Adsorption of Nickel and Cadmium by *Pedomicrobium manganicum* ACM 3067

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Thesis Title	Adsorption of Nickel and Cadmium by *Pedomicrobium manganicum* ACM 3067
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Major Program	Biotechnology

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ชื่อวิทยานิพนธ์ การดูดซับนิกเกิลและแคดเมียมโดยเชื้อ *Pedomicrobium manganicum* ACM 3067

ผู้เขียน นางสาว รัชยาภรณ์ ผลมั่ง

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บทคัดย่อ

จากการเลี้ยงเชื้อ *Pedomicrobium manganicum* ACM 3067 ในอาหารเลี้ยงเชื้อ PSM บนเครื่องเขย่าที่ความเร็ว 150 รอบต่อนาที ณ อุณหภูมิห้อง (30±2 องศาเซลเซียส) เป็นเวลา 8 วัน พบว่าเชื้อเจริญได้สูงสุดหลังจากเลี้ยงเชื้อเป็นเวลา 4 วัน จากการศึกษาการเจริญและความสามารถในการออกซิไดส์แมงกานีสของเชื้อ *P. manganicum* ACM 3067 ในอาหารเลี้ยงเชื้อ PSM และ PC ที่เติมแมงกานีส 0, 2, 6, 12, 20 และ 50 พีพีเอ็ม พบว่าเชื้อเจริญสูงสุดและสามารถออกซิไดส์แมงกานีสได้อย่างสมบูรณ์ในอาหารเลี้ยงเชื้อทั้งสองชนิดที่เติมแมงกานีส 2 พีพีเอ็ม และจากการศึกษาค่าความเข้มข้นต่ำสุดของนิกเกิลและแคดเมียมที่ยับยั้งการเจริญของเชื้อ พบว่า *P. manganicum* ACM 3067 สามารถทนต่อพิษของแคดเมียมได้สูงกว่านิกเกิลและนิกเกิลรวมกับแคดเมียมตามลำดับ นอกจากนี้การเติมแมงกานีสในอาหารเลี้ยงเชื้อส่งผลให้เชื้อต้านทานต่อพิษของนิกเกิลและแคดเมียมได้ดียิ่งขึ้น จากการทดลองตรึงเซลล์บนถ่านกัมมันต์ชนิดเกล็ดในฟลาสก์ พบว่าเชื้อจะถูกตรึงได้สูงสุดเมื่อใช้อาหารเลี้ยงเชื้อ PC ความเข้มข้นครึ่งเท่าที่เติมแมงกานีส 1 พีพีเอ็ม และใช้ถ่านกัมมันต์ชนิดเกล็ดขนาด 400-1,000 ไมโครเมตร ในการตรึง เซลล์ถูกดูดซับสูงสุดหลังจากทำการตรึง 2 นาที โดยประสิทธิภาพการดูดซับเซลล์บนถ่านกัมมันต์ชนิดเกล็ดมีค่าสูงสุดประมาณ 32 เปอร์เซ็นต์ ประสิทธิภาพการชะของเซลล์ออกจากถ่านกัมมันต์ชนิดเกล็ดในอาหารเลี้ยงเชื้อ PC ความเข้มข้นครึ่งเท่าที่เติมแมงกานีส 1 พีพีเอ็ม มีค่าสูงสุดเท่ากับ 33-34 เปอร์เซ็นต์ หลังการชะ 1-2 นาที จากการศึกษาการดูดซับนิกเกิลและแคดเมียมจาก

อาหารเหลวโดย *P. manganicum* ACM 3067 ที่ถูกตรึงบนแมกนีไทต์และถ่านกัมมันต์ชนิดเกล็ดในระบบ continuous recycle fluidized bioreactor (CRFB) พบว่าเชื้อถูกตรึงบนแมกนีไทต์และถ่านกัมมันต์ชนิดเกล็ดได้ 2.55×10^{9} และ 9.54×10^{9} CFU ตามลำดับ คิดเป็นประสิทธิภาพในการตรึงเชื้อเท่ากับ 60.7 และ 50.2 เปอร์เซ็นต์ ตามลำดับ ในอาหารเลี้ยงเชื้อที่เติมโลหะหนักแต่ละชนิด $\leq$ 8 พีพีเอ็ม เชื้อที่ถูกตรึงบนแมกนีไทต์สามารถดูดซับนิกเกิลและแคดเมียมได้ $\geq$ 95% และ $\geq$ 96% ตามลำดับ ในขณะที่เชื้อที่ถูกตรึงบนถ่านกัมมันต์ชนิดเกล็ดสามารถดูดซับนิกเกิลและแคดเมียมได้ $\geq$ 91% และ $\geq$ 94% ตามลำดับ ในอาหารเลี้ยงเชื้อที่เติมโลหะหนักทั้งสองชนิด (แต่ละชนิด $\leq$ 4 พีพีเอ็ม) เชื้อที่ถูกตรึงบนแมกนีไทต์สามารถดูดซับนิกเกิลและแคดเมียมได้ $\geq$ 72% และ $\geq$ 74% ตามลำดับ ส่วนเชื้อที่ถูกตรึงบนถ่านกัมมันต์ชนิดเกล็ดสามารถดูดซับนิกเกิลและแคดเมียมได้ $\geq$ 91% และ $\geq$ 94% ตามลำดับ

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Abstract

Cultivation of *Pedomicrobium manganicum* ACM 3067 in PSM medium was carried out for 8 days on a rotary shaker at 150 rpm at room temperature (30±2°C). The growth was maximum after 4 days cultivation. The growth and manganese oxidizing ability of *P. manganicum* ACM 3067 were studied in PSM and PC media with the addition of 0, 2, 6, 12, 20 and 50 ppm of manganese. The highest growth and the complete manganese oxidation were achieved with the addition of 2 ppm manganese in both media. The minimum inhibitory concentration (MIC) values showed that *P. manganicum* ACM 3067 could tolerate cadmium at higher concentrations than nickel and the combination of nickel and cadmium, respectively. The presence of manganese provided a protective effect against the toxicity of nickel and cadmium. Studies on the immobilization of *Pedomicrobium* cells on granular activated carbon (GAC) particles in flask revealed that the highest immobilized cell was obtained when a half strength PC medium with 1 ppm manganese and 400-1,000 μm GAC were used. The cells were rapidly adsorbed and the maximum cell adsorption was obtained after 2 min of the immobilization with the adsorption efficiency of approximately 32%. The maximum efficiency of cell desorption from GAC to a half strength PC medium with 1 ppm manganese was 33-34% after 1-2 min of the desorption. The adsorption of nickel and cadmium adsorption from an aqueous medium was performed using *P. manganicum* ACM 3067 immobilized on magnetite and GAC in the continuous recycle

fluidized bioreactor (CRFB). The number of immobilized *Pedomicrobium* cells in the CRFB using magnetite and GAC as the supporting materials were 2.55×10^9 and 9.54×10^9 CFU, respectively with the adsorption efficiency of 60.7% and 50.2%, respectively. The *Pedomicrobium* cells immobilized on magnetite particles in the CRFB adsorbed nickel and cadmium ≥ 95% and ≥ 96%, respectively when the influent medium contained ≤ 8 ppm each of nickel and cadmium while the cells immobilized on GAC particles adsorbed nickel and cadmium ≥ 91% and ≥ 94%, respectively. In the presence of both metals (≤ 4 ppm each), the cells immobilized on magnetite particles in the CRFB adsorbed nickel and cadmium ≥ 72% and ≥ 74%, respectively while the cells immobilized on GAC particles adsorbed nickel and cadmium ≥ 91% and ≥ 94%, respectively.

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List of Abbreviations

°C	=	degree Celsius
CFU	=	colony forming unit
CFU/ml	=	colony forming unit per millilitre
CRFB	=	continuous recycle fluidized bioreactor
g	=	gram
×g	=	gravitational force
GAC	=	granular activated carbon
h	=	hour
L	=	litre
L/day	=	litre per day
L/min	=	litre per minute
m^2/g	=	square metre per gram
mg/l	=	milligram per litre
MIC	=	minimum inhibitory concentration
min	=	minute
ml	=	millilitre
ml/min	=	millilitre per minute
mm	=	millimetre
nm	=	nanometre
OD	=	optical density
ppm	=	part per million
rpm	=	revolutions per minute
µm	=	micrometre
%	=	percent
µg/l	=	microgram per litre

Chapter 1

Introduction

The problems associated with heavy metals in wastewaters entering natural waters have been well documented (Florence and Morrison, 1992). A fundamental factor which heightens the concern over the presence of heavy metals in the environment is their nonbiodegradability and consequent persistence. The problems arise from the toxic effect of heavy metals on many aquatic organisms. This is made worse by the bioaccumulation for metals in food chains (McLean, 1994).

The conventional treatment methods for removal of heavy metal include: chemical precipitation, chemical oxidation and reduction, ion-exchange and filtration. Such processes may be ineffective or extremely expensive when the initial heavy metal concentrations are not very high (Marques *et al.*, 1991). The uptake and accumulation of heavy metals by microorganisms and their metabolites are recieving increasing attention in a biotechnological context since microbe-based technologies may provide an alternative or adjunct to conventional techniques of metal removal/recovery from water. Intact microbial cells, living or dead, and derived microbial products can be highly efficient bioaccumulators of both soluble and particulate forms of metals, especially from dilute external concentrations (Marques *et al.*, 1991). For using living biomass, metabolic uptake mechanisms may contribute to the process. As a result of metal toxicity, living cell systems may be inactivated; therefore most living cell systems exploited to date have been used to decontaminate effluents containing metals at subtoxic concentrations (Gadd and White, 1993) such as in the final treatment of heavy metal contaminated effluents, enabling their discharge (Costa and Leite, 1991).

Pedomicrobium manganicum is a manganese-oxidizing bacterium and produces an extracellular polysaccharide (ECP). The oxidized manganese compounds were found to be deposited on the cell (Gebers, 1981), and/or on the ECP (Ghiorse and Hirsch, 1979). This observation suggested a mechanism involving the adsorption of Mn^{2+} to the extracellular polysaccharides followed by its oxidation to manganese oxide. In addition, *P. manganicum* cells were capable of binding and depositing preformed colloidal manganese oxide in the absence of Mn^{2+} (Sly *et al.*, 1990). The heavy metal adsorption by *Pedomicrobium manganicum* has been reported by several researchers (Sly *et al.*, 1993; Sripoaraya, 1993; McLean, 1994). The metal adsorption mechanism of *P. manganicum* was proposed to involve both ECP and hydrous MnO_2 bound to the ECP (Sripoaraya, 1993; McLean, 1994).

This study aims to investigate the ability of *P. manganicum* ACM 3067 on the adsorption of soluble Ni^{2+} and Cd^{2+}, two important environmental pollutants. The efficacy of using a continuous recycle fluidized bioreactor (CRFB) containing the *P. manganicum* cells immobilized on either magnetite or granular activated carbon (GAC) particles to remove Ni^{2+} and Cd^{2+} from aqueous medium was also investigated.

Literature Review

1. Heavy metal in the environment

The species and concentrations of heavy metals in aquatic systems are dependent on the sources of metals that enter the water environment and there can be divided into two main categories: those that originate from human activities and those of natural origin. Effluents from domestic, industrial, mining and agricultural sources are probably the major recognized human activities, while metals from natural origin may come from the atmosphere and storm water runoff (Lester, 1987).

A major fraction of metals entering the aquatic system is found associated with the bottom sediments (Marshall, 1991). At the beginning of a food chain, relatively dilute and apparently safe levels of heavy metals may be taken up and concentrated by algae and seagrasses which can pump heavy metals from sediments through their leaves and into surrounding water. If several of these plants are consumed by a shellfish or small fish the metals will become more concentrated. This metal concentration is again increased if a larger fish eats several of these smaller fish, and so this biomagnification process can continue with every link along the food chain (McLean, 1994). Among the aquatic organisms, only the tolerant species are able to accumulate metals from food, the non-tolerant tending to die. The diet of predators may shift towards tolerant species which may either exclude the metals or accumulate and store them. The latter strategy may serve to enhance the transfer of metals through the food chain (Kelly, 1988). If humans enter this food chain it is generally late in the chain when the concentration of metals is likely to be higher and more dangerous (McLean, 1994). The tragic instance of mercury pollution in Minamata Bay, Japan, is perhaps the best known example (Kelly, 1988).

Any metals introduced into the aquatic environment not only have direct effects on the aquatic organisms and their food chain but also may cause severe economic losses. For example, contamination by excessive levels of manganese and iron in water supplies may give water with an off taste or color, cause staining of laundry, fixture equipment and swimming pools, discoloration of products and reduction of pipeline carrying capacities (Dixon *et al.*, 1989).

2. Physicochemical characteristics and speciation of metals

Because of the possible toxic effects of the heavy metals if present in excessively high concentrations in water, well defined legislation controlling the concentration of metals in industrial effluents released to aquatic systems

have been laid down by many countries. However, it has been proposed that this legislation should include statements referring to the speciation of the metals released. This is a relevant proposal since the bioavailability and toxicity of metals to aquatic organisms are influenced by the speciation of metals (Farrell *et al.*, 1993 cited by McLean, 1994). In addition, there is much circumstantial evidence that speciation in sediments has an important influence on metal uptake by organisms (Kelly, 1988). Speciation of metal ions may be achieved to some extent by separating them according to electrochemical, chromatographic, ultrafiltration or dialysis methods. The forms of metals occurring in water include: free metal ions, inorganic complexes (chelates), metals bound to high molecular weight organic material, highly dispersed colloids and precipitates on organic particles (McLean, 1994). Of these different forms of metal, the most toxic form is free (hydrated) metal ion followed by lipid-soluble metal complex. Aquatic animals such as fish can cope with quite high levels of certain heavy metals in the food chain and in sediments. They cannot, however, tolerate free metal ions or toxic lipid-soluble complexes in the water pumped through their gills. Unpolluted aquatic sources contain very low concentrations of these toxic metal forms, most of the dissolved metal being adsorbed on colloidal particles or combined in non-toxic complexes (Florence and Morrison, 1992).

Besides influencing the metal toxicity, metals speciation in wastewater treatment processes is also essential to control metal chemistry for treatment and recovery. Speciation encompasses both soluble metal species and solid salts formed in the precipitation process (Patterson, 1991). The rate and extent of the speciation reactions, such as adsorption, ion-exchange, precipitation, coprecipitation, oxidation-reduction and complexation, are controlled by environmental parameters, for example, pH and Eh, ionic strength, water hardness, chelating agents, concentration of inorganic and organic ligands and the presence of solid surfaces for adsorption (Lester, 1987; Kelly, 1988).

2.1 Nickel (Ni)

Wastewater containing Ni originates from the metal-processing industries, steel foundries, motor vehicle and aircraft industries, paint and chemical industries (Eckenfelder, 1989). Sulfide and laterite ore which contain Ni at 1-4% and 1-3%, respectively are natural sources of Ni as well (Kelly, 1988).

In natural aerobic waters at neutral pH, Ni is octahedrally coordinated as $(Ni(H_2O)_6)^{2+}$ and ionic Ni is likely to constitute 90% of the total metal. Ni also forms complexes with ligands such as $OH^+ > SO_4^{2} > Cl^- > NH_3$, while its solubility under anaerobic conditions is determined by the presence or absence of sulfide. Ni speciation in surface water is affected by the presence or absence of suspended solid, although estimates of the proportion of total Ni associated with particulates vary from 7 to 99%. In the major world rivers, 0.5% of Ni is in solution, 3.1% is adsorbed, 47% exists as a precipitated coating, 14.9% is associated with organic material, and 34.4% is present in crystalline forms. In wastewater, Ni is usually present as Ni(II) and forms stable soluble complexes with cyanide. Although it has a low affinity for biological wastewater materials than other metals such as copper, Ni has been shown to form complexes preferentially with soluble organic ligands in wastewater effluents and this may contribute to its generally high mobility in aqueous system (Lester, 1987).

The presence of Ni cyanide complexes interferes with both cyanide and Ni treatment. Ni forms insoluble Ni hydroxide upon the addition of lime, resulting in a minimum solubility of 0.12 mg/l at pH 10 and 11. Ni can also be precipitated as the carbonate or the sulfate associated with recovery systems. In practice, lime addition (pH 11.5) may be expected to yield residual Ni concentrations in the order of 0.15 mg/l after sedimentation and filtration. Recovery of Ni can be accomplished by ion exchange or evaporative recovery, providing the Ni concentrations in the wastewaters are at a sufficiently high level (Eckenfelder, 1989).

2.2 Cadmium (Cd)

Cadmium is present in wastewaters from metallurgical alloying, ceramics, electroplating, pigment works, textile printing, chemical industries and lead mine drainage (Eckenfelder, 1989). Cd occurs in an oxidation state of II and this ionic form normally predominates in unpolluted soft waters of relatively low pH value. It can form a range of soluble complexes such as with carbonate, sulfate, chloride and hydroxide ligands and the synthetic chelating agents. The least soluble Cd salt in aerated natural water is Cd carbonate which precipitates between pH 8.5 and 11. Humic complexes are significant in organically loaded waters such as sewage effluent (Lester, 1987).

Cd is removed from wastewaters by precipitation or ion exchange. In some cases, electrolytic and evaporative recovery processes can be employed providing the wastewater is in a concentrated form. Cd forms an insoluble and highly stable hydroxide at an alkaline pH. Cd in solution is pH dependent so that concentrations are approximately 1 mg/l at pH 8 and 0.05 mg/l at pH 10 to 11. Coprecipitation with iron hydroxide precipitation at pH 6.5 will reduce Cd to as low as 0.05 mg/l. Cd is not precipitated in the presence of complexing ions, such as cyanide. In this case, cyanide destruction is necessary prior to Cd precipitation. A hydrogen peroxide oxidation precipitation system has been developed that simultaneously oxidizes cyanides and forms the oxide of Cd, thereby yielding Cd where recovery of Cd is feasible (Eckenfelder, 1989).

3. Effect of heavy metal toxicity on human health

Based on the essentiality and/or toxicity of individual metals, heavy metals can be divided into 3 groups (Lester, 1987) :

(1) Essential: Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, V, Zn and B

(2) Possibly beneficial: As and Ba

(3) No apparent metabolic function/toxic metal: Bi, Cd, Hg, Pb and Ti

However, it is important to note that essential elements may become toxic at high concentration, and that elements notorious for their toxicity may exert beneficial effects at very low concentration levels (Hughes and Poole, 1989). The factors which are capable of potentiating the toxicity of heavy metals include the stage of life cycle, age, species and physiological state of the exposed organism, the physicochemical form or species of the metal to which the organisms is exposed, and the extent to which other elements or compounds interact environmentally and metabolically with the toxicant (Lester, 1987).

3.1 Nickel (Ni)

While Ni has been shown to be essential for normal growth in some animals, its role as an essential element for human has not yet been confirmed. Once absorbed, Ni is transported in the bloodstream bound to serum albumin; mean Ni serum values of unexposed human are of the order of 2 to 3 μg/l. During distribution, Ni becomes complexes with amino acids, peptides and proteins and accumulates in tissues of the lung, kidney, liver, endocrine gland and brain depending on the mode of absorption. It is rapidly excreted from the body in urine (0.7-5.2 μg/l) and also via sweat (up to 52 μg/l) and hair (up to 220 μg/kg) (Lester, 1987).

The most toxic form of Ni known is Ni carbonyl. When inhaled, Ni carbonyl can cause severe lung damage and produces symptoms of headaches, vertigo, nausea, vomiting and insomnia. Oral exposure, however, produces a low toxicological response due to the absorption via this route, and appears to be tolerated at low levels via a homeostatic mechanism. Concentrations of Ni in tissues of exposed workers have been shown to decrease over several weeks once they had left the contact area (Lester, 1987).

Ni is capable of causing mutagenic effects by binding to phosphates and heterocyclic bases of nucleic acids in the place of other elements such as magnesium. This results in destabilization of the double helical structure of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and results in errors in

replication. There is little evidence to suggest that Ni in drinking water may be carcinogenic. Maximum admissible concentrations of Ni in drinking water have been specified by the Council of the European Communities (0.05 mg/l), but not by the World Health Organization (WHO) or the Environmental Protection Agency (EPA) (Lester, 1987).

3.2 Cadmium (Cd)

The absorption of ingested Cd is generally low in humans, ranging from 4.7 to 7.0%, but may increase in cases where dietary calcium is low. Absorption is species dependent, with Cd sulfide and selenide being taken up to a lesser degree than chloride and oxide forms. In humans with average body burdens of 10-60 mg, Cd is excreted in the urine at about 0.5-2.0 µg/l. Anemia is frequently observed in chronic Cd toxicity which results from the metabolic antagonism, between Cd and Fe. Normal metabolism is also disrupted through competition and displacement of Zn in metalloenzymes by Cd. Inhibition of nucleic acid synthesis and protein synthesis can be induced by Cd. In teratogenic studies on animals, Zn and Se were found to be antagonistic towards Cd, whereas Hg and Pb were synergistic. Symptoms of exposure to low concentrations include vomiting, diarrhea and colitis, while continuous exposure causes hypertension, heart enlargement and death. Skeletal thinning and bone breakage were observed in victims of the widespread Cd poisoning incident in Japan. The guideline value for maximum permissible concentration of Cd in drinking water has been specified by the World Health Organization (WHO) as 0.005 mg/l (Lester, 1987).

