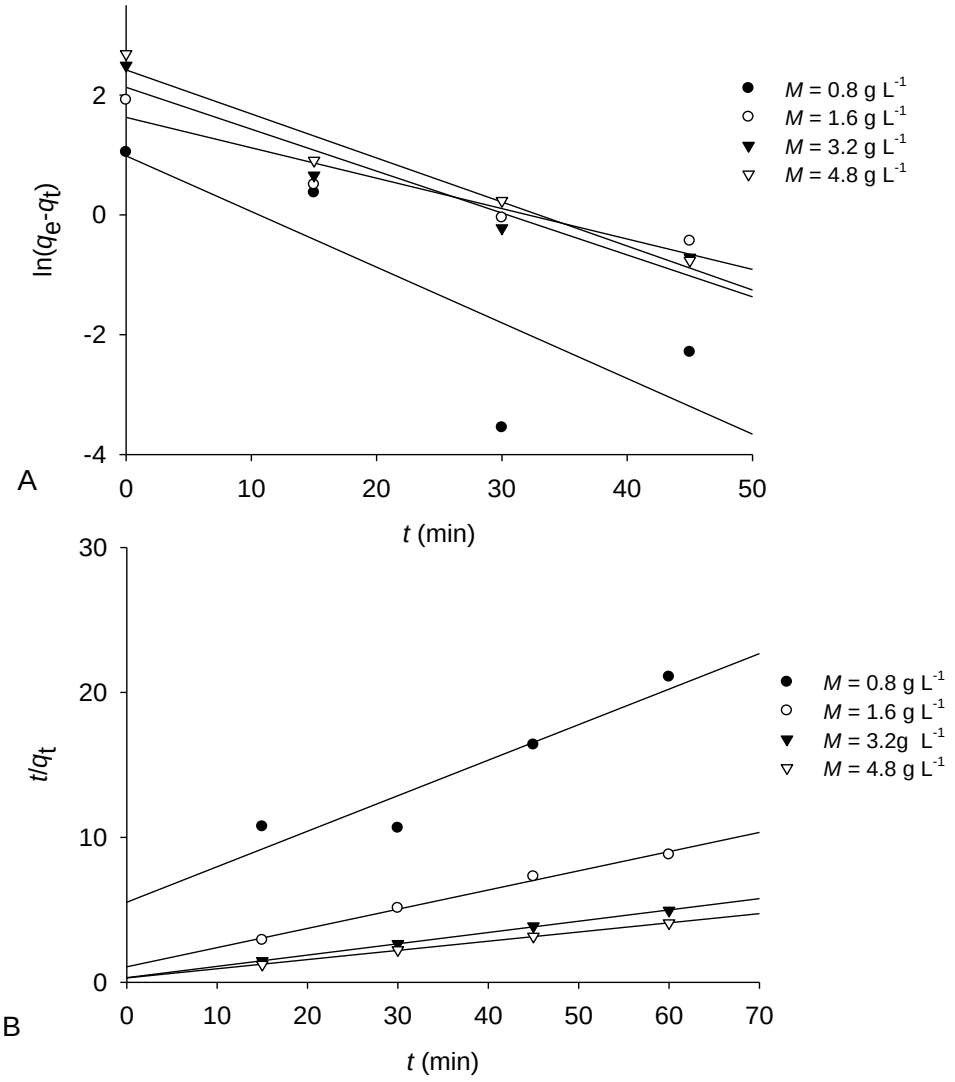

Fig. 3.17. Kinetic plots for the biosorption of Cu(II) by non-viable GM 16 biomass at different concentrations of biomass and 40 °C. Pseudo-first order (A), Pseudo-second order (B).

Table 3.11

Kinetic parameters for the effect of biomass concentration on biosorption of Cu(II).

[Biomass] g L^{-1}	Pseudo-first order			Pseudo-second order				Experimental
	q_e mg g^{-1}	k_1 L min^{-1}	r^2	q_e mg g^{-1}	k_2 g mg^{-1} min^{-1}	h mg g^{-1} min^{-1}	r^2	q_e mg g^{-1}
0.8	2.7	0.16	0.684	4	0.011	0.176	0.900	2.85
1.6	5.1	0.05	0.910	6	0.025	0.914	0.992	6.81
3.2	8.5	0.06	0.922	12	0.021	3.024	0.999	12.10
4.8	11.0	0.02	0.960	15	0.014	3.226	0.999	14.69

NB: The differences in model parameters were statistically significant (*t*-test): $P \leq 0.05$

3.10. **Thermodynamic parameters of Cd(II) and Cu(II) biosorption onto non-viable GM 16 biomass**

The effect of temperature on the efficiency of adsorption was studied at different temperatures in the range of 25-40 °C. The *Gibbs* free energy changes (ΔG^o) were calculated from equation $\Delta G^o = -RT\ln K_c$ and it was found to be negative for the sorption of both Cd(II) and Cu(II) (Table 3.12). Generally, the magnitude of change in *Gibbs* free energy (ΔG^o) increased with increase in temperature. The values of ΔH^o and ΔS^o were determined from the slopes and intercepts, respectively, of van't Hoff plot (Fig. 3.18). Thermodynamic parameters of Cd(II) and Cu(II) biosorptions, *Gibbs* free energy changes (ΔG^o), enthalpy change (ΔH^o), entropy change (ΔS^o) and the correlation coefficients were compared in Tables 3.12 and 3.13. The enthalpy and the entropy changes for the sorption processes were found to be 2.81 kJ mol^{-1} and 14 J mol^{-1} K^{-1}, respectively, for the sorption of Cd(II). For the sorption of Cu(II), the enthalpy and entropy changes were found to be -0.85 kJ mol^{-1} and 2.91 J mol^{-1} K^{-1}, respectively.

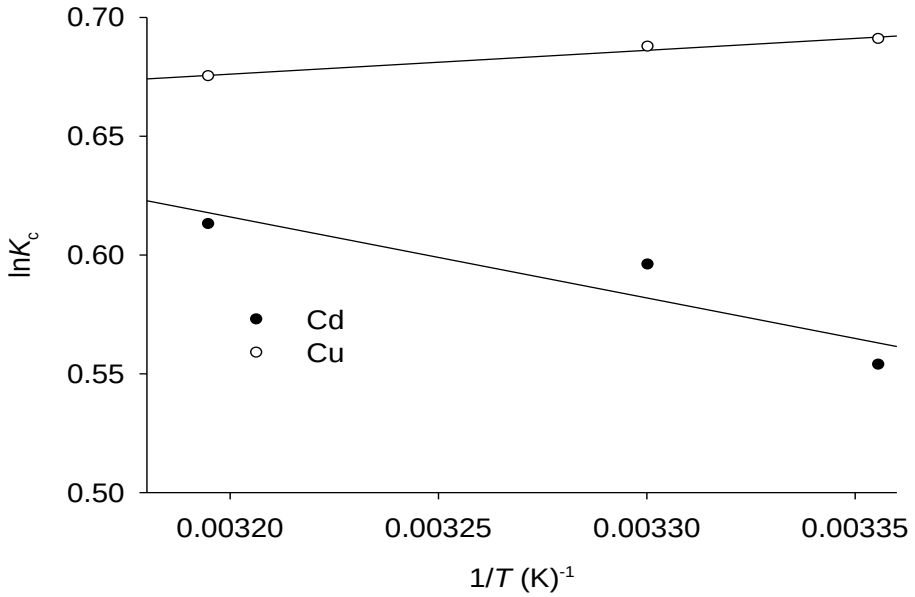

Fig. 3.18. Variations of $\ln K_c$ with $1/T$ (K^{-1}), van't Hoff's plot.