4. *Pedomicrobium manganicum*

P. manganicum is a Gram-negative budding hyphal bacterium (Gebers, 1989). The youngest cell called the "swarmer" cell may be oval to spherical shaped and is usually motile by means of flagella (Fig. 1). The swarmer cell sheds its flagella after a given period of time, normally

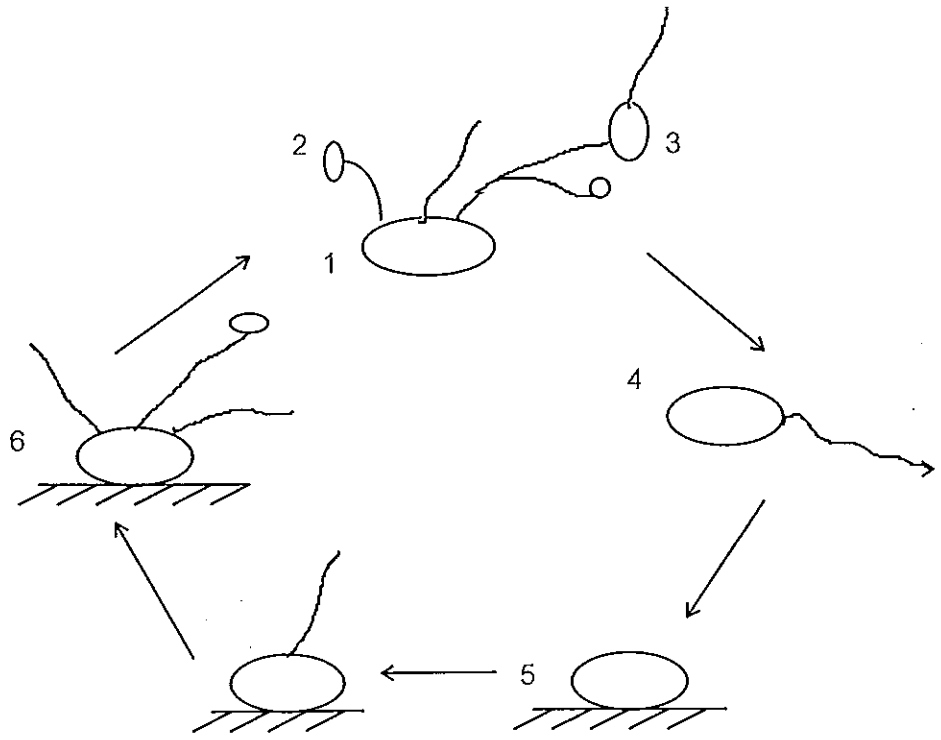


Fig. 1 Life cycle of *Pedomicrobium* sp.

1 mother cell with hyphae and buds
2 young bud
3 mature bud with flagellum
4 swarmer cell
5 young mother cell attached to solid surface
6 mature mother cell with hyphae and beginning bud formation

Source : Gebers (1989)

after attachment, and produces a hypha. A swelling at the tip of the hypha signals the beginning of bud formation. Before the bud is fully mature, flagella are produced to separate the daughter from the mother cell, however, daughter sometimes remain attached (Cox, 1995). Extracellular polymers which are proposed to be anionic polymers (Ghiorse and Hirsch, 1979), produced by the cell can be stained with ruthenium red and sometimes are visible in india ink mounts as thick capsules around mother cells (Gebers, 1989). *Pedomicrobium* has been commonly found in a wide range environments such as soil, freshwater and marine (Gebers and Beese, 1988). Some characteristics of *P. manganicum* are shown in Table 1.

The oxidation of manganese by *P. manganicum* is thought to occur biologically and to be associated with extracellular anionic polymers (acidic polysaccharides) produced by the cell (Cox, 1995). Deposition of manganese from aquatic habitats by *P. manganicum* occurs mainly at solid surfaces and involves the adhesion of the cells to the surfaces. Once firmly attached to the surface, the cells are able to take advantage of the nutrients and soluble manganous ions attracted to the solid-liquid interface. It is likely that the positively charged manganous (II) ions would first adsorb to polymers and then be oxidized by the oxidizing factors present at the cell surface or in the polymer matrix surrounding the cells. Once initiated, the accumulation of manganous (II) ions on both polymers and newly formed oxides would continue, however, their oxidation would depend on the presence of an as yet unidentified oxidation factor proposed to be enzyme which is inhibited by heat, azide and chloramphenicol (partially). The proposed enzyme displays similar characteristics to manganese peroxidases, since its activity was stimulated by hydrogen peroxide (Larsen, 1995). Furthermore, the manganese oxidizing activity displayed by *P. manganicum* is contained in the cell membrane and it was induced by the presence of manganese. However, there is some evidence to suggest that

Table 1 Characteristics of *Pedomicrobium manganicum* isolated from drinking-water distribution systems in South-East Queensland

Characteristic	Isolates
Morphology	
Swarmer cell	
-shape	spherical, oval
-size (μm)	0.4×0.6
-flagellation	polar
Mother cell	
-shape	spherical, oval or pear
-size (μm)	0.4-0.6×0.8-1.2
Number of hyphae	1-4
Position of hyphae	lateral and polar
Diameter of hyphae (μm)	0.1-0.25
Bud shape	oval or bean
Bud orientation	lateral, occ. polar
Growth and nutrition	
Growth on :	
PC agar	
-colony size (mm)	1
-colony color	brown-black
PC broth	
-sediment color	brown-black
PSM agar	
-colony color	cream
PSM broth	
-turbidity	uniform
-adhesion to glass	+
PYEA	NG
Utilization of methanol	-
Growth on nitrogen sources in PSM broth :	
Yeast extract (0.05%)	+
Peptone (0.05%)	+
Vitamin-free casamino acids (0.05%)	+
NO_2^- (10 mM)	-
NO_3^- (10 mM)	-
NH_4^+ (10 mM)	-
Temperature range (°C)	13-37
Optimum temperature (°C)	26-35
pH range	6-10
Optimum pH	9
Physiology	
Aerobic growth	+
Oxidase	+
Catalase	+ (w)
Deposition of Mn (IV)[b]	+
Deposition of Fe (III)[b]	-
Mol% (G+C) DNA	66.0±0.5

NG, no growth; (w), weak reaction; (+), deposition on extracellular polymers

[b] On PC and PSM agar containing 20 mg/l $MnSO_4$ or $FeSO_4$

Source : Sly *et al.*(1988)

manganese oxidation is an unstable character. The loss of the ability to oxidize manganese by some cultures when grown on nutrient medium and the simultaneous loss of plasmid DNA has indicated that manganese oxidation may be related to the presence of a plasmid (Gregory *et al.*, 1982 cited by Cox, 1995).

5. Factors affecting manganese oxidation by microorganisms

A number of bacteria have been found to oxidize Mn (Table 2). Some promote the oxidation directly (enzymatic or organic matrix-mediated), others indirectly (e.g. localized changes in pH or Eh) (Ehrlich, 1990). Oxidation of Mn by these bacteria was affected by some factors such as pH, nutrient level, Mn concentration and oxygen tension etc. These parameters influence the growth of Mn-oxidizers and their oxidizing abilities in different ways (Larsen, 1995).

5.1 Attachment to solid surface

Many Mn-oxidizers, but by no means all, exhibit enhanced oxidation when attached to the solid surfaces. Some marine bacteria oxidize Mn only when solid MnO_2 or other components of marine are added to the growth medium (Nealson and Ford, 1980). In addition, a variety of surfaces, including calcite crystals, sand grains and glass beads, stimulated Mn oxidation by a marine *Bacillus* spp. This stimulation occurred as a function of the total available surface area added, independent of the nature of the surface. In the same way, *Hyphomicrobium* spp. also exhibited this enhanced Mn oxidizing capacity upon attachment to pipeline surfaces (Marshall, 1980).

5.2 Oxygen tension

Manganese oxidation can occur under conditions ranging from aerobic to microaerophilic (Larsen, 1995). Studies on the effect of oxygen tension on the microbially catalyzed Mn oxidation rate in a marine fjord (Tebo and Emerson, 1985) showed that Mn oxidation rate increased with an increase in the oxygen tension. In contrast, growth of marine *Bacillus* spp. was favored by

Table 2 Some bacteria that oxidize manganese

A. Attack of soluble Mn^{2+} enzymatically
1. Derive useful energy
Marine strains SSW_{22}, S13, HCM-41 and E_{13} (all are Gram-negative rods)
Hyphomicrobium manganoxidans
Pseudomonas strain S-36
2. Do not derive useful energy
Arthrobacter siderocapsulatus
Leptothrix discophora
Leptothrix pseudoochracea
Metallogenium
3. Not known if able to derive useful energy
Arthrobacter B.
Arthrobacter citreus
Arthrobacter globiformis
Arthrobacter simplex
Citrobacter freundii E_4
Hyphomicrobium T37
Pedomicrobium
Pseudomonas E_1
Pseudomonas spp.
B. Attack of Mn^{2+} prebound to Mn (IV) oxide or some clays enzymatically
1. Derive useful energy
Arthrobacter 37
Oceanospirillum
Vibrio
Marine strain CEP-11
C. Attack Mn^{2+} nonenzymatically
Pseudomonas manganoxidans
Streptomyces sp.
Bacillus

Source: Ehrlich (1990)

increased oxygen, while Mn oxidation was favored by microaerophilic conditions (Nealson, 1978).

5.3 Growth phase

The ability of some microbes to oxidize Mn also depends on the growth phase (Larsen, 1995). Miyajima (1992) revealed that a dense accumulation of *Metallogenium*-like particles of MnO_2 appeared in the stationary phase of the heterotrophic Mn-oxidizing bacteria collected from lake Biwa in Japan. *Sphearotilus discophorus* also exhibited the ability to oxidize Mn when the cells reached the stationary phase (Haji and Makemson, 1976 cited by Miyajima, 1992). In addition, *Pseudomonas manganoxidan* exhibited the Mn oxidizing ability only at the end of its exponential growth phase (Schweisfurth *et al.*, 1978 cited by Larsen, 1995). In contrast, Boogerd and deVrind (1987) indicated that *Leptothrix discophora* was able to oxidize Mn in the early exponential phase. Similar findings with *L. discophora* were reported by van Veen (1972) and Adam and Ghiorse (1985). For a marine *Bacillus* spp., the binding and oxidation of Mn occurred during sporulation (Rosson and Nealson, 1982).

5.4 Temperature

Temperature is an important variable controlling the Mn oxide formation rate by microorganisms (Sunda and Huntsman, 1987). Tebo and Emerson (1985) found that the optimum temperature for Mn oxidation by bacteria from a marine fjord occurred at about 20°C. For *Leptothrix discophora* ss-1, the Mn^{2+} oxidizing activity exhibited temperature optima at approximately 28°C (Adam and Ghiorse, 1987). The optimum temperature for microbiological manganese oxidation in the River Tamar, England and North Carolina Estuary were 30°C which is close to the optimum for growth of estuarine mesophilic bacterial population (Sunda and Huntsman, 1987; Vojak *et al.*, 1985). Studies on microalgal-facilitated bacterial oxidation of Mn (Stuetz *et al.*, 1996) showed that the optimum temperature for both Mn^{2+} removal and oxide formation was

30°C. Lower oxidation temperatures resulted in lower oxide yields, whereas at higher temperatures oxide yield were unchanged. The temperature of incubation affected Mn binding by spores of marine *Bacillus* spp. in which the initial rate of binding at 45°C was 2-5 fold greater than that at 4°C. The total Mn bound at all temperatures up to 45°C was nearly equal, again suggesting a saturation-type system. At 60 and 80°C, only 50% of the 45°C amount was bound (Rosson and Nealson, 1982).

5.5 pH

Below pH 8 and above an Eh value of +200 mV, Mn is oxidized only by microorganisms under natural conditions (Schweisfurth *et al.*, 1978). The optimal pH for microbial oxidation is usually between pH 6 and 8 (Uren and Leeper, 1978). For *Arthrobacter* sp., oxidation was inhibited by only small changes of 0.1-0.2 of a pH unit outside the pH range of 5.8-7.8 (Bromfield, 1978). The rate of Mn^{2+} binding by spores of marine *Bacillus* spp. increased between pH 6.5 and 8.0. The maximum amount of Mn bound by spores was typically equal to 100% of the initial soluble Mn for pH 7.0 to 8.0, however, the rate of inorganic oxidation was significant above pH 8.0 (Rosson and Nealson, 1982). Oxidation of Mn by *Vibrio* sp. began at pH above 8.0 (Vojak *et al.*, 1988). Optimum pH for Mn oxidation by a soil *Streptomyces* sp. was about 5.0 and it was unable to oxidize Mn at pH 6.5-7.5 (Bromfield, 1979 cited by Larsen, 1995). Oxidation was also completely inhibited at pH 4.0 but not at pH 4.5.

5.6 Manganese concentration

High Mn concentrations are known to inhibit not only Mn oxidation but also the growth of many microorganisms (Green *et al.*, 1992). Manganese oxidation of *Pseudomonas manganoxidans* was inhibited by the concentrations of Mn over 5-10 mg/l (Schweisfurth *et al.*, 1978). Vojak *et al.* (1985) indicated that the maximum rate of Mn oxidation (1.22 µg/l/h) in bacteria occurred at 2 mg/l Mn. When greater amounts of Mn were added, the rate of oxidation was

increasingly depressed and only 0.24 μg of Mn/l/h was oxidized in the presence of 50 mg/l of Mn. Chapnick *et al.* (1982) reported an increase in the rate of Mn binding to insoluble matter up to a Mn concentration of 1.05 mg/l. Concentrations of Mn above 3.6 mg/l inhibited binding. It is possible that the inhibitory effect of high concentration of Mn is due to Mn toxicity effect on Mn oxidizing microorganisms (Vojak *et al.*, 1985).

Manganese removal and oxide formation by microalgal-facilitated bacteria were unrelated over a range of initial Mn concentrations (Stuetz *et al.*, 1996). The removal of Mn from solution increased linearly with increasing Mn, whereas the amount of oxide formed increased to a maximum at 3.3 g/l (60 mM Mn) and then remained constant. Addition of 5 μM Mn stimulated the Mn oxidation rate while addition of 18 μM totally inhibited Mn oxidation in bacteria isolated from a marine fjord (Tebo and Emerson, 1985). Bromfield and David (1976) reported that Mn ion concentrations between 0.5 mM and 6 mM had little effect on the rate of oxidation but higher concentrations became progressively inhibitory and 40 mM was completely inhibitory. No marked effect of Mn concentration on the growth rate of an estuarine *Vibrio* sp. could be detected but higher amounts of Mn correlated with slightly higher cell yields and optimal oxidation (2.9 mg MnO_2/l) occurred at 100 mg Mn/l (Vojak *et al.*, 1988).

5.7 Nutrient level and medium composition

Many studies revealed that as the available nutrient concentration increases, Mn oxidation is inhibited while growth is favored. The mechanism by which this occurs is unclear but it has been observed in soil bacteria and marine bacteria (Bromfield, 1956; Nealson, 1978 cited by Larsen, 1995). Changes in medium composition had a marked effect on algal-bacterial oxidation. The addition of nutrients; yeast extract, peptone and/or sucrose suppressed oxide formation and with increased concentrations, also promoted the precipitation of Mn carbonates ($MnCO_3$) (Stuetz *et al.*, 1996). For MnO_2

production from *Chlamydomonas* sp., urea was optimal at 10 g/l (167 mM) (Green and Madgwick, 1991). Excess urea (>10 g/l) probably leads to metabolic inhibition from ammonia toxicity and protein denaturation. Glucose and other metabolites e.g. acetate, succinate and glycerol strongly inhibited Mn oxidation but not growth in 30 out of 40 strains tested by Nealson (1978). Yeast extract at concentration above 0.1% inhibited Mn oxidation while growth of *Pedomicrobium manganicum* ACM 3067 was inhibited at concentration above 0.5% (Larsen, 1995). Acetate (0.1-1.0 g/l) improved Mn uptake in a continuous recycle fluidized bioreactor but did not increase MnO_2 production, directly. Phosphate above 1-2 mM inhibited Mn oxide production and the CRFB proved resilient to low levels of phosphate (7.4-63.2 μM) likely to be present in wastewater. Miyajima (1992) reported that *Bacillus* A2 and *Leptothrix* B4 showed the highest Mn oxidation efficiency at the bicarbonate concentration of 500 μM while *Bacillus* GPA4 and GNB6 did at 50 μM. However, the addition of 500 μM of phosphate inhibited entirely the Mn oxidation activity of *Bacillus* A2, GPA4 and GNB6 but stimulated the Mn oxidation by *Leptothrix* B4. Stadtman *et al.* (1990 cited by Miyajima, 1992) interpreted this phenomenon by the fact that the complexation of Mn by bicarbonate anion decreases the redox potential of Mn^{3+}/Mn^{2+} equilibrium. A similar explanation may be possible for the case of bacterial Mn oxidation, since the decrease in the redox potential facilitates the oxidation of Mn.

5.8 Salt and salinity

Oxidation was much faster in freshwater and very low salinity water than seawater. The inhibition of Mn oxidation by seawater cations may be due to ionic competition, though the minimum in oxidation rate at an intermediate salinity (10.6%) is not readily explicable by a chemical mechanism of Mn oxidation (Vojak *et al.*, 1985).

The growth rate of *Vibrio* sp. was highest in the range 20-60% of full strength seawater, whilst the cell yield was unaffected from 20% to 100%. Both

of these parameters were lower at 10% salt, and no growth or Mn oxidation occurred in the complete absence of salt. In addition, Mn oxidation exhibited sharp optimum at 20% salinity and was gradually lowered with increasing salt concentration (Vojak *et al.*, 1988).

According to Stuetz *et al.* (1996), addition of varying mineral salt concentrations also suppressed oxide formation in algal-bacterial oxidation whereas when no salts were added a slight drop in oxidation occurred. Oxide inhibition was noted when individual salt was added at high concentration. However, NaCl addition gave comparable amounts of oxidation and Mn removal to algal-bacterial oxidation with mineral salt addition. Low Fe^{2+} concentrations also increased oxide formation, whereas at higher concentrations oxidation was suppressed. Chemical analysis showed that Fe^{2+} addition reduced the total Mn and Mn (IV) contents in the crude precipitates.

Boogerd and deVrind (1987) found that when concentrations of NaCl were added increasingly from 0 M to 1 M, the MnO_2 formation by *Leptothrix discophora* ss-1 decreased from 5.6 to 0.6 nmol/ml/min as well.

6. Adsorption by extracellular polymer

Extracellular polymers (ECP), also known as extrapolymeric substances (EPS), refers to macromolecules excreted outside the cell wall (Geesey and Jang, 1990). ECP are produced by a number of bacteria (Table 3) and different bacteria produce different ECP so that there is not a standard order of affinity for all ECP (McLean, 1994). ECP occur in two forms: a) loose slime which is nonadherent to the cell and b) microcapsules and capsules firmly adhere to the cell wall and can withstand large shear forces.

Although most capsule and slime exopolymers described to date are composed of polysaccharide, considerable amounts of protein are frequently recovered in crude exopolymer preparations. Both form of ECP serve as a buffer between the cell wall and the external environment. Under some

Table 3 Extracellular polymers produced by microorganisms

Microorganism	Polymer
Alcaligenes cupidus KT201	anionic polysaccharide
Alcaligenes latus B-16	anionic polysaccharide
Arcuadendron sp. TS-49	glycoprotein
Bacillus anthracis, *B. natto*, *B. licheniformis*, *B. subtilis*	polyglutamate
B. subtilis PY-90	polyglutamate
Bacillus sp. DP-152	anionic polysaccharide
Enterobacter sp. BY-29	anionic polysaccharide
Nocardia amarae YK-1	protein
Paecilomyces sp.I-1	cationic polysaccharide
Pestalotiopsis sp. KCTC 8637	anionic polysaccharide
Rhodococcus erythropolis S-1	glycolipid
mixed culture of *Oerskovia*, *Acinetobacter*, *Agrobacterium* and *Enterobacter*	anionic polysaccharide

Source : Modified from Dermlim and Prasertsan (1997)

circumstances the exopolymers serve as a barrier to harmful or toxic substances such as bacteriophage, antibiotics and biocides. Many exopolymers also carry a charge which promotes ionic and electrostatic binding of counterions. These interactions may, on the other hand, prevent excess quantities of charged molecules such as heavy metals from approaching the cell surface and yet facilitate the concentration of growth-promoting nutrients present at low concentration in the surrounding environment for subsequent uptake by the cell.

ECP may be neutral or anionic polyelectrolytes and they contain primary binding sites of metal ions. Different metal adsorption sites appear to exist on anionic and neutral polysaccharides (Kasan, 1993). The two most important types of interactions between metal ions and exopolymers are those that involve salt bridges with carboxyl groups on acidic polymers and those that involve weak electrostatic bonds with hydroxyl groups on neutral polymers. In acidic polysaccharides containing uronic acids or pyruvate groups, lone-pair electrons on oxygen atoms of carboxyl groups have a strong tendency to interact with charge-compensating metal ions, whereas oxygen atoms in the ether bonds and hydroxyl groups of sugar subunits act as weak electron donors in both acidic and neutral polysaccharides (Geesey and Jang, 1990). The complexes between metal ions and the capsule of *Zoogloea ramigera* 115 was proposed to involve primarily hydroxyl groups of the glucose subunits even though the polysaccharide contains free carboxyl groups on the ketal-linked pyruvate residue. This type of metal binding is believed to be the most important mechanism of metal removal in activated sludge (Geesey and Jang, 1990).

The release of protons by acidic polysaccharides during exposure to Cu ions has been reported in several instances (Mittelman and Geesey, 1985). Binding of Cu ions resulted in a shift in the pKa of a capsular polysaccharide from 4.90 to 4.05. These data suggested that competition exists between Cu ions and protons at the metal-binding site of acidic exopolymers (Geesey and Jang, 1990). Some polysaccharides contain amino sugars or sugars with amide

linked functional groups. These nitrogen-containing functional groups are capable of reacting with some metals. Kihn *et al.* (1987 cited by Geesey and Jang, 1990) showed that Cu^{2+} was chelated by peptides and proteins extracted from cell walls of *Saccharomyces cerevisiae*. The binding sites were formed by an amide and a strongly complexing, amine-link ligand. In slightly acidic conditions, Cu^{2+} was bound by oxygen of the amide, whereas at basic pH, NHCO became deprotonated and the negatively charged nitrogen bound the metal.

The stability and affinity of interaction between ECP and metal cations is determined by a variety of factors involving the properties of both the cation and the polysaccharide such as charge density of ECP, ionic radius of metal cation, degree of ion hydration, stereochemistry of sugar subunits comprising the polysaccharide, position of hydroxyl groups on ECP and pH of the binding condition etc. The properties of ECP are quite variable and appear to be determined by conditions of the surrounding environment. It is therefore likely that the metal-binding characteristics of the exopolymers also depend on the environmental conditions.

In activated sludge, the bacterium *Zoogloea ramigera* is important in flocculation because of extensive exopolysaccharide production. This has metal-binding properties and a continuous process for metal accumulation using pregrown *Z. ramigera* removed approximately 3 mmol (g dry weight)$^{-1}$ copper at a biomass concentration of < 1 g dry weight/l. This was only 40% of the value recorded for a batch process, a probable reason being the formation of flocs with a high proportion of inaccessible copper binding sites (Gadd, 1992). Binding of metals by other bacterial polymers are shown in Table 4.

Geesey and Jang (1990) reported that purified preparations of the floc polysaccharide isolated from *Zoogloea* spp. bound different amounts of alkaline-earth and transition metal ions and the preference was determined to be Fe > Cu > Co > Ni. Approximately 25% of the floc weight was contributed by

Table 4 Maximum binding abilities of exopolymer-metal complexes

Exopolymer sample	Metal	MBA (nmol/mg)
Thermus sp.		
suspended	Cu	9
attached	Cu	85
Deleya marina	Cu	263
	Mn	556
	Fe	39
	Ni	435
Pedomicrobium manganicum	Fe	13
	Mn	184
Pedomicrobium ferugineum	Mn	409
Zoogloea ramigera 115	Fe	250

MBA, maximum binding ability

Source : Modified from Ford and Mitchell (1992)

bound metal ions. The combined polysaccharide species purified from crude floc produced by cells of *Z. ramigera* 115 was reported to bind 0.25 μmol Fe^{3+}/ mg polysaccharide.

Rudd *et al.* (1984) found different numbers of binding sites on bacterial ECP for each of the metals they studied. When metals were added separately to ECP from *Klebsiella aerogenes,* Ni was found to have the greatest number of binding sites followed by Cu, Cd, Mn and Co in decreasing order. This suggests that several different types of metal-binding functional groups are present, that different metals may associate preferentially with different specific types of functional group, and that the number of effective binding sites may depend to some extent on the relative distributions of these groups.

The application of an emulsifying agent, emulsan, and its derivatives from bacteria such as *Arthrobacter*, *Pseudomonas* and *Acinetobacter* sp., for uranium removal has been described by Gadd (1992). Emulsan has a polysaccharide backbone comprising three amino sugars, D-galactosamine, D-galactosamine uronic acid and an unidentified hexosamine, with linked fatty acids. Metal binding ability is related to the need for divalent cations for full emulsifying activity above pH 6 and this substance is capable of high uranium binding. If emulsan was sonicated and dispersed in water/hexadecane, the emulsanosol product bound more than 800 mg uranium/g (Gadd, 1992).

7. Adsorption by manganese oxide

Manganese oxides are one of the most common types of sedimentary deposits to be found on the surface of the earth. The origin of the various types of manganese oxide surface accumulations continues to be controversial. Both biological and chemical processes have been proposed to originate the various types of MnO_2. For biological process, some Mn-oxidizing bacteria were shown in Table 2. For chemical process, colloidal hydrous oxides of Mn occur in water as products of the oxidation of Mn^{2+}, and are formed as well by

reduction of Mn^{7+} in waters which have been treated with permanganate for control of taste and odor or for oxidative removal of ferrous iron. The dioxide can be also form as a result of the disproportionation of soluble complexes of Mn^{3+} (Posselt *et al.*, 1968).

In aqueous solution, MnO_2 commonly forms a relatively stable hydrous colloid of needle-like shape. The occurrence of the needle form of the material has been evoked to support the contention that the principle mode of formation of the MnO_2 is by disproportionation of Mn_2O_3, a process in which polymerization of $Mn(OH)_4$ would play an important intermediate role. Indeed, $Mn(OH)_4$ and $Mn(OH)_3$ may be two of the principal forms of soluble and colloidal manganese in oxygen-bearing natural waters. The oxide seems to have a poor degree of crystalinity in its δ-MnO_2 form, while the γ-MnO_2 form is more organized or more ordered (Posselt *et al.*, 1968). Loganathan and Burau (1973) indicated that γ-MnO_2 is one of the main hydrous MnO_2 which is an important active form in soil, water and manganese nodules in the ocean and are stable at ordinary conditions of temperature, pressure and Eh.

The surface area of γ-MnO_2 and δ-MnO_2 are 150 (Gabano *et al.*, 1965 cited by Posselt *et al.*, 1968) and 300 m^2/g (Morgan and stumm, 1964b cited by Posselt *et al.*, 1968), respectively. A schematic arrangement of surface atoms for MnO_2 is shown in Fig. 2 assumes that a relatively well ordered crystal, surface-bound hydrogen and hydroxide ions may be exchanged at the surface of MnO_2 in response to changes in the relative activities of these ions in solution phase. Thus at least in the absence of other ionic species H^+ and OH^- function as potential determining ions, and the surface charge of the MnO_2 is largely determined by the pH of the solution, the charge becoming more negative as pH is increased. At very low pH, the H^+ ions bound at the surface predominate and the colloidal MnO_2 bears a net positive surface charge (Posselt *et al.*, 1968). The pH at which the MnO_2 is uncharged is called the point of zero charge (pzc). The pzc of hydrous MnO_2 lies between pH 2.8 to 4.5 (McLean, 1994).

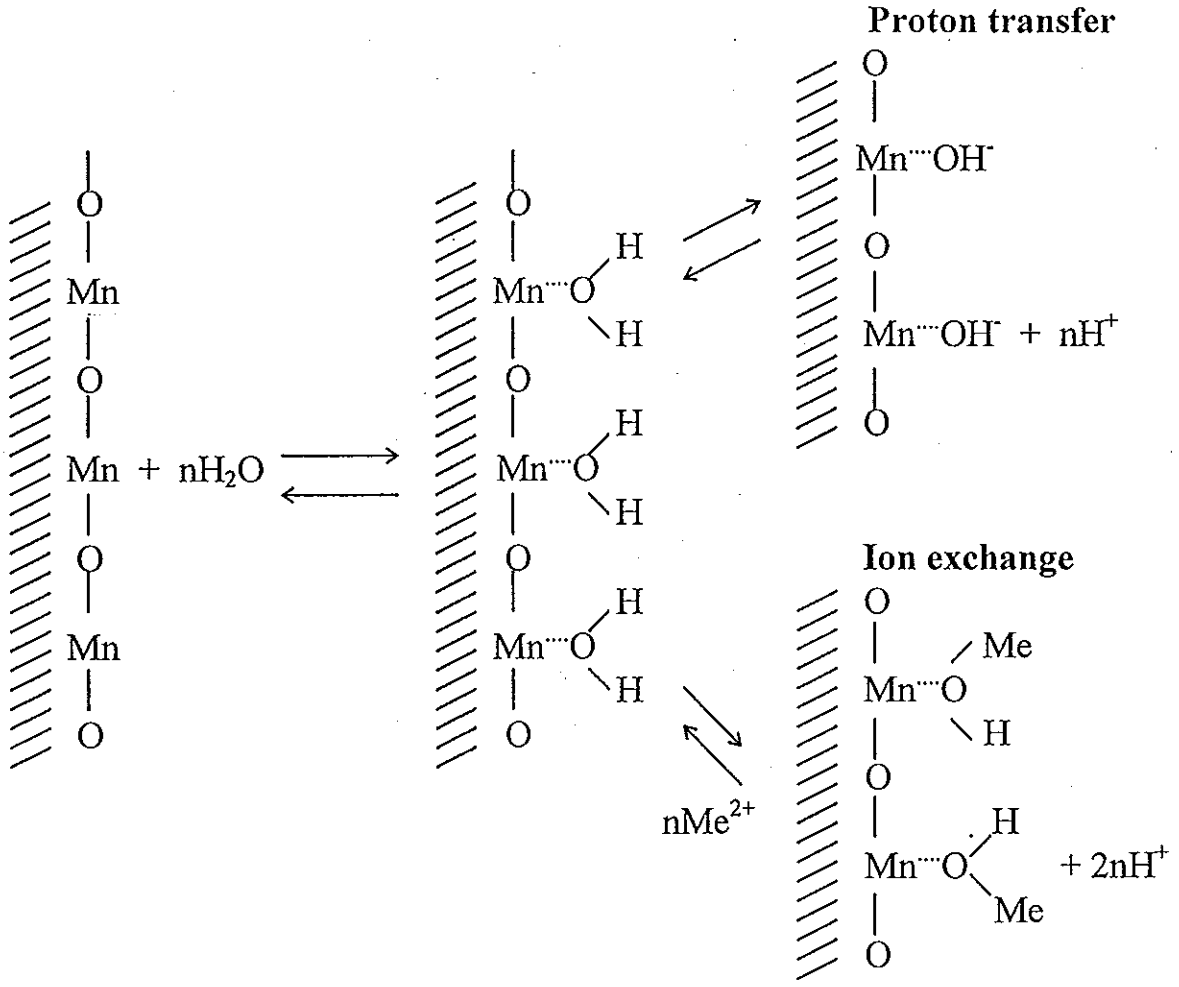


Fig. 2 Schematic representation of the surface structure and surface reactions of manganese dioxide

Source : Posselt *et al.* (1968)

Therefore, it appears quite clear that hydrous MnO_2 exhibits a net negative surface charge within the pH range (5 to 11) of principal interest for natural waters and for water treatment operations.

The hydrous oxides of Mn, generally referred to as γ-MnO_2, furnish the principal controls on the fixation of Mn, Fe, Co, Ni, Cu and Zn in soils and freshwater sediments (Jenne, 1968 cited by Loganathan and Burau, 1973). MnO_2 is used in some water treatment plants and it has been suggested as a sorbent for the removal of radionuclides from drinking water (Gray and Malati, 1979).

The effectiveness of hydrous MnO_2 in binding heavy metals is due to its low solubility, large surface area and high surface charge. MnO_2 readily incorporates metals by adsorption, ion-exchange, coprecipitation and surface complex formation (McLean, 1994). The proposed reaction of metal ions adsorption by MnO_2 involves (Murray, 1975) :

(1) The separation of a proton from the covalent bond at the MnO_2 surface

(2) The association of a solute cation with this site

$$\begin{array}{l} | \qquad\qquad\quad | \\ \text{-Mn-OH}^0 + M^{2+} \rightarrow \text{-Mn-O-}M^{+} + H^{+} \\ | \qquad\qquad\quad | \end{array}$$

Where M^{2+} = metal ions

From the mechanism above, the adsorption of metal ions onto the surface of MnO_2 results in a release of protons which effects a pH drift that can also be used to monitor the kinetics of metal ions adsorption (Murray, 1975). Exchange sorption appears to be the principal mechanism involved in the sorptive uptake of the metal ions and cationic organic solute (Posselt *et al*, 1968). In addition, the adsorption of cations onto MnO_2 is probably accompanied by the exchange with Mn ions from the solid surface which results in the release of Mn ions to

the solution phase (Gray and Malati, 1979). Loganathan and Burau (1973) indicated that during the sorption of Co and Zn by MnO_2 at 24±0.5°C, pH 4, Mn was released to the solution phase. However, Mn release was not detected during the sorption of Ca and Na. From these results, it is proposed that Ca and Na interchanged only with surface bound hydrogen. Zn was postulated to interchanged not only with these bound hydrogen sites, but also with structural Mn^{2+} whereas Co was postulated to interchange with both bound hydrogen and structural Mn^{2+} and additionally, with a third site, structural Mn^{3+}. The cation adsorption by MnO_2 is also influenced by the specific adsorption potentials which are determined from the amount of metal that is absorbed onto the surface at the pH of pzc or below the pzc. The degree of specific adsorption is related to the reversibility of adsorption. Murray (1975) indicated that the higher specific adsorption is reflected in the lower reversibility of adsorption against pH and the degree of specific adsorption potential correlates with the ratio of H^+ release/metal adsorbed.

The rates of adsorption of metal ions onto MnO_2 surface are rapid, with equilibrium attained within a matter of several minutes, for example, the rates of Mg ions (5×10^{-4} M) adsorption onto MnO_2 surface at pH 5 and 8.9 was less than 5 to 10 min to reach an equilibrium condition (Posselt *et al*, 1968).

There are many factors proposed to be involved with metals adsorption by the MnO_2 surface. For example, the size of the hydrated radii, an apparent heat of adsorption, ionic strength, pH, metal ion concentration, competitive adsorption of metal ions and groups of element e.g. alkaline, alkaline-earth and transitions.

The adsorption behavior of cations was attributed to the size of the hydrated radii, $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+}$, and the ability of the smaller ions to approach closer to the active surface of MnO_2 (Kawashima *et al.*, 1986). A higher charge density would result with the smaller ions and this is likely to favor adsorption of anions. Thus the order of adsorption capacity decreases in

the series: Ba > Sr > Ca > Mg. In addition, the adsorption of these cations onto MnO_2 is favored by increasing pH (Kawashima *et al.*, 1986).

Gray and Malati (1979) indicated that the adsorption capacity of MnO_2 at pH 6, 298 K increased in the series: $Ni^{2+} < Co^{2+} < Cd^{2+} \cong Zn^{2+} < Mn^{2+}$. This was nearly the order of decrease in the radii of hydrated ions and the order of decrease in an apparent heat of adsorption.

Gadde and Latinen (1974) indicated that on hydrous MnO_2, the adsorption of Pb, Cd and Zn was favored with increased pH. At any pH in the range 2 to 8, the extent of adsorption lay in the order Pb > Zn > Cd. At pH 6, the adsorption capacities are 0.17, 0.24, 0.28 and 0.56 mol/mol hydrous MnO_2 for the metal ions: Cd, Zn, Ti and Pb, respectively. They also studied the competitive adsorption of Pb with Cd, Zn and Ti and found that the percent adsorption of Cd, Zn and Ti found independently without the competition from Pb.

Posselt *et al.* (1968) reported that there is competition for the adsorption site on MnO_2 surface between Cs and Ag as well as between Ca and Mn. They also indicated that the adsorption capacity decreased proportionately with increasing ionic strength.

Murray (1975) found that transition metal ions were adsorbed much more strongly on hydrous MnO_2 than alkaline-earth and alkali metals, respectively when compared at equal concentrations. The selectivity order observed was: Mg < Ca < Sr <Ba < Ni < Zn ≤ Mn ≤ Co.

However, many researchers have found that some cations showed the complicated binding with MnO_2 surface, such as Cr and Co. Fendorf and Zasoski (1992) indicated that the oxidation of Cr (III) could be mediated by a common naturally occurring form of MnO_2, δ-MnO_2, and was characterized over a range of Cr (III) concentrations and pH values. Cr (III) oxidation was limited as pH and Cr (III) concentration increased. Reaction products, Mn (II) and Cr (VI) did not limit Cr (III) oxidation. Initial Cr (III) oxidation rates were

very rapid at pH 5, but were subsequently followed by a dramatic rate decline. Cr (III) oxidation was dependent on Cr (III) concentration, pH, initial surface area and ionic strength.

According to Manceau and Charlet (1992), the mechanism of Cr (III) oxidation can be depicted as followed :

(1) Cr (III) diffuse toward Mn (IV) vacancies present in the sheet of MnO_6.

(2) The coupled Cr (III) oxidation/Mn (IV) reduction occurs.

(3) Cr (VI) are released into the solution.

Co was sorbed even at pH values lower than the pzc. The specific sorption potential of Co was high due to a surface oxidation of Co^{2+} to Co^{3+} at the oxide-water interface (Murray *et al.*, 1968 cited by Loganathan and Burau, 1973). Mckenzie (1970) found that Mn was released when the heavy metal ions were sorbed. This suggest that the high capacity of δ-MnO_2 for Co was due to Co replacing Mn^{3+} existing in the disordered layer which fits between the main ordered layers in δ-MnO_2.

Generally, MnO_2 has a negative surface charge at near neutral pH and has not been thought to adsorb any anions. There are, however, some pieces of evidence which indicate that in the presence of cations the MnO_2 can adsorb some anions such as arsenate and phosphate. Kawashima *et al.* (1986) reported that the presence of alkaline-earth cations, Ba, Sr, Ca, Mg and transition metal ions, Mn, Co, Ni causes hydrous MnO_2 to strongly adsorb phosphate between pH 6 and 9 depending on the cation. The order of effectiveness of alkaline-earth cations to cause phosphate adsorption was Ba > Sr > Ca > Mg which is the same order as their affinities for the oxide. Changes with time were found in the abilities of the transition metals to cause phosphate adsorption onto MnO_2 and this may be due to conversion of the adsorbed cation to its oxide. This suggests a new potential role for MnO_2 as an adsorbent of phosphate in natural waters. As well as phosphate adsorption, in the solution containing cations such as Mn, Ni and the alkaline-earth metals, arsenate was shown to adsorb at near

neutral pH (Takamatsu *et al.*, 1985 cited by Kawashima *et al.*, 1986). It was suggested that the surface charge on MnO_2, normally negative at near neutral pH, was reversed by exchange of H^+ on MnO_2 surface for cations from solution. A positively charged surface thus resulted leading to the adsorption of arsenate onto the MnO_2 (Murray, 1975).

8. Bacterial immobilization on activated carbon

Activated carbon is a generic term for a family of highly porous carbon materials that are available in various forms, e.g. powder, granular chip and shaped product (extruded into rod-like shape). The various products are employed extensively worldwide in numerous applications, especially used in the removal of unwanted substances from aqueous and in a variety of recovery processes. It is generally conceded that activated carbon owes its adsorptive properties primarily to its large internal surface area, its pore size distribution, its external surface area and the nature of the surface oxides being accorded a minor role. The external surface can provide only as much access as possible to the inner pores and it is likely that the main role of the surface oxides is to give a hydrophilic character to the predominantly hydrophobic carbon skeleton, which would account for the affinity that activated carbon shows towards many polar and non-polar organic and inorganic species (Hassler, 1974).

Activated carbon functions by adsorption, i.e. by the binding of certain substances to the internal surface constituting the walls of the pores. Therefore, the greater the adsorption surface available, the better is the adsorption function. Adsorption occurs because of an imbalance of forces upon the carbon atoms constituting the surface of the pore wall, a phenomenon inherent in all surfaces. In order to rectify this imbalance, molecules are adsorbed from the aqueous phases and are attracted and held to the surface.

There are two types of adsorption processes: physical and chemical adsorption. Physical processes involve weak Van der Waals forces and the

processes are generally reversible, whereas chemical adsorption refers to processes involving homopolar forces (as in ionic or covalent bonds) and such adsorptions are generally irreversible.

Bacterial growth on activated carbon in water is an expected consequence of the favorable environment provided by this material. The surfaces of activated carbon are excellent for colonization by bacteria. Its adsorptive properties serve to enrich substrate and oxygen concentrations, the craggy surface provides recesses that are sheltered from fluid shear forces, the variety of functional groups on the surface can enhance attachment of bacterial (Weber Jr. *et al.*, 1978) and its very high surface area to volume ratio due to its large proportion of internal pores and rough surface texture greatly increase the rate of bacterial colonization (Shimp and Pfaender, 1982). Scott and Karanjkar (1995) indicated that activated carbon immobilization of bacteria can also provide resistance against toxic agents and increase metabolic activity. The attachment of bacteria on the activated carbon particle consists of 2 steps. The first is the initial interactions (adsorption) between the surface of particles and bacteria which are dictated by physico-chemical phenomena. The second step is the beginning of cell growth and multiplication, increasing the cell mass. Over a period, the entire surface of particle is covered with deposits of cells which take the shape of a film of bacterial cells and other material that they produce. Such a film is referred to as a biofilm (Rao, 1985). Bacteria able to be immobilized on granular activated carbon (GAC) are shown in Table 5.

The initial adsorption of bacteria on activated carbon particles is influenced by many factors which can be divided into 3 groups (Marshall, 1980) :

(1) Character of bacteria : species, culture age and cell concentration.

(2) Character of activated carbon : particle size, concentration and nature of the activated carbon.

Table 5 Some bacteria have been immobilized on activated carbon

Bacteria	Capacity of immobilization	Reference
Zymomonas mobilis	-	*Mustranta *et al.*, 1987
Escherichia coli T2	3×10^8 cells/g	Daniels, 1980
Escherichia coli T4	1.6×10^{12} cells/g	Daniels, 1980
Marine bacteria	$\cong10^8$ cell/g	Shimp and Pfaender, 1982
Mixed bacterial cultures (*Alcaligenes eutrophus*, *Pseudomonas putida*, *Bacillus* sp. and *Mycoplana* sp.)	2.52×10^9 cells/g	Rao, 1985
Enterobacter aerogenes	-	Scott and Karanjkar, 1992
Mixed methanotrophs cultures	75.6 g/l	Fennell *et al.*, 1992
Mixed bacterial cultures (*Pseudomonas* spp., *Flavobacterium* spp. and *Achromobacter* spp.)	-	Oh and Tuovinen, 1994
Pseudomonas sp. NCIMB 11592	-	Scott and Karanjkar, 1995
Pseudomonas pictorum	22.4×10^4 cells/g	Chitra *et al.*, 1995
Zoogloea ramigera NRRL 3691	78 g/l	Seker *et al.*, 1995

- , data not shown

* , cited by Toth and Tomasovicova (1989)

(3) Character of environment : composition of immobilization fluid, pH, temperature, time of contact, agitation and inorganic salt concentration.