Table 3.12

Thermodynamic parameters of Cd(II) adsorption onto non-viable GM 16 biomass.

T K	T °C	K_c	ΔG^o ± SD kJ mol^{-1}	ΔS^o J mo^{l-1} K^{-1}	ΔH^o kJ mol^{-1}	r^2
298	25	1.74	-1.372 ± 0.035	14	2.81	0.835
303	30	1.82	-1.501 ± 0.016			
313	40	1.85	-1.595 ± 0.030			

Table 3.13

Thermodynamic parameters of Cu(II) adsorption onto non-viable GM 16 biomass.

T K	T °C	K_c	ΔG^o ± SD kJ mol^{-1}	ΔS^o J mol^{-1} K^{-1}	ΔH^o kJ mol^{-1}	r^2
298	25	1.99	-1.712 ± 0.042	2.91	-0.85	0.979
303	30	1.98	-1.732 ± 0.026			
313	40	1.96	-1.757 ± 0.032			

Chapter 4

4. Discussion

4.1. Identification of bacterial isolates

The results presented in this study have shown two different groups of bacteria, the *Bacillus* as well as members of *Serratia* spp. which have been isolated from samples collected from an antimony mine in South Africa. Gram-positive bacilli (GM 10(1), GM 10(2), GM 15 and GM 16) and Gram-negative cocci (GM 14 and GM 17) bacterial isolates have been identified. All the isolates possessed 16S rDNA sequences with greater than 90 % sequence similarities with those of previously characterized bacterial species with the exception of GM 10(1). Only 72 % sequence similarity of GM 10(1) was obtained when compared with previously identified organisms signifying delineation of this isolate. Isolate GM 16 was identified as a member of *Bacillus* species and is closely related to *Bacillus thuringiensis* and *Bacillus cereus* strains as shown in Fig. 3.3. *Bacillus thuringiensis* is a Gram-positive soil-dwelling bacterium and closely related to *Bacillus cereus* species which is also a soil-dwelling bacterium. Members of the *Bacillus* group have been isolated from heavy metal polluted environments and were found to be highly resistant to heavy metal ions as reported by Kamala-Kannan and Lee (2008).

4.2. Determination of bacterial morphology by Gram-staining

The cell wall of the Gram-positive bacterial isolate GM 16 (identified as a *Bacillus* sp.) suggests that this isolate may possess teichoic acids and peptidoglycan, providing carboxyl and phosphoryl groups that are potential sites for metal binding. Different functional groups (OH^-, COO^-, NH_3^+, PO_4^{2-} and SO_3^-) found on microbial cell walls have been reported to serve as metal sorption sites during biosorption (Mashitah *et al*., 1999; Tereshina *et al*., 1999; Zhou, 1999). The metal

sorption capacities of isolate GM 16 biomass described in this study could be attributed to the nature of its bacterial cell wall. However, in this study, the surface functional groups on isolate GM 16 were not investigated. There are also some studies which have reported the potential uses of some members of the *Bacillus* spp. in the biosorption of Cu(II) (Lo *et al*., 2003; Pan *et al*., 2007; Zheng *et al*., 2008) and Cd(II) (Valentine *et al*., 1996).

4.3. **Biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass**

The effective removal of heavy metals from polluted waste water is among the most important issues for many industrialized countries. Although the biosorption process has been reported to be an efficient and eco-friendly technology for heavy metal removal from polluted waste water, there are several factors that are crucial for the design and application of biosorption process (Vijayaraghavan and Yun, 2008[b]). Most laboratory studies of biosorption have been performed on a small scale but the results might not be applicable in a large scale environment. Therefore, it is important to consider investigating the environmental factors affecting the biosorption process for the design of a specific biosorption reactor. The ability of non-viable GM 16 biomass to adsorb Cd(II) and Cu(II) was investigated in a batch equilibrium process. The results presented in this study showed the effects of environmental factors affecting Cd(II) and Cu(II) biosorption processes using non-viable GM 16 biomass. Although most industrial applications prefer a continuous mode of operation, batch experiments have often been used to evaluate the required fundamental information, such as biosorbent efficiency, optimum experimental conditions, biosorption rate and the possibility of biomass regeneration (Vijayaraghavan and Yun, 2008[b]).

4.3.1. **Effect of contact time on the biosorption of Cd(II) and Cu(II)**

The present study showed that biosorption of both Cd(II) and Cu(II) by GM 16 biomass was rapid during the first 15 min of biomass contact with the metal

solutions and equilibria were reached within 1 h (Figs. 3.4A and B). Many reports on metal biosorption studies have shown that the biosorption process is a fast process and equilibrium is reached within the first few minutes or few hours (Ucun *et al*., 2007; Khani *et al*., 2008; Ozdes *et al*., 2009). The increase in metal sorption in the first few minutes of biomass exposure to the metal solution suggests the availability of the sorption sites and that the metal ions were able to interact easily with the sorption sites. For all the pH values studied, equilibrium was reached within 1 h. However, to make sure that there was sufficient contact between the metal and biosorbent, the sorption experiments were carried out for 140 min (Fig. 3.1A). The observed rapid kinetic phenomenon is practically important as it could facilitate the scale-up of the process in smaller reactor volumes that will ensure economic efficiency. It could also ensure shorter contact time between the metal solution and the biosorbent in continuous process.

4.3.2. **Effect of initial pH of metal solution on the biosorption of Cd(II) and Cu(II)**

The biosorption of both Cd(II) and Cu(II) by the non-viable GM 16 biomass was also a function of pH of the metal solution. There was an increase in metal sorption when the pH of the metal solution was increased until the maximum sorption of metals was reached for both Cd(II) and Cu(II) (Fig. 3.5). The maximum sorption of Cu(II) occurred under more acidic (pH 6) conditions than the sorption of Cd(II) which occurred at pH 7. The maximum metal sorption capacities were found to be 16.7 mg g^{-1} of dry weight of biomass when the initial pH of the metal solution was 7 for Cd(II) and 13.7 mg g^{-1} of dry weight of biomass when the initial pH of the metal solution was 6 for Cu(II). The difference in the pH values at which the maximum adsorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass occurred indicated that the biosorption process of each of the metal ions was highly dependent on the initial pH of the metal solution. Previous studies on biosorption of heavy metals have shown that pH is an important parameter affecting the biosorption process (Aksu, 2001; Esposito *et al*., 2002;

Vijayaraghavan and Yun, 2008[b]). The pH of a metal solution has been reported to influence both the cell surface metal binding sites and metal chemistry in water (Aksu, 2001). At low pH, the overall surface charge on the cells becomes positive, which inhibits the approach of positively charged metal cations (Hanif *et al*., 2007; Özdemir *et al*., 2009). The protons compete with metal ions for binding sites, thereby decreasing the interaction of metal ions with microbial cells (Sag and Kutsal, 1995). However, when the pH is increased, more ligands such as the carboxylic, phosphate, imidazole and amino groups would be exposed and will carry a negative charge with subsequent adsorption of metallic cations and biosorption onto the cell surface (Dönmez *et al.*, 1999; Aksu, 2001). The results on pH effect suggest that an adjustment of pH of wastewater may be necessary for a better sorption of Cu(II) and Cd(II).

In comparison with the pH values at which the maximum sorption of Cd(II) and Cu(II) occurred in this study, several studies have reported that the biosorption of Cd(II) and Cu(II) occurred at pH 7 and 6, respectively, using different biosorbents. Aydin *et al*. (2008) and Kapoor *et al*. (1999) reported the biosorption of copper at pH 6 using biomasses of *Aspergillus niger* or Lentil shell. In addition, the biosorption of cadmium at pH 7 was reported by Zouboulis *et al*. (2004) and Green-Ruiz *et al*. (2008) using *Aspergillus niger* or *Bacillus jeotgali* as biosorbents. Other studies reported biosorption of Cd(II) at pH values above 7 (Valentine *et al*., 1996; Igwe and Abia, 2007) whereas the maximum adsorption of Cu(II) was found between pH 4-8 (Pehlivan *et al*., 2006). Yilmaz and Ensari (2005) reported the biosorption of Cd at pH 7 using *Bacillus circulans* strain EB1.

In the present study, the effect of pH on the sorption of Cd(II) by the GM 16 biomass above the pH of 7 was negligible. The metal sorption capacity remained constant at pH values above 7 ($P \geq 0.05$). However, Aksu (2001) reported a decrease in metal uptake by *C. vulgaris* at pH values beyond the optimum pH of 4 when studying the biosorption of Cd. The different pH sorption profiles for various heavy metal ions could be related to the nature of chemical interactions

of each metal with the different biomass types. The study of pH effect on the sorption on Cd(II) at high pH values could not be done because precipitation of the metal occurred. Furthermore, beyond the optimum pH value of 6 for Cu(II) adsorption, precipitation occurred. Antunes *et al*. (2003) also reported that insoluble copper hydroxide precipitates were formed at pH values above 6, making true biosorption studies impossible. This supports the general view that pH of the solution affects the solubility of the metal ions; hence, it is always important to study the biosorption process when the metal is dissolved in solution. Schiewer and Volesky (1995) reported that it is difficult to distinguish between sorption and metal precipitation during metal sorption studies. This phenomenon has also been observed in other biosorption studies (Aksu, 2001; Pokhrel and Viraraghavan, 2006; Mamisahebei *et al*., 2007).

4.3.3. **Selective adsorption of Cd(II) or Cu(II) in binary [Cd(II) + Cu(II)] metal solutions at optimum pH values for Cu(II) (pH 6) and Cd(II) (pH 7)**

The observation that there were different pH optima for the adsorption of Cd(II) and Cu(II) necessitated the investigation of the adsorption of each metal in the presence of the other at their respective pH optima. The results suggested that the presence of either metal did not have any appreciable effect on the adsorption capacity of the biomass at the respective pH optimum of each metal (Fig. 3.6). These observations corroborate the report of Shi *et al*. (2004) which evinced that the adsorption of a specific metal cation is highly dependent on pH of the metal solution.

4.3.4. **Effect of the speed of agitation of the biosorption of Cd(II) and Cu(II)**

Increase in the speed of agitation caused increase in the biosorption of Cd(II) or Cu(II) until the optimum speed was reached. The optimum speed of agitation was found to be 100 rpm for the uptake of both Cd(II) and Cu(II) on GM 16 biomass (Fig. 3.7). Further increase in speed of agitation beyond the optimum speed (100

rpm) resulted in a decrease in metal sorption capacity. The speed of agitation is reported to facilitate proper contact between metal ions in solution and the biomass binding sites thereby promoting effective transfer of sorbate ions to the sorbent sites (Ahalya *et al*., 2005). However, the biosorption of Cd(II) or Cu(II) by GM 16 biomass decreased at high speeds of agitation (above 100 rpm). As a physical phenomenon, the metal ions seemed to be desorbed from the sorption sites at high speeds of agitation resulting in a low metal uptake. Several studies have reported a positive impact of increase in speed of agitation until the optimum speed is reached where the maximum metal uptake is achieved. Benguella and Benaissa (2002) noticed a reduction in the removal of cadmium by chitin at low (below 100 rpm) and very high speeds (1000-1250 rpm) of agitation. Ahalya *et al*. (2005) also reported low adsorption rate in a non-agitated system and at high speed (180 rpm) of agitation. Generally, the optimum speed of agitation of biosorption processes with different biosorbent materials (bacterial, fungal, algae and other biomaterials used for biosorption) is moderate (0 to 400 rpm) depending on the type of biomass used. Niyogi *et al.* (1998) reported an optimum speed of agitation for the removal of Cr(VI) by *Rhizopus arrhizus* to be 100 rpm. Bai and Abraham (2002) reported the optimum speed of agitation to be 120 rpm for the removal of Cr(VI) by *Rhizopus nigricans*. Ahalya *et al*. (2005) also reported 120 rpm as the optimum speed of agitation for the removal of Cr(VI) using the husk of *Cicer arientinum*.

4.3.5. **Effect of biomass concentration on the biosorption of Cd(II) and Cu(II)**

Another parameter that strongly affects metal uptake is the concentration of biomass. Data on the effect of biomass concentration can be useful for optimization of the process of metal biosorption especially if the metal concentration in metal contaminated effluent is known. Appropriate amount of the biosorbent could be added according to the effective ratio. Therefore, the biomass effect on Cd(II) and Cu(II) biosorption was studied at a constant metal

ion concentration (100 mg L^{-1}), pH 6 (Cu) and pH 7 (Cd), temperature (40 °C), speed of agitation (100 rpm) and varying the biomass concentration from 0.8 to 4.8 g L^{-1}. There was a rapid increase in the biosorption both Cd(II) or Cu(II) when the initial concentration of the biomass was increased from 0.8 to 4.8 g L^{-1} (Fig. 3.8). The rapid increase in the sorption of metals was probably due to the increase in the number of sorption sites for metal sorption when the biomass was increased. Several studies have reported gradual increase in metal uptake when the concentration of the biomass was increased. However, at high biomass concentrations the available metal in solution could be insufficient to completely occupy the available binding sites on the biomass, resulting in low metal uptake. The availability of the metal ions in a solution, the unsaturated binding sites, the interaction of metal ions with the sorbent materials in the biosorption process or the affinity between the binding sites and the metal ions can affect metal uptake (Rome and Gadd, 1987; Fan *et al*., 2008; Febrianto *et al*., 2009).

The results in this study suggest that the biosorption of Cd(II) and Cu(II) by GM 16 cells could be dependent on the number of binding sites available on the biosorbent, the availability of the metal to be adsorbed in a solution and the interaction of the metal ions to be adsorbed with the binding sites. However, Tangaromsuk *et al*. (2002) observed a negative effect of high biomass concentration on the metal uptake. Also, Gadd *et al*. (1988) have suggested that the interference between binding sites is due to increased biomass concentrations as this will result in a low specific metal uptake. Metal sorption efficiency of other biomass types has been reported to decrease due to cell aggregation and reduction in the distance between the metals with increasing biomass concentrations (Rome and Gadd, 1987; Akhtar *et al*., 2004).

4.3.6. **Effect of other cations on the biosorption of Cd(II) and Cu(II)**

Industrial application of a biosorption process must deal with the fact that heavy metal bearing wastewater often contains other metal ions that may interfere with