Adsorption by activated carbon generally decreases with increasing temperature. As the temperature increase, several functional groups on the activated carbon surface such as carbonyl, quinone and carboxylic acid are known to decompose giving off CO or CO_2, thereby changing the reactivity of the carbon surface (Krishnan *et al.*, 1994). In contrast, Rao (1985) found that there was no significant temperature effect on physical adsorption of bacterial mixed culture on granular activated carbon. He also suggested that the overall attachment rate of bacteria can be expected to decrease at lower temperature because of the decrease in metabolic activity and consequently, decrease in the production of extracellular adhesives. However, Fletcher and Floodgate (1973) reported that increase in temperature can alter the quantity or characteristics of the adhesive polysaccharide produced and the strength of an adhesive polymer can decrease with an increase in temperature.

Increasing surface area (decreasing particle size) exerted a positive effect on bacterial colonization of activated carbon (Shimp and Pfaender, 1982). Rao (1985) indicated that reducing support particle size is attractive because of the resulting increase in surface area of the biofilm in the reactor. He also reported that a support particle size of around 275 μm diameter appears to be the most suitable. In the same way, Li and Digano (1980 cited by Shimp and Pfaender, 1982) hypothesized that decreasing particle diameter decreased the diffusional pathlength of organic substances from the internal pores of the carbon to the biofilm and hence increased the availability of adsorbed substrate to attached cells. Thus, growth would be somewhat enhanced in small grain system.

Rao (1985) demonstrated that the adsorption of activity growing bacterial cells in the exponential growth phase was found to be higher than cells in either stationary phase or death phase. However, it is likely that the stationary

phase cells are more efficient in synthesizing the extracellular polymer adhesive materials because of the increased specific metabolic activity under starvation condition. This in turn, may enable starved cells to attach themselves irreversibly more efficiently, although their physical adsorption efficiency is less than that of actively growing cells (Marshall, 1980).

Hassler (1974) indicated that for most of sorbate, the initial adsorption by activated carbon appears to be rapid and generally, complete in less than an hour, after which a very slow sorption continues. This was supported by studies of bacterial mixed culture adsorption on GAC by Rao (1985) which also showed that the adsorption equilibrium was reached within 20 min and a uniform and firm biofilm was observed within 4-5 days later.

Dissolved organic matter tends to concentrate at solid surfaces in aqueous environments and this leads to enhanced bacterial activity and colonization at the solid-liquid interface (Zobell, 1937 cited by Rao, 1985). The adsorbed nutrient ion concentrations appear to play an important role in the subsequent bacterial adsorption, perhaps by altering the surface charge of the support material (Rao, 1985). Studies on the adsorption and desorption of bacteria on clean and presaturated activated carbon with medium by Rao (1985) indicated that the level of adsorption was found to be 28% more on presaturated activated carbon particle, while the rate of desorption was higher in the case of clean activated carbon. The lower adsorption rates on the unsaturated support are, thus, understandable because of the time involved in the adsorption of nutrient ions prior to the adsorption of bacteria. These effects will become negligible over a longer adsorption time, however, presaturation of the support material is likely to minimize the seeding time in the startup of the reactor (Rao, 1985). Different nutrient conditions resulted in changes in physicochemistry of the bacterial surfaces, measured either by liquid contact angles on lawns of cells or by hydrophobic and electrostatic interaction chromatography of cells, and in different levels of attachment. In addition, organic substances can affect

the liquid and solid phases of the attachment interaction through their influence on thermodynamic parameters, e.g. surface tension (McEldowney and Fletcher, 1986). Furthermore, media with different carbon: nitrogen ratios and carbon source had a marked effect on the attachment of bacteria. The four bacterial species investigated, *Pseudomonas fluorescens*, *Enterobacter cloacae*, *Chromobacterium* sp. and *Flexibacter* sp., all reacted differently, with respect to attachment ability and to changes in culture conditions. The results showed that *P. fluorescens*, *E. cloacae* and *Chromobacterium* sp. attached in high numbers after 24 h growth in glucose, lactose and sucrose, respectively.

9. The biological fluidized bed reactor

The biological fluidized bed reactor is a novel biological water and wastewater treatment process (Rao, 1985) which contains an inert or active support media that provided a site for biofilm growth. In these media-based reactors, contaminated water or wastewater is passed upward through the bed at a velocity sufficient to expand or fluidized the bed of immobilized cells beyond the point at which the frictional drag is equal to net downward force exerted by gravity or, in other words, superficial velocity equal to the setting velocity of the particles. Once beyond the point of minimum fluidization, the bed media particles provide a vast surface area for biological growth, in part leading to the development of a biomass concentration that is five or ten times greater than that normally achieved in more conventional bioreactors, such as activated sludge reactors (Sutton and Mishra, 1991).

The development of a fluidized bed bioreactor offers several advantages over most other biological reactors as follows (Rao, 1985; Tavares *et al.*, 1995):

(1) Larger effective surface area per unit volume of reactor

(2) Uniform particle movement (good mixing)

(3) No clogging or channeling problems

(4) Insignificant head losses

(5) Equilibrium between growth and attrition of biofilm possible

(6) Better process control

(7) Lower hydraulic retention time

(8) Lower sludge production

However, it is important to realize that the exact nature of the media particle is far more critical in the design of fluidized beds than packed beds. In fact, it can be shown that once the flow and substrate concentration at the inlet, the required substrate removal, and the biological rate constants have been specified, then the only remaining variables are the size, density and cell concentration of the media particles. Fixing these determines the height and diameter of the bed and thus, its volumetric productivity. So in choosing the support material particle, many factors must be considered (Andrews and Przezdziecki, 1986) :

(1) Small particles are desirable because they give a higher area of biomass and there will be less mass transfer resistance in the particle.

(2) In a fluidized bed, unlike a packed bed, the particle size and density and the flow velocity are not independent variables. Choosing particles with a large settling velocity means that the superficial velocity must be large, so in order to achieve the required contact time the bed must be very tall and will be narrow for a given flow rate. Conversely, a small, light particle leads to a short, wide bed.

(3) A large, heavy particle is associated with a high particle Reynolds number. This has two consequences. First it increases the shear stress on the particle surface and thus, the rate at which biomass washes off. This is not a problem with cohesive, rapidly growing aerobic films, but it may limit the thickness of other types to less than the desired value. Second, increasing Reynolds number increases the mass transfer coefficient at the biomass/liquid interface.

(4) It is generally true that productivity is maximized when the thickest films are exposed to the highest substrate concentrations (an exception is the first-order kinetics case). This can be achieved by choosing small, light particles. For light plastic supports or floc particles, film growth increases the setting velocity and heavily coated particles go to the base of the bed where substrate concentrations are highest. Note that this is a stable stratification since the particles near the base, being exposed to the highest substrate concentrations, will grow fastest and thus retain its setting velocity advantage over the other particles.

To improve reactor productivity, small, dense, porous and adsorptive particles were suggested by Andrews and Przezdziecki (1986).

The biological fluidized bed reactor have been used or proposed for a number of applications especially for the treatment of contaminant water and wastewater. A fluidized bed biofilm reactor using activated carbon particles of 1.69 mm diameter as the support for biomass growth and where molasses is used as the carbon source for wastewater denitrification was developed by Coelhoso *et al.* (1992). The startup of the reactor was successfully achieved in one week using a liquor from garden soil leaching as the inoculum. Coelhoso *et al* (1992) also indicated that activated carbon as the support has the following advantages: good adsorptive characteristics, homogeneous bioflim thickness along the reactor bed and easy restartup of the reactor.

Puhakka and Jaervinen (1992) studied degradation of polychlorinated phenol in continuous flow fluidized bed reactors using celite carrier for immobilized mixed bacterial cultures. Continuous polychlorophenol biodegradation activity was maintain in a fluidized bed reactor for 315 days. Chloride release and chlorophenol removal efficiencies of over 99% were achieved at substrate loading rates of up to 430 g 2,4,6-trichlorophenol/m^3/day and 400 g 2,3,4,6-tetrachlorophenol/m^3/day at 3-5 h hydraulic retention times, respectively.

A fluidized bed reactor, with sand as carrier and ethanol as the carbon and electron source, was investigated for the biological denitrification of ground water by Green *et al.* (1995). Values for the maximum specific nitrate and nitrite removal rates of 11 g and 6 g NO_3^- (g volatile suspended solids)$^{-1}$ day^{-1}, respectively were obtained. These values were used to interpret nitrate and nitrite concentration profiles in an experimental fluidized bed reactor operating at different conditions of hydraulic loading and retention time.

Scott and Hancher (1976 cited by Fukuda, 1995) made a preliminary investigation of a tapered fluidized bed (TFB) using adhering *Pseudomonas* bacteria within and/or on coal particles for biodenitrification. In the TFB there is gradual expansion from a relatively small cross-sectional area of the entry to one that may be several times larger, and this makes the flow relatively stable throughout the reactor. Also, the TBF can either provide flow patterns that have minimal back-mixing, especially at the feed entry point, or operate in a similar way to a multistage unit in which the reactant and product concentrations vary throughout the reactor.

Sly *et al.* (1993) developed a process for the immobilization of manganese oxidizing bacteria on magnetite particles and to use the immobilized cells in a continuous recycle fluidized bioreactor (CRFB) for the removal of manganese from water. A model CRFB was operated for 22 weeks with removal rates of greater than 90% and up to 100% for Mn^{2+} concentrations in the range 0.25 to 8.5 mg/l when operated at a residence time of 21 h. The bioreactor approached maximum removal efficiency within a week. In addition, the CRFB required minimal maintenance, did not clog or bind and therefore did not require backwashing.

Biological fluidized bed reactors using porous media have been employed for the treatment of contaminated groundwater (Massol-Deya *et al.*, 1995) and found that bacteria may remain in place within the porous medium i.e. GAC forming biofilm communities through either attachment or

agglomeration. Under optimum conditions, up to 99% of the total applied organic load can be removed within 5 to 10 min hydraulic retention times.

10. Heavy metals removal by immobilized living microorganisms

Heavy metal toxicity and other extreme properties of wastewater effluents may limit the use of living cell systems. However, living cell systems may allow a greater potential for a long term continuous process, without the need for periodic desorption, if there is continual biomass replenishment (Mccaskie and Dean, 1989 cited by Gadd, 1992). Thus, studies of metal ions removal from aqueous solution by biosorption with immobilized living biomass have been under taken by many researchers (Table 6).

The method most often applied to metal removal is the use of cells immobilized as a biofilm on inert supports. Ideal supports have a large surface area but are sufficiently porous to enable high flow rates and minimal clogging. Materials include those with planar surfaces (glass, metal sheets and plates, plastics), uneven surfaces (wood shavings, clays, sand, crushed rock, coke) and porous materials (foams, sponges) and many of these systems have been used in a variety of bioreactor configurations including rotating biological contactors, trickle filters and fluidized beds and air lift bioreactors. However, immobilized biomass particles appear of greater potential in packed or fluidized bed reactors with benefits including control of particle size, better capacity of regeneration, easy separation of biomass and effluent and recirculation, high biomass loadings and minimal clogging under continuous flow (Gadd, 1992).

In addition to removal of metal ions, living cell biofilms may provide capacity for the removal of other pollutants including hydrocarbons, pesticides and nitrates. For examples, *Pseudomonas aeruginosa* was immobilized on particles of polyvinyl chloride (PVC) and polypropylene webs and used in batch and column reactors for simultaneous denitrification and heavy metal removal from contaminated wastewater (Hollo *et al.*, 1979 cited by Gadd,

Table 6 Metals removal by immobilized living microorganisms

Microorganism	Supporting material	Type of reactor	Target metal	Removal efficiency	Reference
Pedomicrobium manganicum ACM 3067	magnetite	Continuous recycle fluidized bioreactor	Mn	90-100%	Sly *et al.*(1993)
Pedomicrobium manganicum ACM 3067	magnetite	Continuous recycle fluidized bioreactor	Cu Pb Zn	98.9% 98% 99.4%	McLean (1994)
Enterobacter aerogenes	granular activated carbon	fluidized reactor	Cd	60% (1.2-5.2 mg Cd/g)	Scott and Karanjkar (1992)
Pseudomonas sp. NCIMB 11592	granular activated carbon	fluidized reactor	Cu Zn Cd Ni Ag	- - - - -	Scott and Karanjkar (1995)
Microorganisms from waste streams	plexiglass disc	Rotating biological contactor	Fe Mn	98% 86%	Russell and Alleman (1986)
Citrobacter sp.	polyacrylamide gel	Flow-through bioreactor	UO_2^{2+}	90-95% (9 g/g)	Macaskie (1990)
Zoogloea ramigera	Ca-alginate	Packed bed column	Cu	94.3%	Sag *et al.* (1993)
Pseudomonas aeruginosa	Ca-alginate	-	Ni	91%	Asthana *et al.* (1995)
Rhodopseudomonas BHU strain 1	Ca-alginate	-	Ni	72%	Asthana *et al.* (1995)

-, data not shown

1992). Another system used a mixed bacterial culture, mainly *Pseudomonas* sp., immobilized as a film on anthracite particles for denitrification and uranium removal (Shumate and Strandberg, 1985).

Following are the several advantages and disadvantages of metal removal by immobilized living cells (Macaskie, 1990) :

Advantages

(1) Although each cell may become saturated, the system as a whole is self-replenishing due to growth.

(2) The metal is deposited in an altered chemical state and less sensitive to spontaneous desorption.

(3) Metabolic activity may be the only economic way to achieve changes in valency state or degrade organometallic compounds: multi-enzyme steps can be used.

(4) There is the potential for mutant isolation or genetic manipulation to improve the strain, since it is a microbial property rather than a product under exploitation.

(5) Two or more organisms can be employed in a synergistic way.

Disadvantages

(1) Toxicity: often the metal can be presented only at low concentrations, but metal resistant strains have been used.

(2) Necessity to present the flow under physiologically permissive condition

(3) Need for growth nutrients

(4) Disposal of metabolic products and unconsumed growth nutrients.

(5) Metabolic products may form complexes with metals and retain them in solution.

(6) Potential for desorptive metal recovery is limited since metals may be intracellularly bound.

(7) Difficulties in mathematical modeling of a non-defined system.

11. Factors affecting metals removal by biosorption with bacteria

Some factors affecting metal removal have been mentioned by a number of researchers. These include pH, temperature, contact time, cell age, metal species and metal concentration, nutrient and medium composition and other ions.

11.1 pH

The pH of the medium influences metal uptake by bacteria. At acid pH, metal binding is often limited by the increasing tendency of protons to compete with metal ions for anionic binding sites (McLean and Beveridge, 1990). Increasing pH results in an increased negative charge at the surface of the cells, which favors electrochemical attraction and adsorption of metal cations. However, several metals form insoluble oxides and/or hydroxides at alkali pH. A pH between 4.0 and 8.0 is widely accepted as being optimal for metal uptake for almost all types of bacteria (Blackwell *et al.*, 1995).

Gourdon *et al.* (1990a) found that at neutral pH (pH 6-7) Gram-positive cells exhibit approximately 20% more Cd biosorption than Gram-negative cells. This indicated that glycoproteins and teichoic acids on the outer side of the cell wall in Gram-positive bacteria may be responsible for this difference.

The optimum pH for the adsorption of Cu, Cd, UO_2^{2+} and Zn by *Azotobacter vinelandii* were 5.4, 6.0, 6.5 and 6.7, respectively (Cotoras *et al.* 1992).

11.2 Temperature

Temperature effects are confined to metabolic metal accumulation. At low temperature (0-5°C), little metal is sequestered through metabolic processes by viable cells. Most laboratory experiments are carried out in the temperature range 25-35°C, which has been reported optimal for metal accumulation (Blackwell *et al.*, 1995). Metal binding through biosorptive action is unchanged across the temperature range 4-25°C (White and Gadd, 1987 cited by Blackwell *et al.*, 1995).

Gourdon *et al.* (1990a) studied Cd uptake by Gram-positive and Gram-negative bacteria from activated sludge, and found that when the temperature was raised from 5°C to 40°C, Cd uptake also increased, but to a much smaller extent. This suggested that passive and active adsorption mechanism occurred simultaneously. At 5°C, Cd uptake resulted exclusively from a passive adsorption mechanism. This was confirmed by the fact that no extracellular protein production was observed then, indicating very slow or no biological activity at this temperature. When the temperature was raised to 40°C, the metabolic activity of the sludge strongly increased, as shown by the marked increase in extracellular protein production.

11.3 Time course of metal adsorption

Gourdon *et al.* (1990b) found that Cd uptake by bacteria from activated sludge increased rapidly over 5-10 min after the addition of Cd and decreased only slightly thereafter. More than 95% of total biosorption was achieved within 5 min metal-sludge contact time. The rapid initial phase of Cd uptake seemed more consistent with a mechanism of passive adsorption to the cells rather than with metabolically mediated uptake. The slight decrease in Cd concentration in solution after the rapid initial phase may be related to a slow phase of metabolically mediated uptake.

Similarly, Scott and Karanjkar (1992) reported that *Enterobacter aerogenes* immobilized on granular activated carbon rapidly adsorbed Cd within 5 min indicating physical adsorption, followed by slower uptake indicative of at least in part active metabolically sponsored accumulation. The initial uptake is due to metal ion sorption onto the external surfaces of the biofilm. For active uptake, Cd ions are transported through the cell membrane and into the cytoplasm where precipitation, often associated with sulfides, can occur (Scott and Palmer, 1990). In addition, the time taken to reach metal uptake equilibrium is related to the initial metal ion concentration. As the metal

concentration increases, the time decreases as sites available for direct metal adsorption are rapidly taken up (Scott and Karanjkar, 1992).

11.4 Culture time or cell age

Methods employed to prevent metal cations entering the cell include extracellular precipitation of metals by means of excreted metabolites such as extracellular polymer and siderophores. Thus, to receive the maximum removal efficiency, it would be worthwhile to ensure that when the excretion of these metabolites has reached a maximum (Blackwell *et al.*, 1995). In addition, the characteristic life cycle of many bacteria exhibit morphological changes, so cell age may have an effect on metal binding capacity (Scott and Palmer, 1990). *Arthrobacter viscosus* cultures were found to be 75-80% rods after 24 h but by 48 h, approximately 50% rods and 50% cocci. After 72 h the cocci cells dominated with very few rods present and 96 h cultures were completely composed of cocci. Biomass recovered at 12 and 24 h was found to be less effective than older samples for Cd accumulation. Scott and Palmer (1990) suggested that this was due probably to sub-maximal exopolysaccharide synthesis. In contrast, Marques *et al.* (1991) found that the amounts of uranium removed did not change substantially with the age of *Pseudomonas* sp. EPS-502. A similar result was found in the studies of uranium removal using *Streptomyces viridochromogenes* (Horikoshi *et al.*, 1981)

11.5 Metal species and metal concentration

Studies on the biosorption of Cd, Co, Ni and Sr by *Bacillus simplex* ZAN-044 indicated that the relative amounts of cations bound by the strain was Cd > Ni ≥ Co > Sr. Binding of these metals at pH 6 by *B. simplex* ZAN-044 was increased linearly from 0.01 μM to 100 μM, whereas at 1000 μM metal, biosorption was no longer linear (Valentine *et al.*, 1996). A similar result was observed in the studies of Cd biosorption by *Streptomyces pimprina* (Puranik *et al.*, 1995) and uranium uptake by *Streptomyces viridochromogenes* (Horikoshi *et al.*, 1981).

Gourdon *et al.* (1990b) reported that besides having effect on a limiting adsorption capacity, high concentration of Cd also affect the respiration of bacteria from activated sludge. As the concentration of Cd was raised from 0 to 50 mg/l, bacterial respiratory activity was reduced by 65%.

11.6 Nutrients and medium composition

The addition of carbon source, nitrogen source, sulfur and phosphate to the medium affected Cd adsorption by bacteria from activated sludge (Gourdon *et al.*, 1990b). In the presence of nutrients, Gram-positive bacteria accumulated 37% more Cd than Gram-negative bacteria. This indicated that some metabolic uptake did occur where Cd was taken into Gram-positive bacterial cells, whereas it is possible that Gram-negative cells had enough carbon source within themselves to support the low metabolic uptake of Cd (Gourdon *et al.*, 1990a).

11.7 Other cations and anions

Cations and anions additional to the ion of interest have a generally detrimental impact on metal uptake. Decreased metal uptake is thought to be a response to increased competition between like charged species for binding sites, with preference given to particular ions (Blackwell *et al.*, 1995).

Cotoras *et al.* (1992) studied biosorption of uranium by *Azotobacter vinelandii* and found that Cu inhibited the UO_2^{2+} biosorption whereas Mg and K had no inhibitory effect. In term of binding selectivity the metal can be ranked: $UO_2^{2+} > Cu > Zn > Cd \cong Ca$.