the uptake of heavy metals of interest (Babarinde *et al*., 2008; Hernainz *et al*., 2008). Therefore, batch equilibrium experiments were conducted to evaluate the effect of some common cations (Mg^{2+}, K^{+}, Na^{+} or Ca^{2+}) on the sorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass. These cations (Mg^{2+}, K^{+}, Na^{+} and Ca^{2+}) were chosen on the fact that some they are some of the naturally occurring elements that could be found at mining sites as well as being constituents of polluted wastewater/effluent. Some metal ions (Mg^{2+}, K^{+}, Na^{+} and Ca^{2+}) have been used as eluants of metals from biosorbents (Gupta *et al*., 2000). The sorption experiments were performed using equimolar concentrations of the metal ions (Cd^{2+}, Cu^{2+}, Mg^{2+}, K^{+}, Na^{+} or Ca^{2+}). The biosorption of Cu(II) on the biomass was slightly decreased in the presence of these metal cations (Table 3.2). A decrease in the sorption of Cu(II) in the presence of all the cations tested is an indication that the cations might have competed with Cu(II) for the available binding sites in the cell walls of GM 16 biomass. Metal counter-ions have been reported to affect the adsorption of targeted heavy metals on biosorbent materials (Mahvi *et al*., 2008). Calcium and potassium ions have been shown to decrease the sorption of Zn, Cd and Cu in some studies (de Franca *et al*., 2002; Hashim and Chu, 2004; Ozdes *et al*., 2009). Other studies have reported competitive sorption of the desired metal ions at increasing concentrations of the metal counter-ions (Hashim and Chu, 2004; Ozdes *et al*., 2009) and in all cases, the increasing concentration of metal counter-ions decreased the sorption of desired metal ions. For example, Hashim and Chu (2004) reported the reduction in cadmium sorption to less than 65 % on the biomass *Sargassum bacularia* (seaweed) in the presence of calcium ions. In the present study, divalent cations (Ca^{2+} and Mg^{2+}) inhibited the biosorption of Cu(II) more than monovalent cations (K^{+} and Na^{+}). Ca^{2+} inhibited Cu(II) sorption by 6.4 % whereas Mg^{2+} showed 4.7 % inhibition. K^{+} and Na^{+} inhibited the uptake of Cu(II) by 1.6 and 1.9 % respectively. The atomic weight of a metal ion has also been reported to play a role in the biosorption of a metal ion (Al-Rub *et al*., 2006). Heavy metal ions, especially those with high atomic numbers, tend to bind to some functional groups as indicated by Nies (1999). In comparison with the metal counter ions, the atomic

weight of copper (63.546) is higher than as opposed to Mg^{2+} (24.305), Ca^{2+} (40.078), K^{+} (39.098) and Na^{+} (22.989) (de Laeter *et al*., 2003). Hence the possibility of Cu(II) adsorption on GM 16 biomass would be more than that of the counter ions.

The sorption of Cd(II) by GM 16 biomass was enhanced in the presence of K^{+}, Na^{+} or Ca^{2+} (Table 3.2). The adsorption of Cd(II) on GM 16 biomass was increased in the presence of K^{+}, Na^{+} or Ca^{2+}. Monovalent cations (K^{+} and Na^{+}) were more potent enhancers of Cd(II) adsorption than divalent cations (Ca^{2+} and Mg^{2+}). K^{+} and Na^{+} enhanced the uptake of Cd(II) by 12.3 and 8.7 % respectively, whereas Ca^{2+} enhanced the uptake of Cu(II) by 3.2 %. The fate of metal cations (Mg^{2+}, K^{+}, Na^{+} and Ca^{2+}) was not measured in this study since the focus was in the biosorption of Cd(II) and Cu(II). Factors such as the initial pH (7) of the metal solution might have played a role in the selective adsorption of Cd(II) over the other metal counter ions. This study was performed at the optimum pH (7) for the sorption of Cd(II). Similar to Cu(II), the atomic weight of Cd(II) (112.411) is higher than that of metal counter-ions; Mg^{2+} (24.305), Ca^{2+} (40.078), K^{+} (39.098) and Na^{+} (22.989), hence the possibility of Cd(II) adsorption on GM 16 biomass would be more than that of the counter-ions. The data presented in this study suggest that, under the same conditions used in this study, GM 16 biomass was able to biosorb Cd(II) and Cu(II) from heavy metal polluted water even in the presence of the metal ions investigated.

4.3.7. **Effect of temperature on the biosorption of Cd(II) and Cu(II)**

Increase in temperature caused marginal increases in the adsorption of Cd(II) and Cu(II) at all the initial metal ion concentrations (40-120 mg L^{-1}) investigated (Fig. 3.9). There was no significant ($P \geq 0.05$) increase in the biosorption of Cd(II) and Cu(II) by GM 16 biomass when the temperature was increased from 25 °C to 40 °C. The effect of temperature on the metal biosorption processes found in literature presents diverse behaviors (Gupta and Rastogi, 2008). Some

temperature-independent biosorption processes have also been reported (Aksu and Kutsal, 1990; Tuzun *et al*., 2005; Lodeiro *et al*., 2006). There are, however, reports showing decreases in Cd uptake capacities of some biosorbents (*Chlorella vulgaris*, *Sargassum* sp., *Oedogonium* sp, etc.) with increases in temperature (Aksu, 2001; Cruz *et al*., 2004; Gupta and Rastogi, 2008). Even a more complex process was described by Benguella and Banaissa (2002) for the adsorption of cadmium by chitin where an initial increase in adsorption capacity was followed by subsequent reduction. It is known that temperature increases the kinetic energy of the molecules thereby facilitating the interaction between the biomass and the metal ions (Vijayaraghavan and Yun, 2008[b]; Özdemir *et al*., 2009).

Generally, similar observations were made by other researchers about the effect of temperature on the biosorption of Cd and Cu using different biososrbents. Özdemir *et al*. (2009) observed that temperature in the range of 30-80 °C had insignificant effect on the biosorption of Cd, Cu and other divalent metal cations by non-viable *Geobacillus* spp. Iqbal and Edyvean (2004) also found no significant differences in specific Cu uptake values at different temperatures (10-50 °C) by *Phanerochaete chrysosporium* fungal biomass immobilized on loofa sponge. Furthermore, temperature did not affect the removal of cadmium by immobilized *Spirulina platensis* on alginate gel (Rangsayatorn *et al*., 2004). The temperature of the biosorption solution could be important for energy dependent mechanisms in metal binding processes (Congeevaram *et al*., 2007; Green-Riuz *et al*., 2008). Energy-independent mechanisms are less likely to be affected by temperature, since the processes involved in metal removal are largely physico-chemical in nature (Zouboulis *et al*., 2004).

4.3.8. **Effect of initial metal ion concentration on the biosorption of Cd(II) and Cu(II)**

The effect of the initial metal ion concentration on Cd(II) and Cu(II) biosorption was studied at a temperature range of 25-40 °C. These are the normal temperature ranges that most researchers use for studying biosorption processes (Rangsayatorn *et al*., 2004; Dursun, 2006; Özdemir *et al*., 2009). Most biosorption processes have been studied using initial metal ion concentrations in the range of 50 to 1000 mg L^{-1} (Gupta and Rastogi, 2008; Baysal *et al*., 2009; Ozdes *et al*., 2009). The biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass were performed using concentration ranges between 40 to 120 mg L^{-1}. The non-linearized Langmuir adsorption isotherms (graph of q_e versus C_e) of the metal ions adsorbed by the GM 16 biomass showed that the amount of either Cd(II) or Cu(II) adsorbed increased when the equilibrium concentration was increased in solution (Fig. 3.9A and B). The adsorption isotherm for the sorption of Cd(II) by the biomass reached equilibrium when the equilibrium concentration (C_e) was lower (20 mg L^{-1}) than that of Cu(II) where equilibrium was reached when the equilibrium concentration in a solution was 35 mg L^{-1}. The increase in the biosorption of metals was due to an increase in initial metal ion concentration. However at high initial metal ion concentrations (80 to 120 mg L^{-1}) there was a gradual increase in the biosorption of metals.

4.4. **Linearized adsorption isotherms**

Although the non-linearized isotherm analysis showed that temperature had little effect on the sorption of Cd(II) and Cu(II), the linearized Langmuir and Freundlich adsorption models were used to characterize the adsorption performance of the biosorbent material (non-viable GM 16 cells) for Cd(II) and Cu(II) biosorption. Analysis of equilibrium data is important in characterizing, designing and to predict or explain the biosorption processes. The Langmuir and Freundlich adsorption models are important in describing adsorption performance of a

biosorbent material for the sorption of metals, to describe the biosorption data and to elucidate the possible sorption mechanisms (Özdemir *et al*., 2009). In this study, Linearized Langmuir and Freundlich adsorption isotherm models were used to interpret the data.