Studies on uranium binding by *Pseudomonas aeruginosa* CSU indicated that Fe^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Cr^{3+} and Pb^{2+} inhibited uranium binding. The order of inhibition to uranium binding was $Fe^{3+} > Fe^{2+} > Al^{3+} > Pb^{2+} > Cu^{2+} > Cr^{3+} > Cd^{2+}$. Fe^{3+} caused a severe abatement of uranium removal which is consistent with the competition between the two metals for reducing equivalents (Hu *et al.*, 1996). Horikoshi *et al.* (1981) examined whether carbonate (CO_3^{2-}) interfered with the uptake of uranium by *Actinomycetes* by adding various

concentration of sodium hydrogencarbonate, pH 8 to the uranium solution. The result showed that sodium hydrogencarbonate scarcely affected the uptake of uranium by *Actinomycetes*, however, as the concentration of sodium hydrogencarbonate increased, the amounts of uranium adsorbed by both *Actinomyces levoris* and *Streptomyces viridochromogenes* decreased. It was also found that 3 mM sodium hydrogencarbonate completely prevented uranium adsorption by the cells.

12. Conclusion

Microorganisms are capable of accumulating heavy metals by a process called biosorption. Various mechanisms exist for the biosorption process ranging from adsorption to different negatively charged groups on the cell surfaces, to mechanism dependents on metabolism such as the active transport and internal compartmentation. However, the mechanism responsible for biosorption can vary depending on the microorganism used and the metal adsorbed. The biosorption process is very complex and influenced by a number of physicochemical, biological and environmental factors.

The application of the immobilized living biomass on supporting materials in a variety of bioreactor configurations to remove heavy metals from solutions has many advantages over the use of suspended cells. One of the advantages is the ease of regeneration and recovery of biosorbents and metal. Another is that the immobilization process requires only minimal solid-liquid separation operations. The high efficiency for the removal of Mn (Sly *et al.* ,1993), Cu, Pb and Zn (McLean, 1994) by the immobilized cells of *Pedomicrobium manganicum* ACM 3067 on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) was previously reported. Sly *et al.* (1993) reported that the immobilized *P. manganicum* ACM 3067 could remove Mn^{2+} between 90-100% for Mn^{2+} concentration in the range of 0.25 mg/l to 8.5 mg/l. Furthermore, McLean (1994) found that the removal

efficiency for Cu, Pb and Zn were 98.9%, 98.0% and 99.4%, respectively. The metal adsorption mechanism of *P. manganicum* ACM 3067 was proposed to involve both ECP and MnO_2 bound to the ECP (Sripoaraya, 1993; McLean, 1994). Ni and Cd, the two important environmental pollutants, were the other two metals which were highly tolerated by manganese-oxidizing cells of *P. manganicum* ACM 3067 (Sripoaraya, 1993).

The supporting material used in the CRFB was magnetite which have the necessary density and surface characteristics for being a suitable support particle (Sly *et al.*, 1990). Magnetite rapidly adsorbed 95-99% of various microbial cells from aqueous suspension, moreover, microbial cells attach strongly to magnetite without diminishing their capacity to function microbiologically MacRae and Evans, 1983). Besides magnetite particle, the granular activated carbon (GAC) is an interesting one which has excellent surface for microbial colonization. Its adsorptive properties serve to enrich substrate and oxygen concentrations, the craggy surface provides recesses that are sheltered from fluid shear forces and different functional groups on the surface can enhance attachment of microorganisms (Weber Jr. *et al.*, 1978). GAC immobilization of microorganisms can also provide resistance against toxic agents and increase metabolic activity (Morren and Rehm, 1990 cited by Scott and Karanjkar, 1995). Furthermore, an activated carbon especially from coconut shell is cheap and also available in Thailand.

Objectives

1. To study the effect of manganese concentration on culture growth and manganese oxide production by *P. manganicum* ACM 3067

2. To determine the minimum inhibitory concentrations of nickel and cadmium on *P. manganicum* ACM 3067

3. To study the effect of some factors on the immobilization of *P. manganicum* ACM 3067 on granular activated carbon

4. To determine nickel and cadmium removal by *P. manganicum* ACM 3067 immobilized on both magnetite and granular activated carbon in the continuous recycle fluidized bioreactor

Chapter 2

Materials and Methods

Materials

1. Microorganism

The microorganism used in this study was *Pedomicrobium manganicum* ACM 3067. This strain was isolated from a water distribution system with manganese-depositing biofilm (Sly *et al.*, 1993).

2. Media

Pedomicrobium standard medium (PSM) (Gebers, 1981) was used to maintain the culture.

PC medium (Tyler and Marshall, 1967) was used to examine the Mn-oxidizing ability of the culture.

Half strength PC medium with 1 ppm Mn (McLean, 1994) was used in the experiment of metals removal by the CRFB.

The composition of these media is given in Appendix 1.

3. Metal salts

Metals tested for the adsorption by *P. manganicum* ACM 3067 were Mn^{2+}, Cd^{2+} and Ni^{2+}. They were prepared as follows :

$MnSO_4 \cdot H_2O$ (as Mn^{2+}), 1,000 ppm stock solution

$CdSO_4 \cdot 8/3H_2O$ (as Cd^{2+}), 4,380 ppm stock solution

$NiSO_4 \cdot 6H_2O$ (as Ni^{2+}), 4,472 ppm stock solution

4. Supporting materials

Magnetite particles which had been treated by eight alternating magnetic field cycles (8AMF) (Sly *et al.*, 1993) were used in this study (Appendix 2).

Granular activated carbon (GAC) from Eurocrab Ltd., England (Appendix 2).

5. Immobilization medium

Suspending fluid (MacRae and Evans, 1983) (Appendix 1) and half strength PC medium with 1 ppm Mn were used for cell immobilization on magnetite particle and GAC particle, respectively.

6. The Continuous Recycle Fluidized Bioreactor (CRFB)

6.1 Using magnetite particle as the supporting material

The medium was pumped into the CRFB (a glass column with 60 mm diameter × 600 mm height) in an upward direction to fluidize 1.2 L of magnetite particles (210-300 μm). The 50% expanded fluidized bed was maintained by recirculating a 3.3 L volume of the medium through the column and a 2.1 L mixing vessel (as shown in Fig. 3 and 4; previously described by Sly *et al.*,1993). A pH probe was included in the mixing vessel , however the pH was not controlled since the pH in the CRFB was in the range of 7.8-8.0. The temperature was monitored at 28°C. Dual synchronised peristaltic pumps were used to recirculate the medium at a rate of 1.00-1.125 L/min through the column and mixing vessel. Another peristaltic pump was used to pump in fresh medium and remove effluent from the mixing vessel at a rate of approximately 1 residence volume i.e. 3.3 L/day.

6.2 Using GAC as the supporting material

The adapted CRFB using GAC as the supporting material was

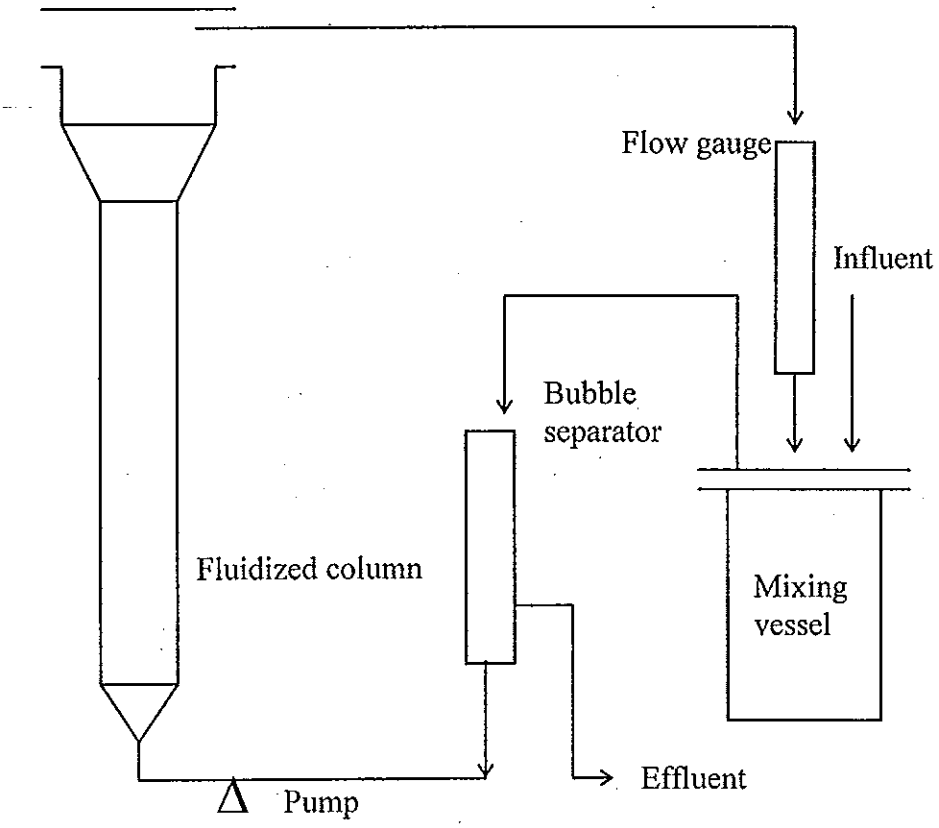


Fig. 3 Schematic of the continuous recycle fluidized bioreactor (CRFB)

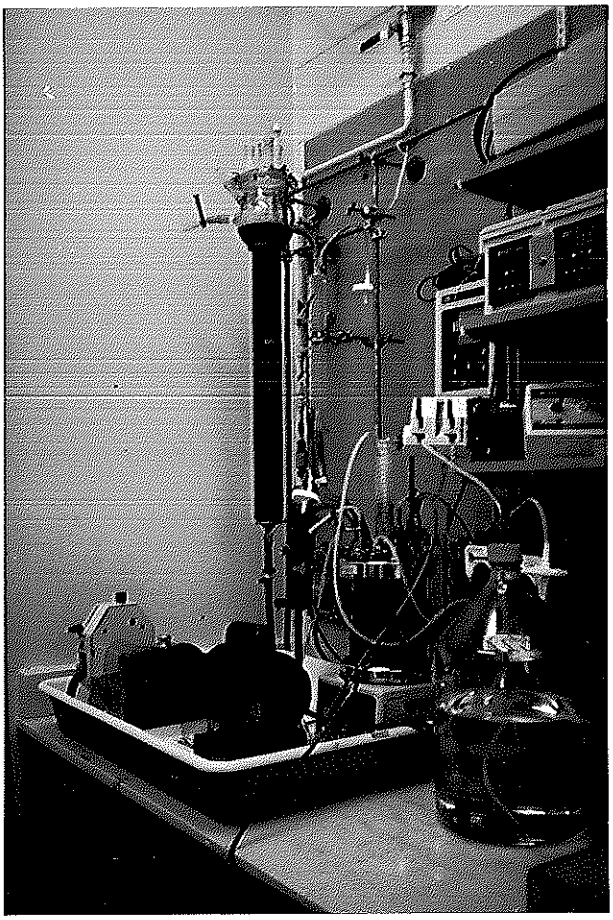


Fig. 4 Photograph of the continuous recycle fluidized bioreactor (CRFB) using magnetite as the supporting material

constructed conforming to the design of the original CRFB previously described in 6.1. The model of this CRFB consisted of an acrylic column (57 mm diameter × 600 mm height), a 2.2 L stirred mixing vessel, a flow gauge and a bubble separator (Fig. 5). The column contained 1 L of 400-1,000 μm diameter GAC particles which was fluidized by recirculating 3.2 L volume of medium to expand the GAC bed to 50%. Probes for pH and temperature were connected to the mixing vessel to maintain pH and temperature at 8.0 and 28°C, respectively. Stirrer speed in the mixing vessel was controlled at about 400 rpm. The pump was used to recirculate the medium at a rate of 0.6-0.8 L/min through the column and mixing vessel. A peristaltic pump was used to pump in fresh medium and remove effluent from the system at a rate of 2.4 ml/min. Thus, the calculated residence time and residence volume were approximately 22 h and 3.46 L/day, respectively.

7. Instruments

Instruments	Model	Company
pH meter	HM-7E	TOA Electronic
Rotary shaker	-	Lab-Line
Spectrophotometer	U-2000	Hitachi Koki
Refrigerated centrifuge	RC-5B	Sorvall
Refrigerated incubator	-	Sanyo
Light microscope	Alphaphot-2YS_2	Nikon
Scanning electron microscopy	SEM JSM-5800LV	JEOL
Inductively coupled plasma atomic emission spectroscopy	Plasma-1000	Perkin Elmer

Fig. 5 Photograph of the continuous recycle fluidized bioreactor (CRFB) using granular activated carbon (GAC) as the supporting material

Methods

1. Time course on growth of *P. manganicum* ACM 3067 in PSM medium

Starter preparation

The 10 days old culture of *P. manganicum* ACM 3067 on PSM agar plate was inoculated (2-3 loops) into 100 ml of PSM. Cultivation was performed on a rotary shaker at 150 rpm at room temperature (30±2°C) for 4 days. The culture broth was diluted with sterile deionized water to adjust the absorbance reading at 540 nm to 0.3-0.4 and used as the starter.

Growth curve determination

The 10 ml starter was inoculated into 100 ml of PSM in 250 ml flasks. Cultivation was performed on a rotary shaker at 150 rpm at room temperature (30±2°C) for 8 days. Samples were taken every 24 h to measure growth (OD_{540}) and viable cell count.

2. Effect of Mn concentration on growth and MnO_2 formation by *P. manganicum* ACM 3067 in PSM and PC media

The 10 ml starter was inoculated into 100 ml each of PSM and PC medium. Mn^{2+} was added into PSM and PC media at the concentrations of 0, 2, 6, 12, 20 and 50 ppm. Cultivation was performed on a rotary shaker (150 rpm) at room temperature (30±2°C) for 7 days. Samples were taken every 24 h to measure pH, OD_{540} and MnO_2 content (Kessick *et al.*, 1972 cited by Johnson, 1991)

3. Determination of the minimum inhibitory concentration (MIC) of Ni and Cd

Plate preparation

PC(Mn) and PC agar plates were prepared in the presence (6.5 ppm

Mn^{2+}) and absence of Mn^{2+}, respectively. Prior to the agar plate preparation, the serial dilution of $NiSO_4 \cdot 6H_2O$ (Ni^{2+}) and $CdSO_4 \cdot 8/3H_2O$ (Cd^{2+}) were added into the agar medium to obtain the various concentrations as specified in Table 7.

Preparation of cell suspension

The 10 days old culture on PSM plate was inoculated into 50 ml of PSM and cultivation at room temperature (30±2°C) on a rotary shaker (150 rpm) for 4 days. After centrifugation at 10,000 rpm (7,600×g), the supernatant was discarded and the cells were resuspended in sterile deionized water to reach an OD_{540} reading of 0.3-0.4.

Determination of MIC on agar plate

A 0.1 ml volume of cell suspension was spread over the agar plate containing each metal (Ni and Cd) concentration (Table 7). The plate was incubated at room temperature (30±2°C) for 10 days. Growth on PC agar and PC (Mn) agar plates was recorded. The MIC was calculated as the minimum concentration of metal ions completely inhibiting growth.

Determination of MIC in liquid medium

The cell suspension, as 10% inoculum, was inoculated into the PC (Mn) and PC broth which contained various concentrations of Ni and Cd (Table 7). Cultivation was performed on a rotary shaker (150 rpm) at room temperature (30±2°C) for 24 h. Triple flasks were sampled at 0 and 24 h and measured for OD_{540} and pH.

4. Removal of heavy metals by the CRFB using magnetite as the supporting material

Bioreactor setup

A packed bed of 1.2 L of magnetite was autoclaved in the bioreactor column, along with the tubing and the mixing vessel containing 2.1 L deionized

Table 7 Range of concentrations of Ni, Cd and a combination of Ni and Cd (Ni+Cd) used in PC agar plates and broths

Ni (ppm)	Dilution	Cd (ppm)	Dilution
4472	stock solution	4380	stock solution
0.100*	1:44676	0.100*	1:43756
0.501*	1:8925	0.502*	1:8721
0.903*	1:4947	0.904*	1:4845
0.996*	1:4488	0.998*	1:4385
2.039*	1:2193	1.997*	1:2195
3.023	1:1479	3.053	1:1427
3.985	1:1122	4.089	1:1070
4.871	1:918	5.051	1:867
6.236	1:714	6.134	1:714
7.307	1:612	7.156	1:612

* Concentrations that were also applied in the combination plate and broth

water. After sterilization, the system was aseptically assembled and flushed with sterile deionized water until the cloudiness due to contaminating clays and fine magnetite disappeared from the effluent. The apparatus was then drained and 1.2 L of suspending fluid was added to the bed of magnetite.

4.1 Immobilization of *P. manganicum* cells in the CRFB

P. manganicum was cultivated in 5 L PSM on a rotary shaker for 4 days at 28°C. The cells were harvested by centrifugation at 10,000 rpm (7,600 ×*g*) for 20 min and resuspended in suspending fluid for immobilization. The concentrated cell suspension was added aseptically to the mixing vessel to a final volume of 2.1 L, and mixed at 500 rpm to break up any cell clumps. Then, the recirculation pump was turned on to fluidize the supporting magnetite material and to circulate the cells through the system. A 10 ml sample was taken at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10 and 15 min to measure for the absorbance reading at 540 nm and for determination of the viable cell count on PSM agar plates. The adsorption and growth of the culture on the surface of magnetite particles in the CRFB was also examined by Scanning Electron Microscopy (SEM) (Appendix 3).

4.2 Removal of heavy metals

The influent medium used was half strength PC with 1 ppm Mn and various concentrations of Ni (as $NiSO_4 \cdot 6H_2O$) and Cd (as $CdSO_4 \cdot 8/3H_2O$) were added individually and in combination. For individual metal addition each was added at the concentration of 0, 1, 2, 4, 6 and 8 ppm. For combination metal addition, 0, 1, 2 and 4 ppm of each metal was used. Samples (approximately 50 ml) were taken from the mixing vessel every 12 h in the morning and the afternoon until the metal concentration in the CRFB tends to be stable which is approximately 3 days for each influent metal concentration. The total and soluble metal concentrations were analyzed using the Atomic Absorption Spectrophotometer (AAS) (Appendix 3).

5. Immobilization of *P. manganicum* ACM 3067 on GAC particles in flask

The adsorption of *P. manganicum* cells on GAC particles was studied in a flask prior to immobilization in the CRFB. A 10 g sample of GAC particles was added to 100 ml of cell suspension in the immobilization medium in a 250 ml flask and shaken at 200 rpm for 15 min. After cell adsorption, the GAC particles were separated from the medium by filtration. Dilutions of cell suspension were made before and after adsorption and triplicate drop plates on PSM agar were carried out to determine the number of unadsorbed cells. The agar plates were incubated at 28°C for 10 days. The following factors were studied for their effect on the immobilization of the *P. manganicum* cells :

5.1 Immobilization medium

The medium used for immobilization was suspending fluid (MacRae and Evans, 1983) and half strength PC medium with 1 ppm Mn (Tyler and Marshall, 1967).

5.2 Particle size of GAC

The size of GAC particles used was varied as 400-1,000 μm, 1,000-1,400 μm and >1,400 μm diameter.

5.3 Time course of the adsorption

Cell suspension and supporting material were mixed by shaking at 200 rpm for 40 min and the samples were taken at 1, 2, 6, 10, 20, 30 and 40 min to determine the number of cells adsorbed.

5.4 Time course of the desorption

A certain number of the immobilized cells on a 10 g of GAC was desorbed in 100 ml half strength PC medium with 1 ppm Mn, shaken at 200 rpm for 40 min. Samples were taken at 1, 2, 6, 10, 20, 30 and 40 min to determine the number of cells remaining adsorbed.

6. Removal of heavy metals by the CRFB using GAC as the supporting material

The CRFB using GAC as the supporting material was as described in the material section 6.2.

Bioreactor setup

A 1 L volume of 400-1,000 μm GAC particles, mixing vessel, bubble separator, flow gauge and tubing were steriled by autoclaving, whereas, the acrylic column was flushed with 30-50% H_2O_2. After sterilization, the system was aseptically assembled without adding GAC particles in the column. The CRFB was flushed again with 30-50% H_2O_2 for 1-2 h. The system was drained and flushed with sterile deionized water for several days to wash H_2O_2 from the system. After that, 1 L GAC particles was added into the column and the system was again flushed with steriled deionized water to remove fine GAC particles from the system. Then, the system was drained and the immobilization medium was continuously fed into the system for several days to saturate the particles with the medium until immobilization was carried out.

6.1 Immobilization of *P. manganicum* cells in the CRFB

The method of cell immobilization in the CRFB on GAC as the supporting material was the same as that previously described for cell immobilization on the magnetite in the CRFB.

6.2 Removal of 1 ppm Mn by the CRFB operated in batch and continuous mode after cell immobilization

To allow the culture to growth and to determine the steady state of the CRFB, the half strength PC medium containing 1 ppm Mn was filled into the system, after the unadsorbed cells were removed. The CRFB was operated in a batch mode for 4 days, then, operated in a continuous mode for 12 days. Samples were taken from the mixing vessel every 12 h in the morning and the afternoon to determine soluble and total Mn remaining in the medium.

6.3 Removal of heavy metals

The method was the same as that previously described for metals removal by the CRFB using magnetite as the supporting material except that the metal concentrations were analyzed using the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Appendix 3).

Chapter 3

Results and Discussions

1. Time course on growth of *Pedomicrobium manganicum* ACM 3067 in PSM medium

The growth of *P. manganicum* ACM 3067 was determined using absorbance reading at 540 nm (OD_{540}) and viable cell count. No lag phase was observed from the absorbance readings and the viable cell count due to the use of the starter (Fig. 6). The growth increased rapidly and reached the maximum after 4 days cultivation in both methods. The maximum cell count on the fourth day cultivation was 3.50×10^8 CFU/ml. After that, the growth decreased slowly and tended to be stable after 6 days cultivation indicated by the absorbance readings, whereas it sharply decreased as indicated by the viable cell count. Furthermore, the culture tended to clump and precipitate after 4 days cultivation. This phenomenon was previously observed by Sly *et al.*(1988) who indicated that the clumps of *P. manganicum* cells occurred by holding together of hyphae, manganese oxide and polysaccharides. Most observations of a wide variety of natural microorganisms showed that microorganisms usually grow as floc due to glycocalyxes consisting of acidic polysaccharides which bind cells together and are also capable of binding metals (Ghiorse, 1986). For the viable cell count, effect of cells clumping resulted in the fluctuation of the number of *P. manganicum* colony forming in each replication at a certain sampling times so that a wide range of the standard deviation (SD) was obtained after 3-4 days of cultivation. From the result, the 4 days old culture was chosen as a starter for the following experiments.

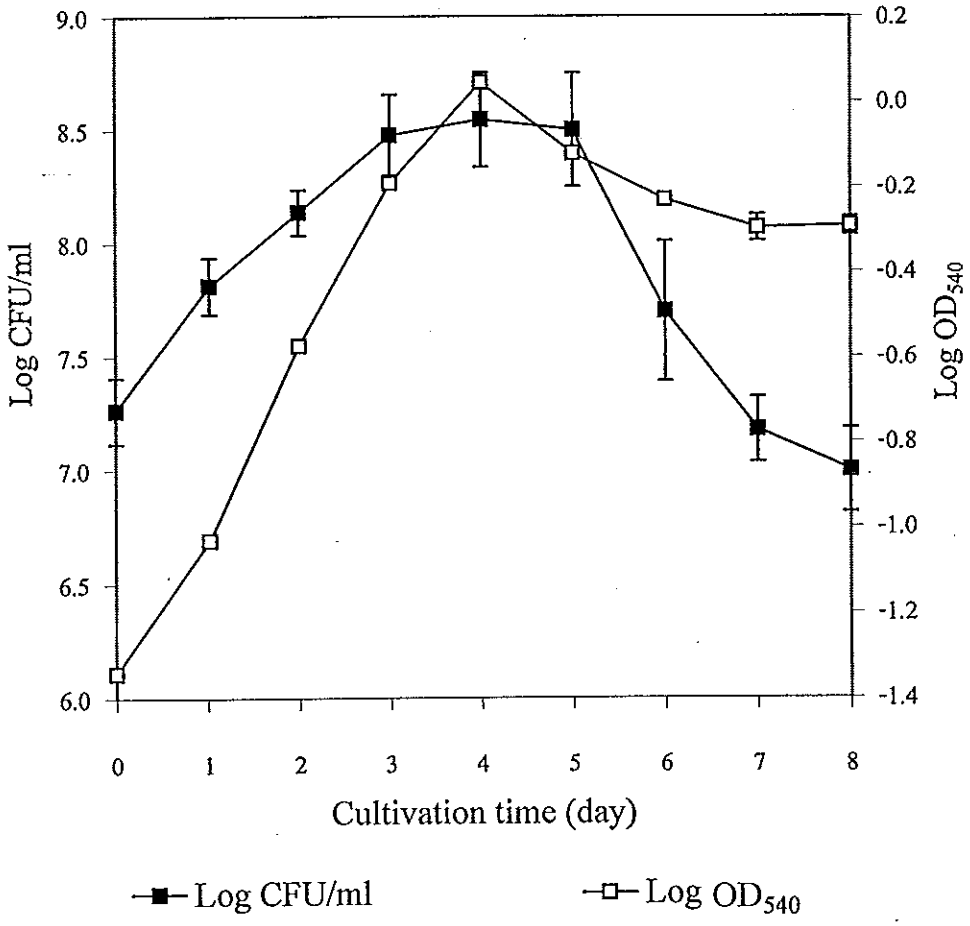


Fig. 6 Time course on growth of *Pedomicrobium manganicum* ACM 3067 cultivated in Pedomicrobium standard medium (PSM) shaken at 150 rpm at room temperature ($30 \pm 2^{\circ}C$) for 8 days

2. Effect of Mn concentration on growth and MnO_2 formation by *P. manganicum* ACM 3067 in PSM and PC media

2.1 Effect of Mn concentration on growth of *P. manganicum* ACM 3067 in PSM and PC media

In PSM medium without the addition of manganese, the culture grew rapidly and the maximum absorbance reading was reached after 5 days cultivation. The absorbance reading on the fourth day and fifth day however were very closed and slowly dropped thereafter (Fig. 7). In the medium containing 2, 6 and 12 ppm Mn, the growth was maximum after 4 days cultivation and the highest growth was obtained in the medium with 2 ppm Mn (OD_{540} = 1.112) followed by 6 ppm (OD_{540} = 1.089) and 12 ppm (OD_{540} = 0.923), respectively. Slow growing of the culture was found in the medium containing 20 and 50 ppm Mn, the growth of both treatments were maximum on the fifth day and decreased slowly thereafter. Thus, the highest growth was obtained in the presence of 2 ppm Mn followed by 0, 6, 12, 20 and 50 ppm Mn, respectively.

In PC medium, the culture growth was highest in the presence of 2 ppm Mn (Fig. 8). In the medium with 0, 2, 6 and 12 ppm Mn, the culture growth indicated by the absorbance reading was maximum in the first 24 h cultivation and slowly decreased thereafter. It seems unusual that maximum growth was achieved after one day in PC medium (with 0, 2, 6 and 12 ppm Mn) compared with five days in PSM medium but the result was obtained again when the experiment was repeated. The result was curious as the composition of PC medium is less complex than PSM medium. On the other hand, in the medium with 20 and 50 ppm Mn, the culture growth increased slowly and reached the maximum absorbance reading on the fourth (OD_{540} = 0.104) and fifth (OD_{540} = 0.110) day cultivation, respectively. Therefore, the highest growth was found in the medium containing 2 ppm Mn followed by 0, 6, 12, 50 and 20 ppm Mn,

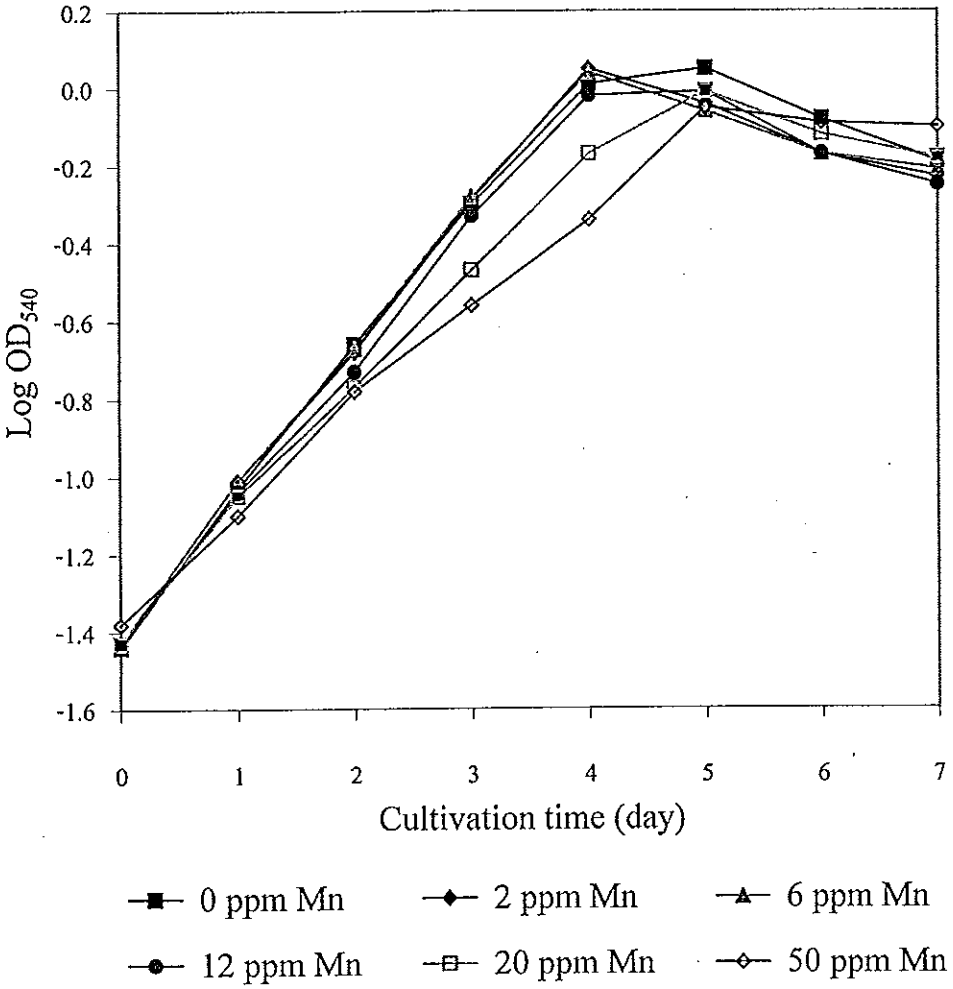


Fig. 7 Effect of Mn concentration on growth of *Pedomicrobium manganicum* ACM 3067 in PSM medium

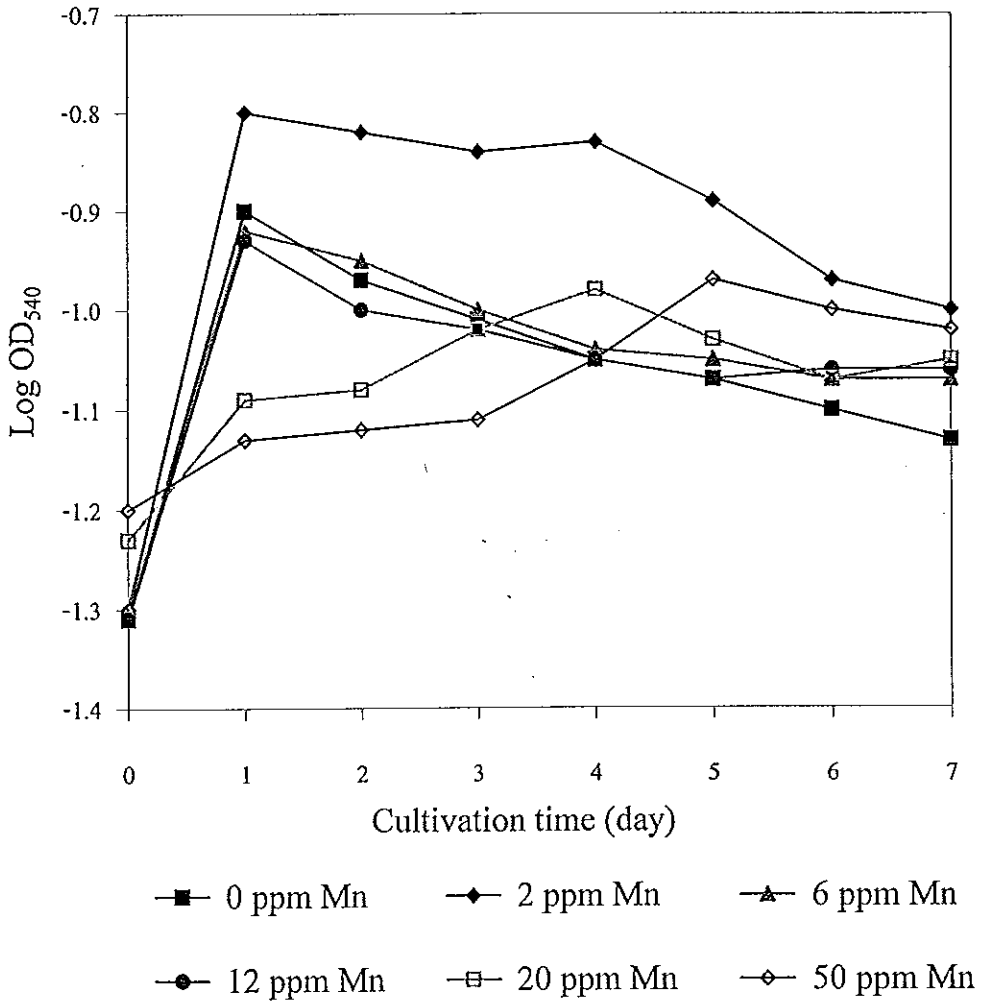


Fig. 8 Effect of Mn concentration on growth of *Pedomicrobium manganicum* ACM 3067 in PC medium

respectively. It should be noted that the culture growth in PC medium tended to be slower when the Mn concentration was higher.

From the result of the culture growth in both PSM and PC media, it was found that *P. manganicum* ACM 3067 preferred low concentration of Mn and the growth was highest at 2 ppm Mn. Growth stimulation by low Mn concentration was also reported by Ehrlich (1990) and Ali and Stokes (1971 cited by Ghiorse, 1984). High concentration of Mn, 20 and 50 ppm Mn, in PSM and PC media showed the inhibition effect on the culture growth. Numerous reports indicated that the elevated concentration of Mn inhibited the heterotrophic growth of Mn-depositing bacteria. For examples, Hajj and Makemson (1976 cited by Miyajima, 1992) found that approximately 18 ppm Mn depressed the heterotrophic growth rate and the cell yield of *Leptothrix discophora* strain 35R. Adam and Ghiorse (1985) reported that elevating the concentration of Mn from 3 to 50 ppm in PYG medium decreased the cell yield and growth rate of *L. discophora* strain ss-1. This inhibitory effect may result from the interference of Mn with physiological process requiring divalent cations such as Mg^{2+} for which Mn^{2+} can substitute. For example, Mn^{2+} may substitute for Mg^{2+} and decrease the fidelity of DNA replication. In addition, elevating Mn level probably exerted other non-specific toxic effects by binding to the functional groups of enzymes or otherwise interfering with vital metabolic reactions (Adam and Ghiorse, 1985). However, the extent of inhibition is dependent on the microorganisms, their origin and the culture medium (Green *et al.*, 1992).

The presence of 20 and 50 ppm of Mn in both PSM and PC media extended the time at which the maximum growth was reached, thereafter, the growth of the absorbance reading dropped slower than in medium without Mn added. This indicated that Mn can prolong viability of the culture. Similar result was found by Adam and Ghiorse (1985) who revealed that elevating the concentration of Mn from 3 to 50 ppm Mn resulted in the decrease of cell yield

and growth rate but the survival of the culture was prolonged. One possible explanation for enhanced survival of mineral-oxidizing heterotrophic bacteria is that the oxidation might provide energy to maintain cells during the stationary phase (Adam and Ghoirse, 1985). This possibility was stated by Ehrlich (1978) to explain the enhancement of stationary phase survival of a strain of *Alcaligenes faecalis* which could oxidized arsenite. Similarly, Kepkay and Nealson (1982) proposed that energy from Mn oxidation was responsible for the enhanced survival of a marine *Pseudomonas* in chemostat cultures growing at low dilution rate. Another possible explanation for Mn-enhanced survival was that Mn participates in the destruction of toxic O_2 and H_2O_2. Dubilina (1978 cited by Ehrlich, 1990) proposed that oxidation of Mn by *Leptothrix pseudochraceae*, *Arthrobacter siderocapsulatus* and *Metallogenium personatum* was catalase mediated to decrease H_2O_2 levels and, therefore, avoid toxicity during growth on glucose or organic substances.

The result also indicated that the growth of the culture in PSM medium was significantly higher than PC medium in all concentrations of Mn added. This was because PSM medium is the enrichment medium (Gebers, 1981) whereas PC medium is the Mn oxidizing medium (Tyler and Marshall, 1986) which containing low nutrients and low buffer capacity. Moreover, at high Mn concentrations in PSM medium, the more organic nutrients can act as Mn chelator, thus altering Mn availability in this medium (Larsen, 1995).

2.2 Effect of Mn concentration on MnO_2 formation by *P. manganicum* ACM 3067 in PSM and PC media

In PSM medium (Fig. 9), MnO_2 concentration slowly increased during the first 3 to 4 days cultivation in all treatments and rapidly increased thereafter, except in the medium with 2 ppm Mn in which its MnO_2 curve tended to decrease slowly after 4 days cultivation. The maximum MnO_2 content in PSM medium containing 2, 6, 12, 20 and 50 ppm Mn were 1.625, 2.265, 2.819, 4.135 and 5.102 ppm Mn (as MnO_2), respectively.

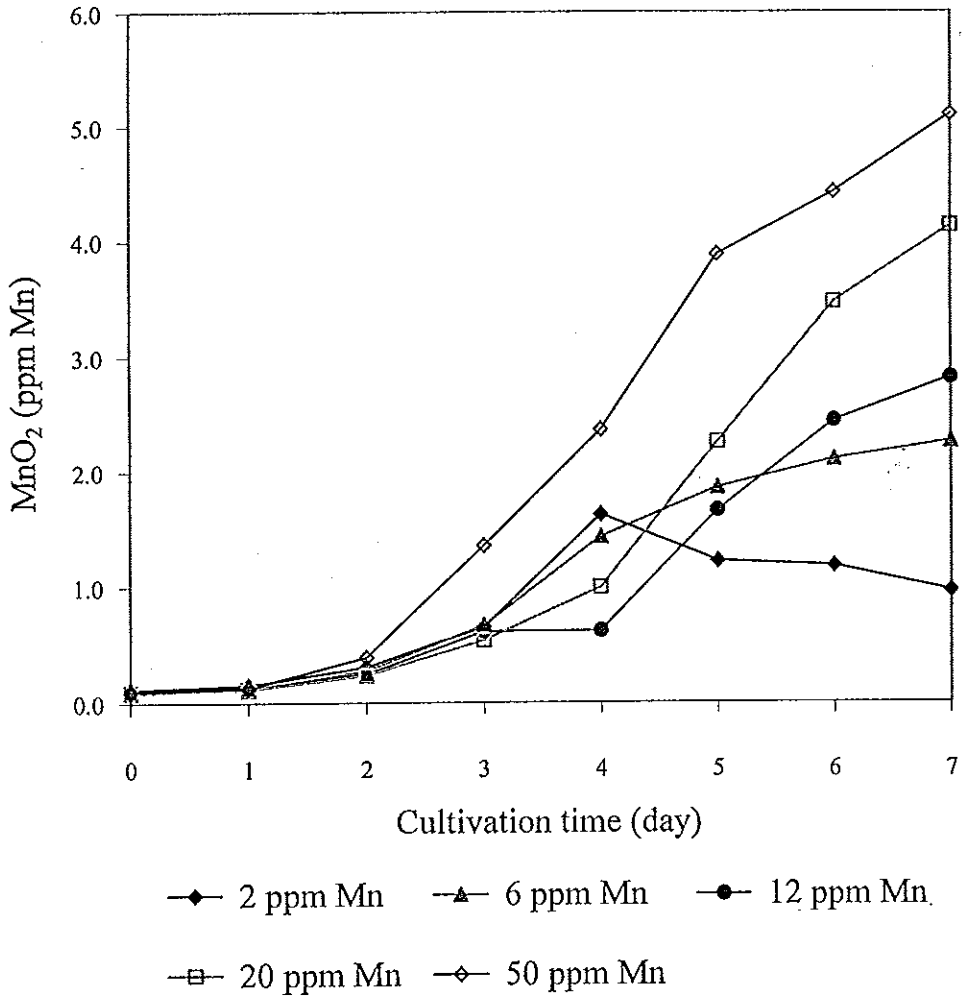


Fig. 9 Effect of Mn concentration on MnO_2 formation by *Pedomicrobium manganicum* ACM 3067 in PSM medium

Fig. 10 showed the effect of Mn concentration on the MnO_2 formation by *P. manganicum* in PC medium. It was found that MnO_2 produced by the cell in PC medium containing 2 ppm Mn occurred rapidly within 1-2 h after Mn was added and continuously increased until about 1.8 ppm MnO_2 was reached in the first day of cultivation and it was stable thereafter. In the medium containing 6 and 12 ppm Mn, MnO_2 content produced by the cell seemed to be stable during the first 5 days cultivation and slightly increase thereafter. The sharply increase of MnO_2 content was observed after the slow formation of MnO_2 in the first 5 days cultivation in the medium with 20 and 50 ppm Mn. The maximum MnO_2 content in PC medium containing 2, 6, 12, 20 and 50 ppm Mn were 1.889, 0.393, 0.393, 1.067 and 2.780 ppm Mn (as MnO_2), respectively. Although MnO_2 content in the 50 ppm Mn treatment was higher than that of the 2 ppm Mn treatment, the MnO_2 content in the 2 ppm Mn treatment was produced faster. It should be noted that in both PSM and PC media, only the 2 ppm Mn treatment can completely oxidize Mn^{2+} to MnO_2.

The result in both PSM and PC media revealed that MnO_2 produced by the culture in the presence of 2 ppm Mn were highest when the culture growth reached maximum. For the other Mn treatments in the PSM medium, the increase of MnO_2 content was observed when the culture reached the maximum growth and still keep increasing although the growth ceased. This could be also observed in the PC medium containing 20 and 50 ppm Mn. This indicated that the oxidizing factor proposed to be an enzyme produced by the cell was produced when the culture reached the logarithmic phase of growth and able to keep oxidizing after the growth ceased. Moreover, the results also indicated that the high concentration of Mn as 50 ppm was still not affected the inhibition of the oxidizing ability of the culture.