The data fitted well to the Langmuir adsorption isotherm model for the sorption of Cd(II). Therefore, biosorption process of Cd(II) by non-viable GM 16 biomass could be considered as monolayer adsorption as in the case of the biosorption of Cu by the *Bacillus* sp. isolated from metal polluted soil (Tunali *et al*., 2006). The values of q_{max} obtained from the Langmuir model for the sorption of Cd(II) on GM 16 biomass were close to the experimental values. However, the differences in the Langmuir adsorption constant (K_L) values at the different temperatures used (25-40 °C) were not statistically significant ($P \geq 0.05$) (Table 3.3). The K_R (equilibrium parameter) values calculated for Cd(II) with initial concentrations used (40-120 mg L^{-1}) were in the range of 0.054-0.153 at a constant GM 16 biomass concentration (4 g L^{-1}) indicating a favorable biosorption process (Table 3.5). For a favorable biosorption process the value of K_R must be greater than zero and less than unity (Ucun *et al.*, 2007; Ozdes *et al*., 2009).

The equilibrium adsorption isotherm of Cu(II) by the GM 16 biomass fitted to both Langmuir and Freundlich adsorption isotherm models with fairly good regression correlation coefficients ($r^2 > 0.900$). The maximum metal sorption capacity (q_{max}) values determined from the Langmuir isotherm for the sorption of Cu(II) increased with increase in temperature although not significantly ($P \geq 0.05$). A large value of K_L (0.041) at 40 °C implied strong binding of Cu(II) ions onto the non-viable GM 16 biomass (Table 3.4). When the Freundlich isotherm model was used for the data analysis, there were slight decreases in the biosorption capacity (K_F) and the intensity of adsorption (n) for the sorption of Cu(II) on GM 16 biomass when the temperature was increased from 25 to 40 °C but the differences were not significant ($P \geq 0.05$). The adsorption of Cu(II) on GM 16 biomass was favorable as indicated by the Freundlich constant, $n > 1$.

Furthermore, the K_R values calculated for Cu(II) at all initial metal ion concentrations used (20-120 mg L^{-1}) were in the range of 0.178-0.472 (Table 3.5) at a constant GM 16 biomass (4 g L^{-1}) indicating a favorable biosorption process. The K_R values also indicated that sorption was more favorable for the higher initial Cd(II) and Cu(II) concentrations than for the lower ones. Furthermore, a more favorable sorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass at 40 °C as indicated by the K_R values. Similar observation were made by Ho (2003) for the favorable sorption of copper on tree fern (K_R values were closer to zero at higher initial concentrations used).

4.5. **Kinetic modeling**

Adsorption equilibria studies are important to determine the efficacy of adsorption. It is also necessary to identify the type of adsorption mechanism in a given system. To investigate the mechanism of biosorption and its potential rate-controlling steps, kinetic models have been used to test the experimental data. The information on the kinetics of metal uptake is required to select the optimum conditions for full-scale batch metal removal processes (Febrianto *et al*., 2009). Kinetic studies have been carried out in batch reactions using various initial metal and biomass concentrations, agitation speeds, pH values and temperature along with different sorbent and sorbate types (Febrianto *et al*., 2009). Although there are several adsorption kinetic models used to understand the adsorption kinetics and rate-limiting steps, pseudo-first order and pseudo-second order kinetic models are the most widely-used models used to study the biosorption kinetics of heavy metals (Liu and Liu, 2008). Kinetic studies on adsorption of numerous biological materials have been done using pseudo-first order and pseudo-second order kinetic models (Febrianto *et al*., 2009). Pseudo-first order and pseudo-second order kinetic models were used in this study to investigate the effect of temperature, initial metal ion and biomass concentrations on kinetics and adsorption capacities of biomass for Cd(II) and Cu(II) biosorption. For pseudo-first order, a linear plot of $\ln(q_e - q_t)$ versus t suggests the applicability of this

kinetic model (Aksu, 2001). The pseudo-second order equation is also based on the sorption capacity of the solid phase and it has been frequently employed to analyze biosorption data obtained from various experiments using different adsorbates and adsorbents (Ho *et al*., 2000). A linear plot of t/q_t against t suggests the applicability of this model (Aksu, 2001).

Generally, the sorption processes of Cd(II) and Cu(II) at different temperatures, increasing initial metal ion and biomass concentrations fitted well to the pseudo-second order kinetic model. This was indicated by the regression correlation coefficients which were greater than 0.990. The pseudo-first order kinetic model showed non-linear relationships and the equilibrium metal sorption capacity (q_e) values were not in agreement with the experimental data and they were also found to be unreasonable when compared with experimental q_e values. Similar results were reported for the sorption of Cd(II) by nonliving algal biomass of *Oedogonium* sp. from aqueous phase (Gupta and Rastogi, 2008) and the sorption of Pb(II) onto *Candida albicans* biomass (Baysal *et al*., 2009), where pseudo-second order model described the adsorption kinetics of the metals well whereas pseudo-first order kinetic model did not. As reviewed by Febrianto *et al*. (2009), disagreement often occurs for most systems, at which the adsorption capacities (q_e) are not equal to the experimental q_e values, indicating the inability of pseudo-first order model to fit the kinetic data of heavy metal biosorption. Similarly, the trend showed that the predicted q_e values for the sorption of Cd(II) and Cu(II) by GM 16 biomass seemed to be lower than the experimental values when using the pseudo-first order kinetic model. A number of metal biosorption studies have been reported to fit pseudo-second order more than pseudo-first order (Febrianto *et al*., 2009). The results obtained in this study suggest that the biosorption system studied followed pseudo-second order more than the pseudo-first order kinetic model as indicated by the correlation coefficients (Tables 3.8 to 3.11). The relatively high correlation coefficients for pseudo-second order kinetic model were caused by a good fit of the experimental for the sorption of Cd(II) and

Cu(II), however, as the adsorption time was increased, the pseudo-second order model deviated from the data and the predicted q_e values were overestimated.

The changes in q_e, h and the pseudo-second order kinetic constant (k_2) were slightly higher when the temperature was increased from 25 °C to 30 °C for the sorption of Cd(II) than when the temperature was increased from 30 °C to 40 °C (Table 3.6). This suggests that 30 °C could be recommended for the biosorption of Cd(II) on GM 16 biomass. Marginal increases in the kinetic parameters (q_e, h and k_2) for the sorption of Cu(II) from 25 °C to 40 °C were observed (Table 3.7). Generally, the increases in the initial sorption rates (h) and equilibrium adsorption capacity (q_e) values of both Cd(II) and Cu(II) were not significant ($P \geq 0.05$) at all temperatures studied.

The increase in the initial Cd(II) and Cu(II) ion concentrations also affected the initial sorption rates (h), equilibrium constants as well as the q_e values. The pseudo-second order kinetic model showed linear relationships with high correlation coefficients ($r^2 > 0.990$). Generally, the initial sorption rates (h) and pseudo-second order kinetic constants (k_2) for the sorption of Cd(II) decreased when the initial metal ion concentration was increased from 100 to 120 mg L^{-1} (Table 3.8). The observations suggest that the sorption of Cd(II) fitted well to the pseudo-second order kinetic model at lower initial metal ion concentration (80 mg L^{-1}) where the high rate of sorption was observed ($P \geq 0.05$). The equilibrium Cd(II) and Cu(II) sorption capacities (q_e) of GM 16 biomass also increased with increase in initial metal ion concentrations (Table 3.8 and 3.9). The increase in initial metal concentrations was also found to increase the initial sorption rates (h) and pseudo-second order kinetic constants (k_2) when the initial metal ion concentrations were increased from 80 to 120 mg L^{-1}. In a recent review, Plazinskiet *et al*. (2009) have discussed the effect of the initial solute concentration and other factors such as pH of the metal solution, temperature and agitation on the pseudo-second order rate constant (k_2). Generally, these authors reported that the k_2 decreases with increasing initial solute concentration.

They further pointed out that in only a few systems, the k_2 is independent of the initial solute concentration or the k_2 increases.

Finally, the results showed that the increase in biomass concentrations also significantly increased the initial sorption rates (h) and the equilibrium metal sorption capacities (q_e) suggesting a better sorption at high biomass concentrations (Tables 3.10 and 3.11). The data fitted better to the pseudo-second order kinetic model with high correlation coefficients; however, the q_e values obtained were overestimated. Although the correlation coefficients obtained for pseudo-first order kinetic model were lower than the correlation coefficients for pseudo-second order model, the estimated q_e values were in agreement with the experimental data.

4.6. Thermodynamic parameters of Cd(II) and Cu(II) biosorption onto non-viable GM 16 biomass

Thermodynamic properties of the biosorption of Cd(II) and Cu(II) indicated spontaneous nature of adsorption as shown by negative *Gibbs* free energy changes (ΔG^o). Furthermore, ΔG^o values were more negative with increase in temperature, which suggest that the spontaneity of the biosorption process increased with increase in temperature. The small changes in ΔG^o values observed in the temperature range studied (25-40 °C) suggest that the effect of temperature on biosorption of both metals was minimal.

The enthalpy change for the sorption of Cd(II) was slightly positive (Table 3.12). Although the value was very small (2.81 kJ mol^{-1}), the positive value of ΔH^o indicated that the biosorption of Cd(II) was an endothermic adsorption process, which was reflected in the slight increase in Cd(II) sorption capacity (q_{max}) of the sorbent with rise in temperature (Table 3.12). Similar observations were reported in some studies where the spontaneous adsorption of metal ions occurred as shown by negative values of ΔG^o, whereas the values of ΔH^o were positive

(Dursun, 2006; Liu and Liu, 2008; Baysal *et al*., 2009). Liu and Liu (2008) suggested that the heat evolved during physical adsorption is of the same order of magnitude as the heat of condensation (i.e. 2.1-20.9 kJ mol^{-1}). Therefore it seems that Cd(II) biosorption by the non-viable GM 16 biomass could be attributed by a physical adsorption process.

The enthalpy change for the sorption of Cu(II) was slightly negative (-0.85 kJ mol^{-1}) which indicated a slight exothermic type of a sorption process (Table 3.13). As shown in Table 3.4, changes in the maximum adsorption capacities (26-26.4 mg g^{-1}) of GM 16 biomass with increase in temperature were very small during Cu(II) biosorption, suggesting that temperature effect on Cu(II) biosorption was less than that of Cd(II). The adsorption processes with high enthalpy changes are temperature-sensitive whereas those with low enthalpy changes are relatively temperature insensitive (Uslu and Tanyol, 2006). The enthalpy change for the sorption of Cd(II) (2.81 kJ mol^{-1}) by GM 16 biomass was higher than that of Cu(II) (-0.85 kJ mol^{-1}), hence, the slight differences in maximum adsorption capacities (q_{max}) of biomass when temperature was increased from 25 to 30 °C during the sorption of Cd(II). In addition, the magnitude of ΔH^o indicates whether the sorption of metal ions on the biomass is physical or chemical (Liu and Liu, 2008). The enthalpy for physical adsorption usually falls within the range of 2.1-20.9 kJ mol^{-1} and for chemical adsorption generally falls into the range of 80-200 kJ mol^{-1} (Khormaei *et al*., 2007; Liu and Liu, 2008; Ozdes *et al*., 2009). The magnitude of ΔH^o for Cd(II) or Cu(II) biosorption suggests that the sorption of both Cd(II) and Cu(II) on GM 16 biomass could involve physical adsorption. It can be pointed out that, although the sorption of Cd(II) and Cu(II) involved a physical process, the non-viable GM 16 biomass could be suitable for the biosorption of Cu(II) at low temperature (25 °C) whereas the biosorption of Cd(II) on the biomass could be done at a slightly higher temperature to facilitate the biosorption process.

The entropy change (ΔS^o) for the sorption of Cd(II) (14 kJ mol^{-1} K^{-1}) was significantly higher ($P \leq 0.05$) than that of Cu(II) (2.91 kJ mol^{-1} K^{-1}). The low

value of ΔS^o for Cu(II) may imply that there was no remarkable change in entropy during the biosorption of Cu(II), whereas the entropy change for Cd(II) biosorption by GM 16 biomass was high. Also, the positive values of ΔS^o suggest an increase in randomness at the solid/solution interface during the sorption of both Cd(II) and Cu(II). Similar results were found for the biosorption of Cu(II) and by *Aspergillus niger* (Dursun, 2006) and Cd(II) by algal biomass of *Oedogonium* sp. (Gupta and Rastogi, 2008).

4.7. Comparison of the biosorption of Cd(II) and Cu(II) by non-viable GM 16 biomass with the viable GM 16 cells

Most biosorption studies have been performed using non-viable biomasses (Dursun, 2006; Gupta and Rastogi, 2008; Baysal *et al*., 2009). In achieving one of the aims of the present study, the biosorption capacities of the non-viable GM 16 for the sorption of Cd(II) and Cu(II), reported by Sekhula *et al*. (2005), were compared with those found in this study (Table 4.1 and 4.2). The percentage of Cd(II) removed by non-viable GM 16 biomass (65%) was significantly higher ($P \leq 0.05$) than that by the viable biomass (48%) when the initial metal ion concentration in the aqueous solution was 100 mg L^{-1} in each case.. However, the difference between the percentage removal of Cu(II) by non-viable (67%) and viable biomass (65%) was insignificant ($P \geq 0.05$). These observations suggest that the non-viable GM 16 biomass could be considered for the removal of Cd(II) and Cu(II) from contaminated aqueous solutions. Moreover, Dönmez and Asku (2001) suggested that although the use of living microbes may allow the development of a single process for removal of most pollutants from industrial effluents, there are significant practical limitations including sensitivity of the systems to extreme pH, high metal/salt concentration and the requirement of external metabolic energy.

Table 4.1. Comparison of % Cd(II) adsorbed by non-viable GM 16 biomass with the previously reported % Cd(II) adsorbed by viable GM 16 biomass

Biomass type	Cd(II)				
	C_i	pH	Temperature	% adsorbed*	Reference
Viable	100 mg L^{-1}	7	37 °C	48	Sekhula *et al*., 2005
Non-viable	100 mg L^{-1}	7	40 °C	65	Present study

*NB: % Cd(II) adsorbed for the different biomass types was statistically significant (*t*-test): $P \leq 0.05$

Table 4.2. Comparison of % Cu(II) adsorbed by non-viable GM 16 biomass with the previously reported % Cu(II) adsorbed by viable GM 16 biomass

Biomass type	Cu(II)				
	C_i	pH	Temperature	% adsorbed*	Reference
Viable	100 mg L^{-1}	7	37 °C	65	Sekhula *et al*., 2005
Non-viable	100 mg L^{-1}	6	40 °C	67	Present study

*NB: % Cu(II) adsorbed for the different biomass types was not statistically significant (*t*-test): $P \geq 0.05$

To date, biosorption has been regarded as an effective technology for the removal of soluble heavy metals from aqueous solutions. Liu and Liu (2008) suggested that the use of biosorbents for the removal of dissolved heavy metals from wastewater should be able to provide high metal uptake capacities and rate of sorption, robustness to harsh operating conditions and reliability without the release of harmful materials from the biosorbent. The effects of the different environmental conditions such as the initial pH of the metal solution, increase in biomass concentration and the initial metal ion concentration increased the metal adsorbed by the GM 16 biomass. These findings suggest that thenon-viable GM 16 biomass had effectively removed Cd(II) and Cu(II) from aqueous solutions. These findings could be considered for the use of non-viable GM 16 biomass in biosorption technology to bioremediate Cd(II)- and Cu(II)-contaminated industrial effluents before discharge into natural water sources.