However, the high concentration of Mn and high pH during the culture growth could also promote the chemical Mn oxidation in this experiment. This was described by the work of Uren and Leeper (1978) which indicated that

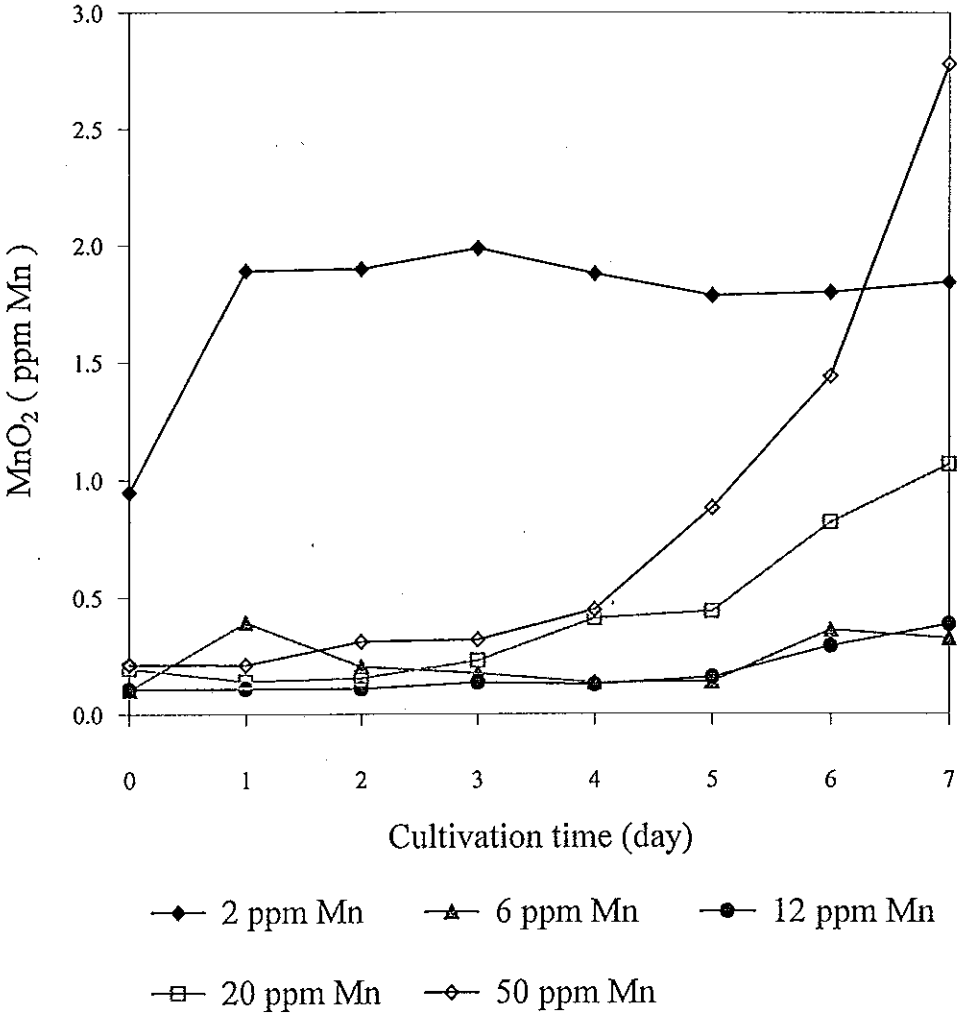


Fig. 10 Effect of Mn concentration on MnO_2 formation by *Pedomicrobium manganicum* ACM 3067 in PC medium

increasing of Mn concentration, O_2, Eh and OH^-(alkaline pH) favored the forward chemical oxidation of Mn. Morgan and Summ (1965 cited by Larsen, 1995) also reported that the minimum pH requirement for oxidation of Mn via inorganic process was approximately 8.6.

Comparison of the MnO_2 content formation in PSM and PC media, it was found that MnO_2 content produced in PSM medium was higher than that in PC medium in almost all treatments except for the 2 ppm Mn treatment in which the MnO_2 formation in PC medium was faster and higher than that in PSM medium. Many studies illustrated that as the available nutrient concentration increases, Mn oxidation is inhibited while growth is favored (Larsen,1995). The addition of yeast extract above 0.01% and below 0.005% inhibited Mn oxidation in cultures of soil Mn-oxidizers (Bromfield, 1965 cited by Larsen, 1995). Nealson (1978) reported that glucose and other metabolites e.g. acetate, succinate and glycerol strongly inhibited Mn oxidation but not growth (Man and Quastel, 1946 cited by Larsen, 1995). Therefore, the above pieces of evidence were contrast to the result in this experiment. The reason why MnO_2 content in PC medium was lower than that in the PSM medium (in which the nutrient composition is more complex than the PC medium) may involve the chemical Mn oxiation that might occur simultaneously with the biological Mn oxidation by the culture in the medium. When the culture grew in PSM medium, the pH of the medium changed to 9.3-9.5 (Fig. 11) so that the high pH may stimulate the chemical Mn oxidation in this medium.

2.3 Effect of Mn concentration on changes of pH during the cultivation of *P. manganicum* ACM 3067 in PSM and PC media

During the cultivation of *P. manganicum* ACM 3067 in PSM and PC media at various concentrations of Mn, the pH of all treatments in PSM and PC media increased from about 7.8 to 9.3-9.5 and to 8.3-8.6 at the end of cultivation, respectively (Fig. 11 and 12). In both PSM and PC media, the increasing of pH of each treatment was not significantly different. However, in

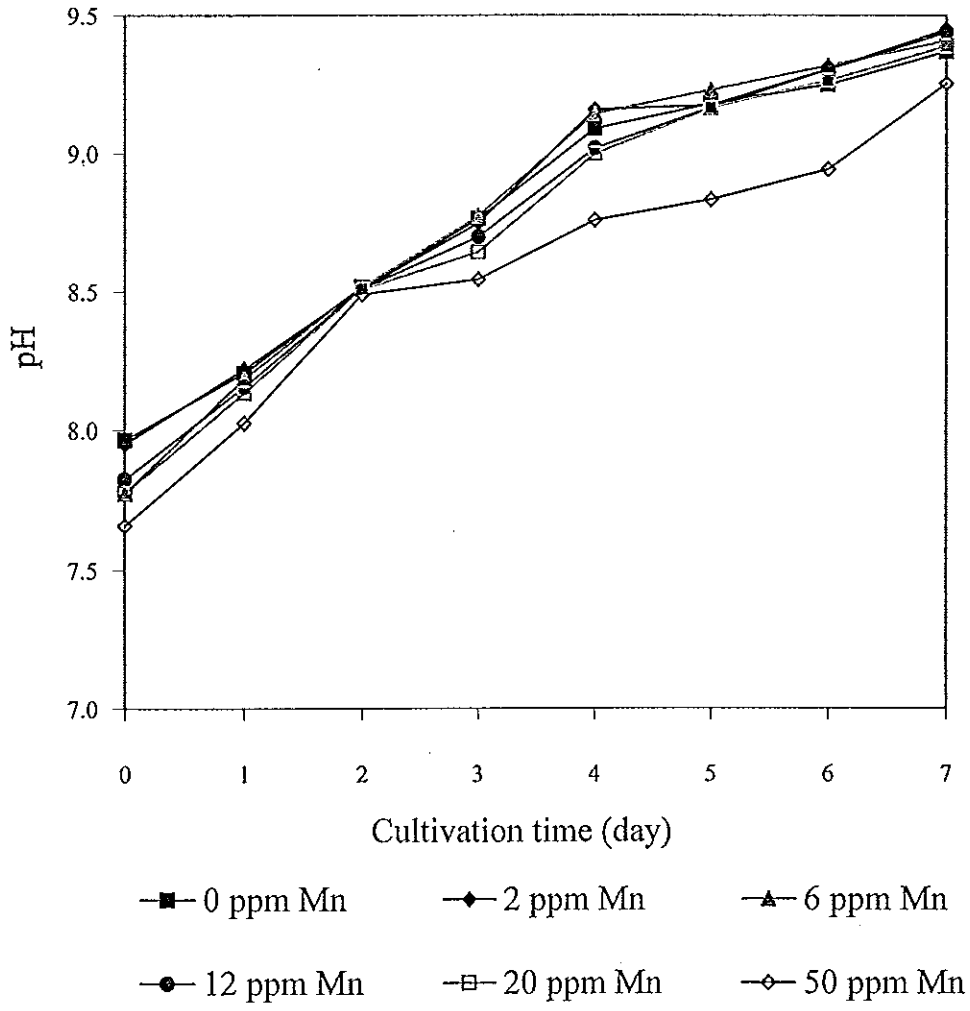


Fig. 11 Changes of pH during the cultivation of *Pedomicrobium manganicum* ACM 3067 in PSM medium with different concentrations of Mn

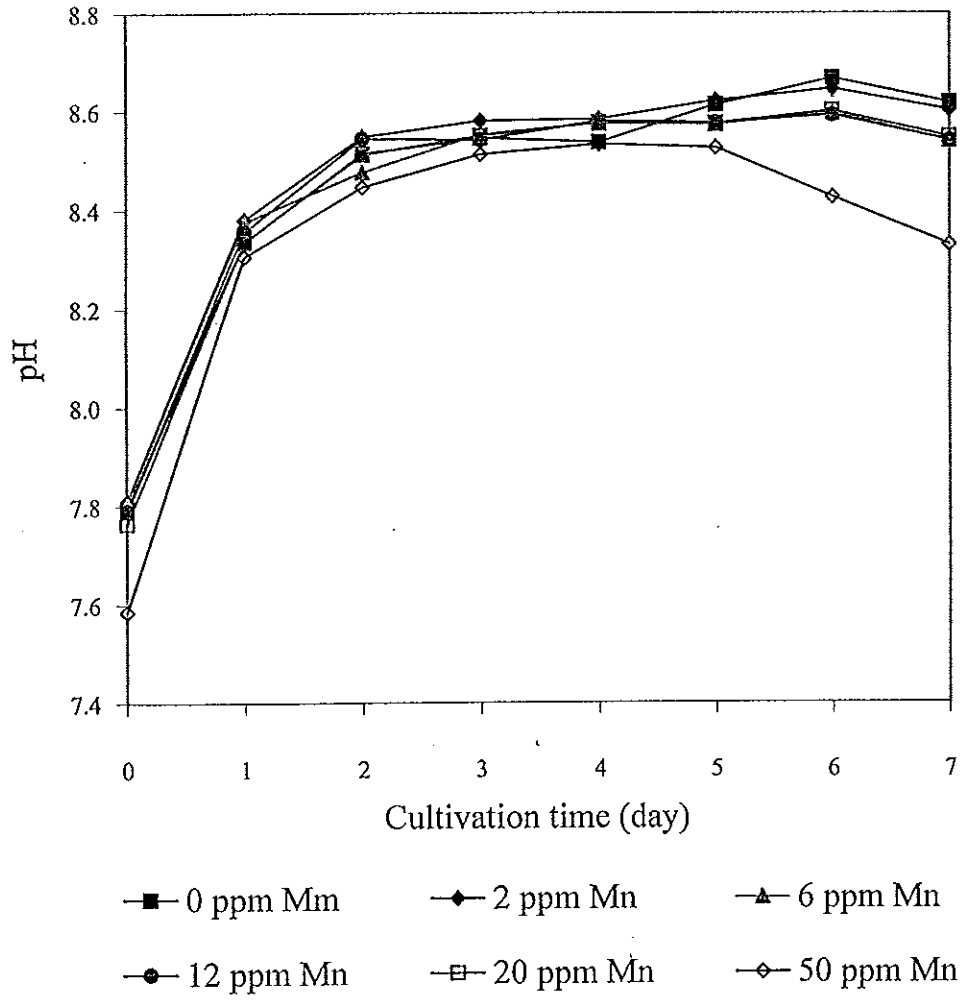


Fig. 12 Changes of pH during the cultivation of *Pedomicrobium manganicum* ACM 3067 in PC medium with different concentrations of Mn

the presence of high concentration of Mn such as 20 and 50 ppm, the pH values were slightly lower than those of low Mn concentrations (treatments; 2 and 6 ppm Mn). The increase of pH by *P. manganicum* was probably due to proton consumption in conjunction with acetate utilization by the cells or due to biodegradation, releasing OH^- (Larsen, 1995) or resulting from the decarboxylation of amino acids producing amines (Cox, 1995).

Although the pH of both PSM and PC media increased as the culture grew, the increased pH throughout this experiment was still in the pH range from 6 to 10 at which *P. manganicum* can grow (Sly *et al.*, 1988). Thus, there was no inhibitory effect of pH changes on the culture growth throughout this experiment. In addition, growth of the culture in PSM medium (Fig. 7) reached a maximum on the fourth and fifth day in which the pH of the medium was about 9 (Fig. 11). This was supported by the previous work of Sly *et al.*(1988), which indicated pH 9 as the optimum pH for growth of *P. manganicum*. The results of increase of pH as illustrated in Fig. 11 and Fig. 12 also indicated the growth of the culture and although the growth curves tended to decrease, the pH curves tended to stable in both PSM and PC media.

Since *P. manganicum* can grow in a pH range from 6 to 10, the proper pH for Mn oxidation by this culture should occur in this pH range as well as Mn oxidation by *P. manganicum* involves an oxidation factor proposed to be protein or enzyme produced by the cell (Larsen, 1995). However, the precise pH range for Mn oxidation of *P. manganicum* was not defined clearly. Studies in soil bacteria revealed that Mn oxidation occurred near neutral pH and the optimum pH for microbial oxidation is usually between pH of 6 to 8 (Uren and Leeper, 1978).

Furthermore, many researchers reported that alkali pH promotes the oxidation of Mn by chemical mechanism. Morgan and Stumm (1965 cited by Larsen, 1995) revealed that the minimum pH requirement for oxidation of Mn via inorganic process was approximately 8.6. Diem and Stumm (1984) also

demonstrated that autooxidation of Mn was favored when Eh, pH and Mn concentration were greater than 500 mV, 8 and 0.01 ppm, respectively. Therefore, this indicates that changes of pH to high pH in both PSM and PC media may result in the simultaneous formation of MnO_2 by both *P. manganicum* cells and the chemical mechanism. However, colloidal MnO_2 formed by chemical oxidation can bind with the ECP, and MnO_2 deposited on the ECP of *P. manganicum* cell as well as Mn^{2+} ion (Sly *et al.*, 1988).

3. The minimum inhibitory concentration (MIC) of Ni and Cd

The minimum inhibitory concentration (MIC) of Ni and Cd on the growth of *P. manganicum* ACM 3067 on PC agar plates and in PC medium (broth) with and without Mn was investigated.

The result (Table 8) indicated that the strain could tolerate higher concentration of Cd than Ni and combined Ni+Cd, respectively in both solid and liquid medium with and without Mn. According to Battistoni *el al.* (1993 cited by Sripoaraya, 1993), each metal is preferentially adsorbed on a different part of the cell, for examples, Cd and Ni were adsorbed on extracellular polymer and Ni was also adsorbed on cell wall. Additionally, Scott and Palmer (1990) revealed that Cd adsorption by different microorganisms involved different parts of cells such as *Klebsiella aerogenes* adsorbed Cd by capsular exopolysaccharides whereas *Arthrobacter viscosus* and *Pseudomonas putida* adsorbed Cd by intracellular accumulation in the cytoplasm. In addition, *Pseudomonas aeruginosa* removed Cd by precipitation on the cell wall (Wang *et al.*, 1997). Studies on the mechanism of Cd uptake by activated sludge revealed that metabolic uptake of Cd was lower than adsorption to the surface of the bacterial cells (Gourdon *et al.*, 1990b). For *P. manganicum*, the ECP produced by the cell acts as the effective adsorbent for heavy metals such as manganese (Sly *et al.*, 1993) and zinc (Sripaoraya, 1993). Therefore, the ECP which is the outermost layer around the cell may act as the adsorbent for Ni and

Table 8 The minimum inhibitory concentrations (MIC) of heavy metals for *Pedomicrobium manganicum* ACM 3067 grown on PC medium with and without manganese

Metals	Plate (ppm)		Broth (ppm)	
	PC	PC (Mn)	PC	PC (Mn)
Ni	0.501	0.996	0.996	2.039
Cd	3.053	4.089	3.053	5.051
Ni + Cd	0.501+0.502	0.501+0.502	0.903+0.904	0.903+0.904

Cd as well. This suggestion was supported by the works of Rudd *et al.*(1984) and Mittleman and Geesey (1985), which revealed that the ECP from *Klebsiella aerogenes* and a freshwater sediment bacterium can adsorb both Ni and Cd effectively, however, Ni adsorption seemed to be more preferential than Cd. Besides the ECP, the cell wall might possibly contribute to the adsorption of Ni and Cd by *P. manganicum* as well.

The result (Table 8) also showed that the tolerance of the strain in the presence of Mn on the toxic effect of Ni and Cd was generally higher than in the absence of Mn. However, its tolerance to Ni+Cd was not affected by the presence of manganese. According to the previous studies by Sripaoraya (1993), the MIC study in *P. manganicum* indicated that the presence of Mn also provided a protective effect against the toxicity of a second heavy metal cation in mixtures and the tolerance to the toxicity of heavy metals of the strain in the presence of Mn increased in the series: (Ag, Pb, Hg) < Cu < Ni < Co < Cr < Cd < Zn. This protective effect of Mn on the *P. manganicum* cell is because *P. manganicum* can oxidize Mn to MnO_2 deposited on the ECP produced by the cell and the MnO_2 which possess the negative charge at pH higher than 4 (pI) can adsorb additional metal cations by the charge transfer mechanism. McLean (1994) studied metal removal by *P. manganicum* immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) and also found that MnO_2 produced by *P. manganicum* functions as the effective adsorbent for Zn, Pb and Cu. Previous studies of Ni and Cd adsorption by MnO_2 revealed that the adsorption capacity of MnO_2 increased in the series; Ni < Co < Cd ≤ Zn < Mn (Gray and Malati, 1979). A similar result was found in the work by Balistrieri and Murray (1986), in which it was shown that an increase in the solid Mn content enhanced the ability of the Panama Basin sediments to bind certain metals e.g. Zn, Pb, Co, Cd and Ba while the binding ability for other metals; Cs, Bs, Sc, Sn, Ni and Fe was not significantly affected. Thus, this indicated that the MIC value of Ni and Cd in the presence of Mn for

P. manganicum ACM 3067 was highly affected by MnO_2 deposited on the ECP, but the higher MIC value improvement of Cd than that of Ni in the medium with Mn added might be due to the high adsorption affinity of Cd on the MnO_2 surface.

Studies on the MIC value of the combination of Ni and Cd in solid and liquid PC medium revealed that the MIC value depended on the higher toxicity of Ni more than Cd. Similar finding in *Lemna polyrhiza* L. was reported as the extracellular Cd binding was inhibited by the presence of Ni (Noraho and Gaur, 1995). An antagonistic interaction which involved the screening or competition for the binding sites on cellular surfaces resulted in the metal ions mutually alleviating their individual toxic effect was generally observed when the metals presented in combinations (Noraho and Gaur, 1995). In this experiment, the antagonistic effect was observed in both solid and liquid PC medium in the presence of Mn in which the MIC value of Ni+Cd was lower than the MIC of each Ni and Cd. The toxicity and uptake of combined species of heavy metal ions in microorganisms is interesting since normally natural waters and effluent always contain a mixture of metal ions.

4. Removal of heavy metals by the CRFB using magnetite as the supporting material

4.1 Immobilization of *P. manganicum* cells in the CRFB

The efficiency of the immobilization of *P. manganicum* ACM 3067 on magnetite (Fig. 13) (the immobilization was carried out at the Department of Microbiology, the University of Queensland) was assessed by the absorbance reading at 540 nm and the viable cell count. Unfortunately, the viable cell count data was not completely reliable but there was insufficient time to repeat the research. The clump nature of *P. manganicum* growth often causes irregular viable counts as the clumps break up. The absorbance data has therefore been used mainly to interpret the immobilization.

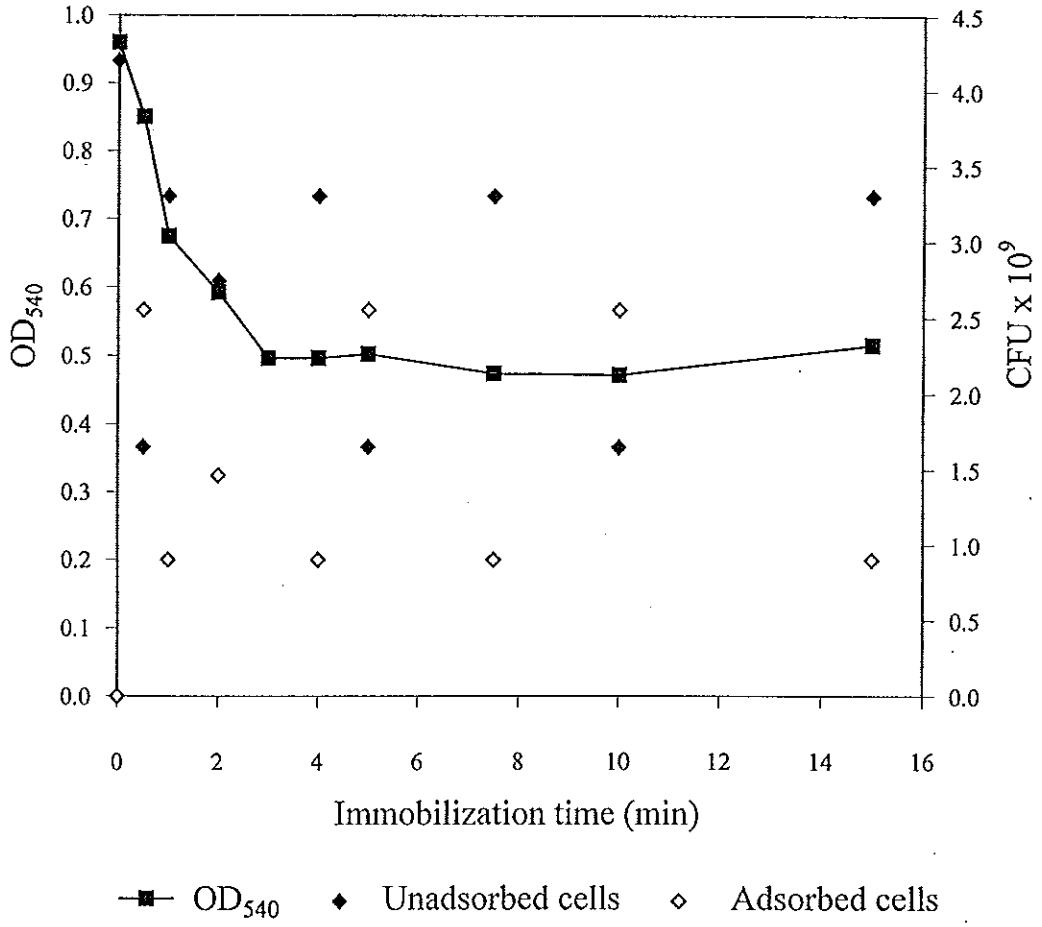


Fig.13 Immobilization of *Pedomicrobium manganicum* ACM 3067 on magnetite particles in the continuous recycle fluidized bioreactor (CRFB)

Immobilization of 4.20×10^9 CFU was completed within 3 and 5 min indicated by the absorbance readings and viable cell count with the maximum adsorption efficiency of 2.12×10^6 CFU/ml magnetite (60.7%). The number of cells immobilized in this experiment was quite lower than the expectation when compared to the previous work by Sly *et al.* (1993) in which the adsorption efficiency was 1.33×10^8 CFU/ml magnetite (73%). The reused magnetite may have caused the reduction of the surface charge of magnetite particle and resulted in the lower amount of immobilized *P. manganicum* cell in this experiment. However, in the work of Sly *et al.* (1993), an initial inoculum of 10^{11} cells was used and this probably accounts for the higher level of immobilization.