Different *Bacillus* species have been shown to have different metal biosorption capacities. The biosorption capacity (20 mg g^{-1}) of the non-viable GM 16 isolate for the sorption of Cd(II) was found to be lower than the biosorption capacities reported for *Bacillus circulans* (26.5 mg g^{-1}), *Bacillus subtilis* (101 mg g^{-1}) and *Bacillus jeotgali* (53.7 mg g^{-1}) (Green-Ruiz *et al*., 2008; Vijayaraghavan and Yun

2008[b]). However, the biosorption capacity (26.4 mg g^{-1}) of the isolate GM 16 was found to be higher than the biosorption capacities reported for *Bacillus* sp. (ATS-1) (16.3 mg g^{-1}) and *Bacillus subtilis* IAM 1026 (20.8 mg g^{-1}) for the sorption of Cu(II). In addition, high biosorption capacity for *Bacillus* sp. F19 (244 mg g^{-1}) was reported for the sorption of Cu(II) (Nakajima *et al*., 2001; Tunali *et al*., 2006; Zheng *et al*., 2008).

Chapter 5

5.1. Conclusions

In conclusion, the study presents two different groups of bacteria, the *Bacillus* as well as members of *Serratia* group which have been isolated from samples (Section 2.2 above) collected from an antimony mine. The external environmental factors investigated in this study affected the biosorption capacity of GM 16 biomass for the sorption of Cd(II) and Cu(II). From the results, some of the environmental factors affecting the biosorption of Cd(II) were different from those for the biosorption of Cu(II).

The following conclusions were drawn from the present study:

- Isolate GM 16 was identified as a *Bacillus* sp.
- The initial biosorption of Cd(II) and Cu(II) onto the non-viable GM 16 biomass was rapid and equilibrium was reached within 1 h of biomass contact with the aqueous metal solutions.
- The results strongly indicated that the biosorption of Cd(II) and Cu(II) on GM 16 biomass was a function of initial pH of the metal solution. The maximum adsorption of Cd(II) and Cu(II) occurred at pH 7 and 6, respectively.
- The presence of each metal did not affect the adsorption the other at their respective pH optimum for adsorption under the conditions tested.
- The non-viable GM 16 biomass could be used for the removal of Cd(II) and Cu(II) from heavy metal polluted waste-water in the presence of other metal cations (Mg^{2+}, Ca^{2+}, K^{+} and Na^{+}) under the same conditions used in this study. The effects of divalent metal ions (Mg^{2+} and Ca^{2+}) on the biosorption of Cd(II) and Cu(II) were higher than that of monovalent cations (K^{+} and Na^{+}).

- The speed of agitation of the biosorption experiments influenced the biosorption of both Cd(II) and Cu(II). The optimum speed of agitation for the biosorption of Cd(II) or Cu(II) under the same conditions (optimum pH, 4 g L^{-1} biomass concentration, 100 mg L^{-1} initial metal ion concentration, and 40 °C) by GM 16 biomass was 100 rpm.
- Increase in biomass concentration increased the Cd(II) or Cu(II) sorption on GM 16 biomass although the saturation level was not reached.
- Increase in initial metal ion concentrations (40-120 mg L^{-1}) increased the equilibrium metal sorption capacity (q_e) of biomass for both Cd(II) and Cu(II).
- The data presented in this study suggested favorable adsorption of both Cd(II) and Cu(II) onto the non-viable GM 16 biomass. The data for the biosorption of Cd(II) could be described better with the Langmuir adsorption model, whereas the biosorption of Cu(II) could be described using both Langmuir and Freundlich adsorption models. The data for the sorption of Cu(II) fitted to both Langmuir and Freundlich adsorption models. Increase in temperature caused marginal increases in maximum metal sorption capacities (q_{max}).
- The kinetics of biosorption followed pseudo-second order model for the biosorption of both Cd(II) and Cu(II) by GM 16 biomass, indicating that kinetics of Cd(II) and Cu(II) biosorption could be described better using pseudo-second order than the pseudo-first order kinetic models. The equilibrium sorption capacities obtained for the pseudo-second order model were overestimated when compared with equilibrium sorption capacities of the experimental data. Increase in biomass and initial metal ion concentrations increased remarkably the initial sorption rates of the metal ions on the biomass but temperature effect was negligible.
- The negative values of ΔG^o confirmed the spontaneous nature of Cd(II) or Cu(II) adsorption, with increased randomness at the solid-solution interface as indicated by positive values of ΔS^o.

- Although the ΔH^o value was very small (2.81 kJ mol^{-1}), the positive value indicated that the biosorption of Cd(II) was a slightly endothermic type of adsorption process. The enthalpy change for the sorption of Cu(II) was slightly negative which indicated a slight exothermic type of sorption process.
- The magnitude of ΔH^o for Cd(II) and Cu(II) biosorption suggested that the sorption of both Cd(II) and Cu(II) on GM 16 biomass could involve a physical process.

5.2. **Recommendations for future studies**

- The biosorption of Cd(II) and Cu(II) was investigated in pure Cd(II) or Cu(II) polluted water of known concentrations. It would also be necessary to investigate the biosorption of these metals in wastewater or effluent from point sources (heavy-metal polluted rivers, industrial effluent or wastewater treatment plant samples) in order to asses the feasibility of Cd(II) and Cu(II) adsorption by GM 16 biomass in a actual situation.
- It would also be necessary to study the biosorption of Cd(II) and Cu(II) using a continuous biosorption process at a small scale for determining the effects of the different environmental factors affecting the biosorption process since most industrial applications prefer a continuous mode of operation.
- The kinetics of biosorption followed pseudo-second order model for the biosorption of both Cd(II) and Cu(II) by GM 16 biomass, indicating that the kinetics of Cd(II) and Cu(II) biosorption could be described better using pseudo-second order kinetic model. However, the biosorption of both Cd(II) and Cu(II) should be carried out within 15 min in order to avoid the overestimation of equilibrium sorption capacities (q_e) and to also capture biosorption reaction. The equilibrium sorption capacities obtained for the pseudo-second order model were overestimated when compared to equilibrium sorption capacities of the experimental data.

- The industrial effluents or wastewater normally contain other heavy metals (cations and anions) at varying concentrations. Therefore, it would be necessary to investigate the effect of other metal cations and anions at different concentrations on the biosorption of Cd(II) and Cu(II). The fate of counter ions need also be investigated.
- The present study of the biosorption of Cd(II) and Cu(II) by the non-viable GM 16 biomass focused on the residual concentration of metals in supernatant solutions in order to calculate the metal sorption capacity (*q*). It is also necessary to determine the amount of metal biosorbed on the biomass.
- The effects of the environmental factors on the biosorption of Cd(II) and Cu(II) was studied using a 100 mg L^{-1} metal solution, which has been used in most biosorption experiments. Although the removal of Cd(II) and Cu(II) by GM 16 biomass was not complete (100 %), the reduction in the concentration of these metals present in contaminated water was found to be above 50 %. Therefore, the purpose of biosorption in this case would not be for the potable use of water; rather, it would be for the treatment of highly polluted industrial effluents before discharge in natural waters. For the application of non-viable GM 16 biomass in the biosorption of Cd(II) and Cu(II) from wastewater which would finally be intended for consumption, would have to be investigated in polluted water with heavy metals at low concentrations.
- From literature, it has been found that the pre-treatment of biosorbents with alkalines or acids may increase the biosorption performance. Thus, modifications of the biosorbent to improve the physical property of the biomass by means of chemical, physical or combination of pre-treatments can be studied.
- The increase in biomass concentration increased the sorption of Cd(II) and Cu(II) by the non-viable GM 16 cell, however, the concentration of biomass should be increased to reach the saturation level .

- The feasibility of biosorption cycles can be tested by following biosorption-desorption processes, by washing heavy metal loaded biomass with a desorbing agent for biomass regeneration and be recycled in biosorption.
- Non-viable GM 16 biomass could also be investigated for the biosorption of other metal ions (Zn, Co, Ni, Cr, etc.) which are commonly found in polluted water.
- Identification of the organisms to the species names using different protocols for identification (BIOLOG, API systems or chemical testing) could also be done.
- Although the free or suspended cells have been used in most biosorption studies, immobilized biomasses have also proven to be effective in the removal of heavy metals from polluted water. It also reduces time and effort to separate suspended biomass from treated water. Therefore, the sorption of Cd(II) and Cu(II) could also be investigated using immobilized GM 16 biomass. Immobilization of biomass will allow the use of a fixed packed-bed reactor which will allow a more feasible pilot-and industrial scale application.
- Therefore, cellular characterization would be necessary:
    - Cell motility for adhesion onto matrix for immobilized biosorbents.
    - Morphology and cell size for surface area of metal sorption.
    - FTIR spectra analysis of functional groups for metal sorption.

Chapter 6

6. References

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Appendix

Reagents preparation

MH broth: Dissolve 23 g of MH broth in a liter of deionized water and sterilize by autoclaving at 121 °C for 30 minutes.

TYG agar (plus sodium arsenite): In a liter of deionized water, dissolve 5.0 g of tryptone, 3.0 g of yeast extract, 1.0 g glucose and 16 g of agar powder. Adjust the medium to pH 6.5 with 1 M hydrochloric acid and 1 M sodium hydroxide and sterilize by autoclaving at 121 °C for 30 minutes. Cool the medium to 45 °C and add 1 M of $NaAsO_2$ to a final concentration of 100 µM.

TYG broth: Dissolve 5.0 g of tryptone, 3.0 g of yeast extract and 1.0 g of glucose in a liter of deionized water. Adjust the medium to pH 6.5 with 1 M hydrochloric acid and 1 M sodium hydroxide and sterilize by autoclaving at 121 °C for 30 minutes.

Preparation of stock solutions

Agarose gel for electrophoresis: Dissolve 0.4 g agarose powder in 50 mL TAE buffer, cool and add 3 µL of 10 mg mL^{-1} ethidium bromide and allow it to set.

AIX agar plates: Prepare LB broth, autoclave at 121 °C and cool to 65 °C and add ampicilin (to final concentration of 50 µg mL^{-1}), X-gal (final concentration of 20 µg mL^{-1}) and IPTG (final concentration of 0.2 mM), mix by swirling and pour into Petri dishes.

Ampicillin: Prepare 100 mg mL^{-1} by dissolving 2 g of ampicilin in 20 mL of distilled water.

$CaCl_2$ (Mr = x g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

$CdCl_2.H_2O$ (Mr = 228.34 g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

$CuCl_2.2H_2O$ (Mr = 170.5 g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

DNA isolation buffer: Mix 5 mL 1M Tris-HCL (pH 8.0), 5 mL 0.5 M EDTA (pH 8.0) and 5 mL 10 % SDS and fill to a final volume of 50 mL using distilled water.

EDTA (372.24 g mol^{-1}): Dissolve 3.72 g in a 15 mL of distilled water, adjust to pH 8 with NaOH and add to a final volume of 20 mL.

IPTG (238.31 g mol^{-1}): Dissolve IPTG in water to a final concentration of 200 mg mL^{-1}. Sterilize solution using a 0.2 µm filter, and store at -20 °C

KCl (Mr = 74.55 g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

LB-Broth: Dissolve 10 g tryptone, 5 g yeast extract, 5 g NaCl and 15 g agar into a liter of deionized water, autoclave at 121 °C and cool to 65°C.

$MgCl_2$ (Mr = 95.211 g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

$NaAsO_2$ (Mr = 129.9 g mol^{-1}) : Dissolve 6.495 g in 50 mL of deionized water to form 1 M stock solution and filter sterilized using 0.2 µm pore-size filter paper (Millipore).

NaCl (Mr = 58.44 g mol^{-1}): Dissolve 1 g in a 1000 mL of deionized water to form 1000 mg L^{-1} sock solution.

TAE buffer: It contains 0.04 M Tris, 0.001 M EDTA (pH 8) and 0.021 mM glacial acetic acid.

Tris (121.14 g mol^{-1}): Dissolve 0.484 g in 90 mL of distilled water, adjust to pH 8 with hydrochloric acid and fill the volume to 100 mL.

X-gal (408.6 g mol^{-1}): Dissolve X-gal in DMSO to a final concentration of 40 µg mL^{-1}. Sterilize solution using 0.2 µm filter and store at 20 °C.

Table A

The concentrations of the metal ions expressed in mg L^{-1} and in mM concentrations.

Metal ion	Molecular weight (g mol^{-1})	50 mg L^{-1}	100 mg L^{-1}
		Concentration in mM	
Cu^{2+}	170.5	0.293	0.586
Cd^{2+}	228.34	0.218	0.438
Co^{2+}	237.93	0.210	0.420
Zn^{2+}	136.3	0.366	0.734
Ni^{2+}	237.7	0.210	0.421

Table B

The concentrations of the metal ions expressed in mg L^{-1} and in mM concentrations.

Metal ion	Molecular weight (g mol^{-1})	50 mg L^{-1}	100 mg L^{-1}
		Concentration in mM	
Cu^{2+}	170.5	0.293	0.586
Cd^{2+}	228.34	0.218	0.438

Table C

Metal ion concentrations (mg L^{-1}) used in the effect of other metal cations on Cd(II) and Cu(II) biosorption experiments (performed using 0.5 mM concentrations of each metal ions).

Metal ion	Molecular weight g mol^{-1}	[metal] mg L^{-1}
Ca^{2+}	147.02	73.51
Cd^{2+}	228.34	114.17
Cu^{2+}	170.5	85.25
K^{+}	74.56	37.28
Mg^{2+}	203.3	101.65
Na^{+}	58.44	29.22

Table D

NanoDrop spectrophotometric measurements (mean of three readings) of approximately 50 ng μL^{-1} gDNA of the GM bacterial isolates.

Sample ID	[DNA] ng μL^{-1}	A_{260}	A_{280}	A_{260}/A_{280}
GM 10(1)	46.9	0.940	0.454	2.08
GM 10(2)	51.9	1.038	0.487	2.13
GM 14	44.2	0.884	0.449	1.96
GM 15	43.2	0.864	0.442	1.95
GM 16	47.2	0.945	0.464	2.04
GM 17	47.1	0.943	0.490	1.92

Table F

NanoDrop spectrophotometry readings (mean values of at least 3 readings) of 16S rDNA PCR product recovered from Zymoclean™ Recovery kit.

GM isolate	Excised gel (g)	ADB buffer (µL)	DNA recovered (ng μL^{-1})
10(1)	0.41	1230	15.19
10(2)	0.31	930	12.67
14	0.32	960	23.82
15	0.30	900	22.19
16	0.31	930	19.04
17	0.34	1020	17.11