Many microorganisms capable of oxidizing Mn are well adapted to an attached mode of growth such as *Metallogenium*, *Leptothrix*, *Hyphomicrobium*, *Sidercapsa* and *Siderocystis*, and they have been exploited in biologically active sand filters for the removal of Mn from drinking water (Czekalla *et al.*, 1985). The cell surface components involved in adsorption of microorganisms onto solid surfaces are holdfast and extracellular fibrous material for adsorption of *Sphaerotilus natan* and *Hyphomicrobium* sp., respectively (Corpe, 1980). For *P. manganicum*, extracellular polymer is involved in its attachment, moreover, the attachment of the young mother cell is also part of the species lifecycle (Gebers, 1981). Besides this strain being well adapted to an attached mode, magnetite has also been shown to adsorb a wide range of bacteria effectively. Magnetite can adsorb bacteria which generally have a negatively charged cell surface above the isoelectric point (pI = 2.0-3.0), by its positive surface charge at pH values lower than its isoelectric point (pI = 8.5 for treated magnetite). The efficiency of bacterial removal by the magnetite was commonly found to be in excess of 99% for a contact time of 10 min (MacRae and Evans, 1983).

To determine the immobilized cells on the magnetite particles, samples were taken from the top and the middle of the fluidized magnetite bed in the column of the CRFB. These samples were examined using SEM (Scanning Electron Microscopy) (Fig. 14 and 15). The figures showed that larger numbers of the *P. manganicum* cells were found on the surface of the top magnetite particles than that of the middle one. The low density of the magnetite particles in the expanded bed (top of the column) resulted in the low shear forces between each particle of magnetite, so allowed well growing of the cells on its surface. The middle sample from the CRFB showed that *P. manganicum* cells were predominantly found in crevices and indentations on the magnetite particle. This may be due to these areas of the particle offering the cells protection from the shear forces of the recirculating aqueous media and collision of the particles (McLean, 1994).

4.2 Removal of heavy metals

4.2.1 Ni and Cd removal when adding each metal individually

When 0-5.468 ppm Ni was added to the medium, the soluble and total Ni concentrations in the effluent were ≤ 0.098 ppm and ≤ 0.135 ppm, and the efficiency of soluble and total Ni removal were about 98% and 95%, respectively (Fig. 16 and 17). These Ni concentrations (0-5.468 ppm) had no effect on the pH of the medium in the CRFB (pH 8.0). Likewise, Ni concentration ≤ 5.468 ppm had also no effect on the soluble and total Mn removal which were stable at about 89% and 69%, respectively. The increase of pH to 8.32 was found when 7.328 ppm Ni was added in the influent medium. This may indicate a toxic effect on the *P. manganicum* cells since normally the pH of the CRFB was controlled by the culture growth at about 7.8-8.0 and moreover, the adsorption of metal cations to the adsorption site (ECP and MnO_2) affected the decrease of pH because of the releasing of H^+ from the adsorption site (Murray, 1975; Geesey and Jang, 1990). Higher pH in the CRFB might result in the increase of the autooxidation of Mn in the medium and

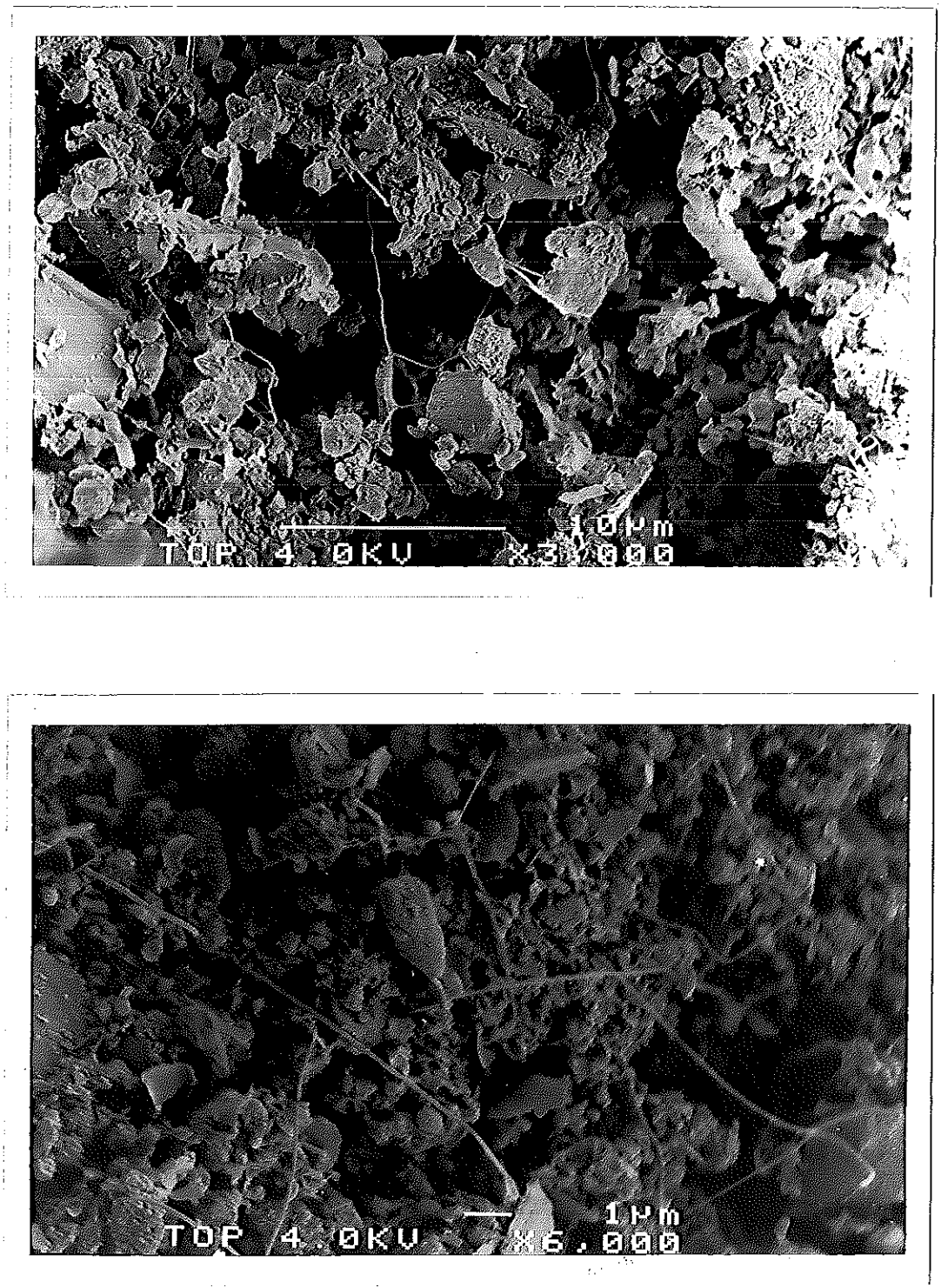


Fig. 14 SEM micrographs of *Pedomicrobium manganicum* cells immobilized on magnetite particles taken from the top of the fluidized magnetite bed in the continuous recycle fluidized bioreactor (CRFB)

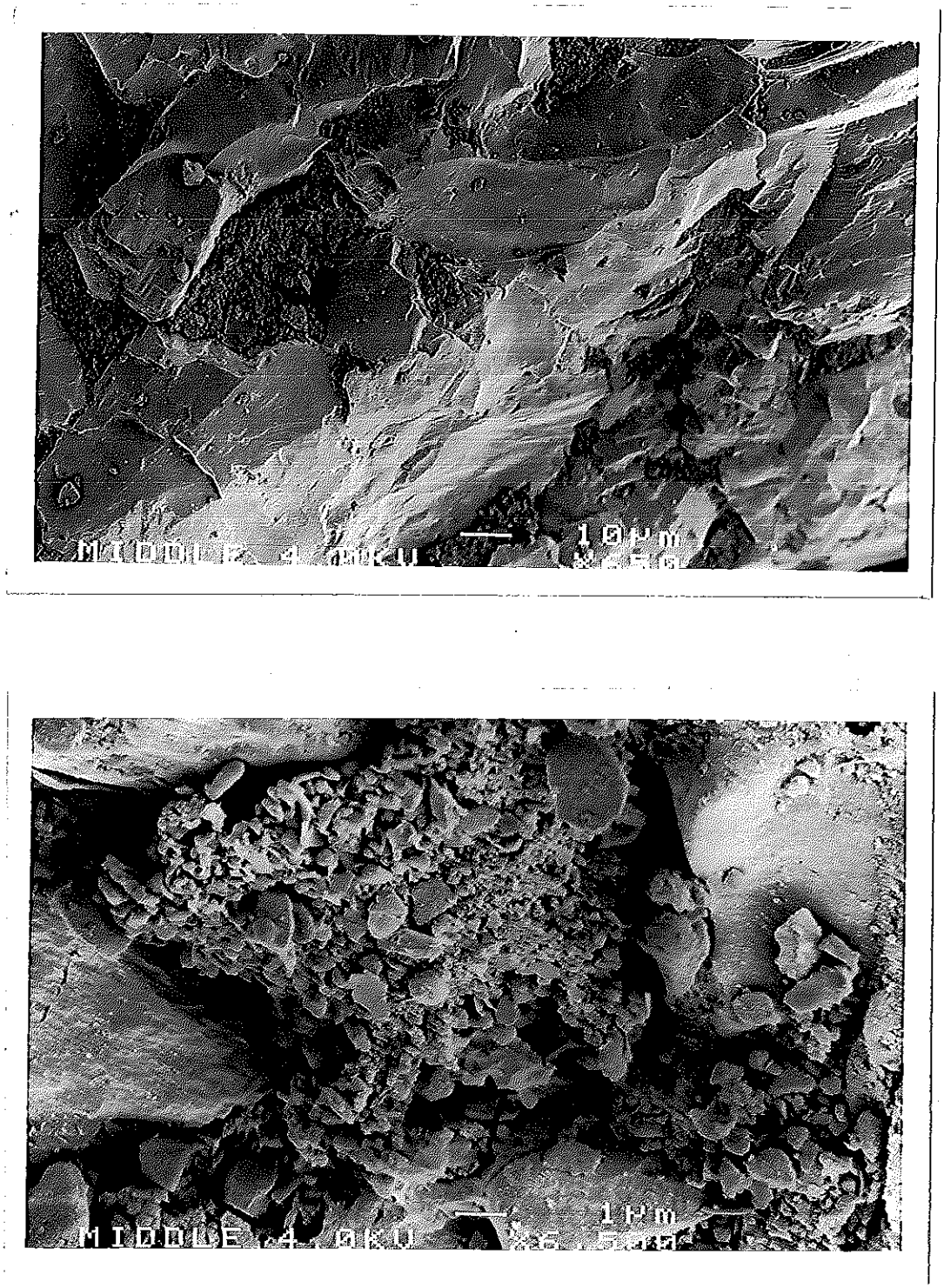


Fig. 15 SEM micrographs of *Pedomicrobium manganicum* cells immobilized on magnetite particles taken from the middle of the fluidized magnetite bed in the continuous recycle fluidized bioreactor (CRFB)

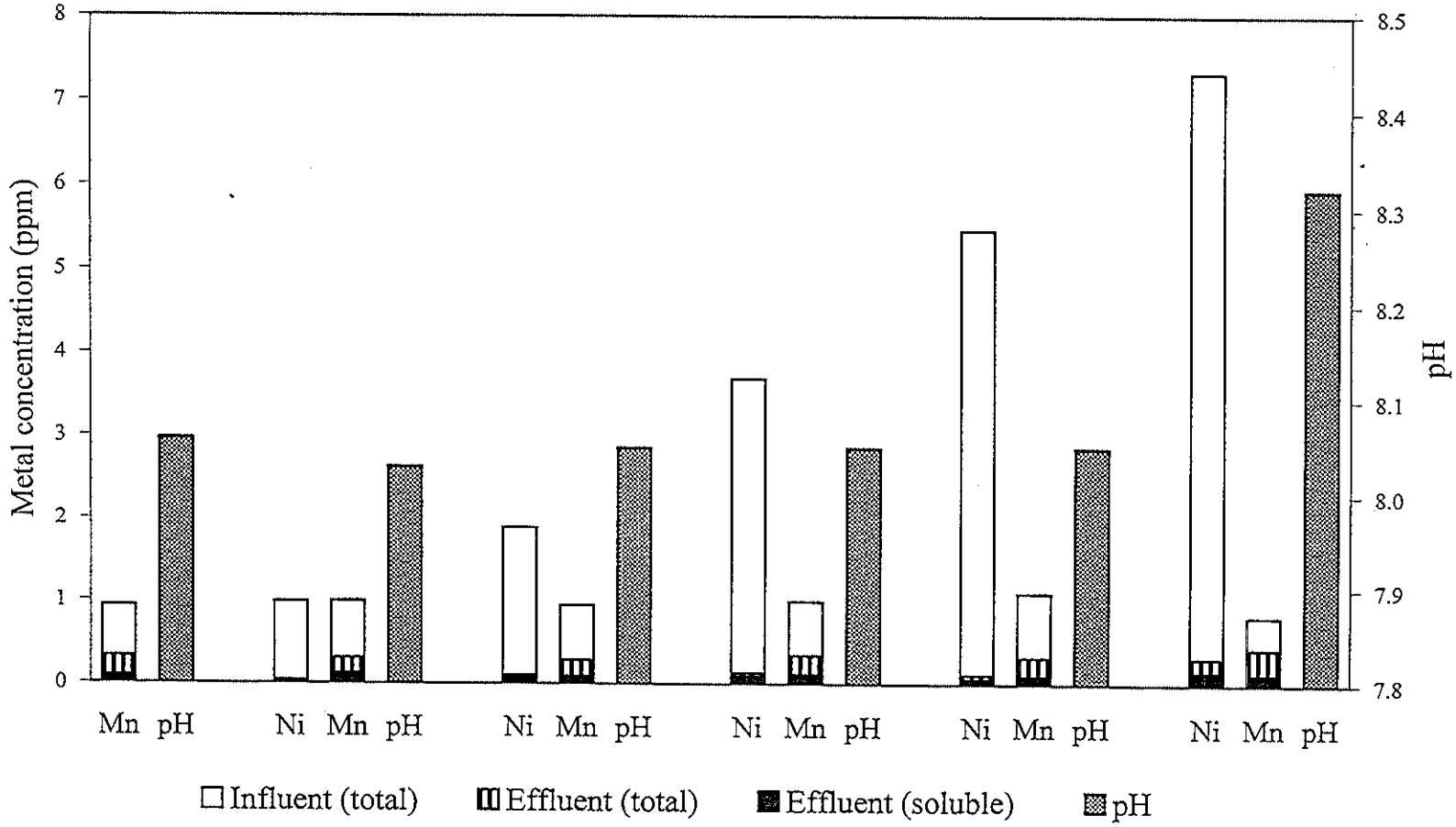


Fig. 16 Removal of Ni and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB)

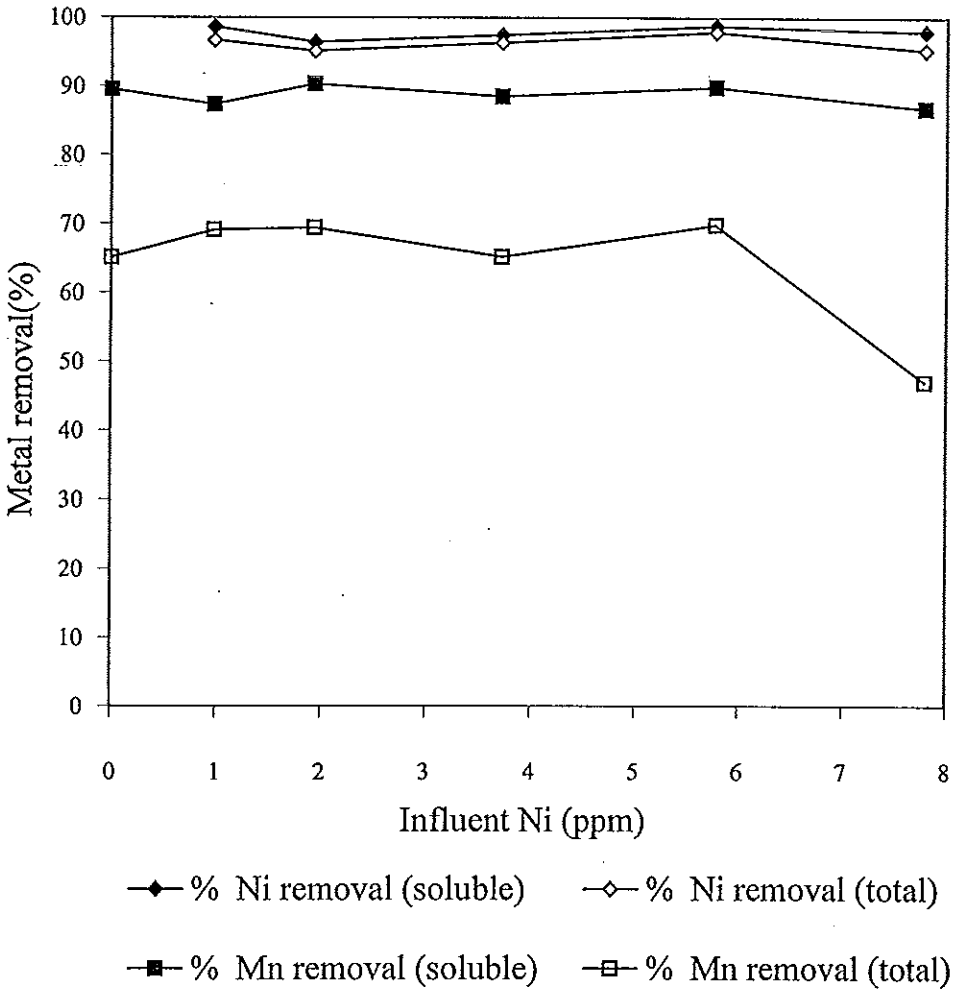


Fig. 17 The percentage of Ni and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Ni concentration

consequently, the higher amount of insoluble Mn in the effluent (0.433 ppm total Mn) and the dropping of the efficiency of total Mn removal to 47% were observed. However, the soluble and total Ni removal efficiency remained high as 98% and 95.29%, respectively.

For Cd removal (Fig. 18 and 19), it was found that as the concentration of Cd in the influent increased in steps from 0.927 ppm to 8.050 ppm, the soluble and total Cd concentrations in the effluent increased slowly from 0.001 ppm to 0.099 ppm and from 0.013 ppm to 0.256 ppm, respectively. The pH of the CRFB decreased from 8.33 to 7.87 as the influent Cd concentration increased in steps from 0 ppm to 8.050 ppm. The reason for this pH drop might be caused by the release of protons as metal ions are adsorbed to the surface of hydrous MnO_2 deposited or the ECP (Murray, 1975; Geesey and Jang, 1990). Alternatively, toxic effects of Cd may have inhibited metabolic activity which leads to the lower pH values. The concentration of soluble Mn in the effluent rose from 0.171 ppm before Cd was added to 0.319 ppm when 8.050 ppm Cd was added to the influent. The concentration of total Mn in the effluent, however, fluctuated throughout this experiment and was apparently unrelated to the concentrations of the influent Cd. The cause of the fluctuating level of insoluble Mn is not clearly known. However, when Cd was again removed from the influent, the concentration of soluble and total Mn in the effluent dropped to 0.219 ppm and 0.334 ppm, respectively. Therefore, the reason for the decrease of Mn adsorption as the influent Cd concentration increased may be due to competition for adsorption site (McLean, 1994).

P. manganicum can adsorb heavy metals by various parts of the cell such as MnO_2, ECP, cell wall, etc. However, with respect to the outermost layer of the cell surface which is the region the cell first make contact with its environment, the MnO_2 and ECP should be the two important binding factors for the strain. Adsorption of Ni and Cd by MnO_2 have been studied by several researchers. Gray and Malati (1979) revealed that the adsorption capacity of

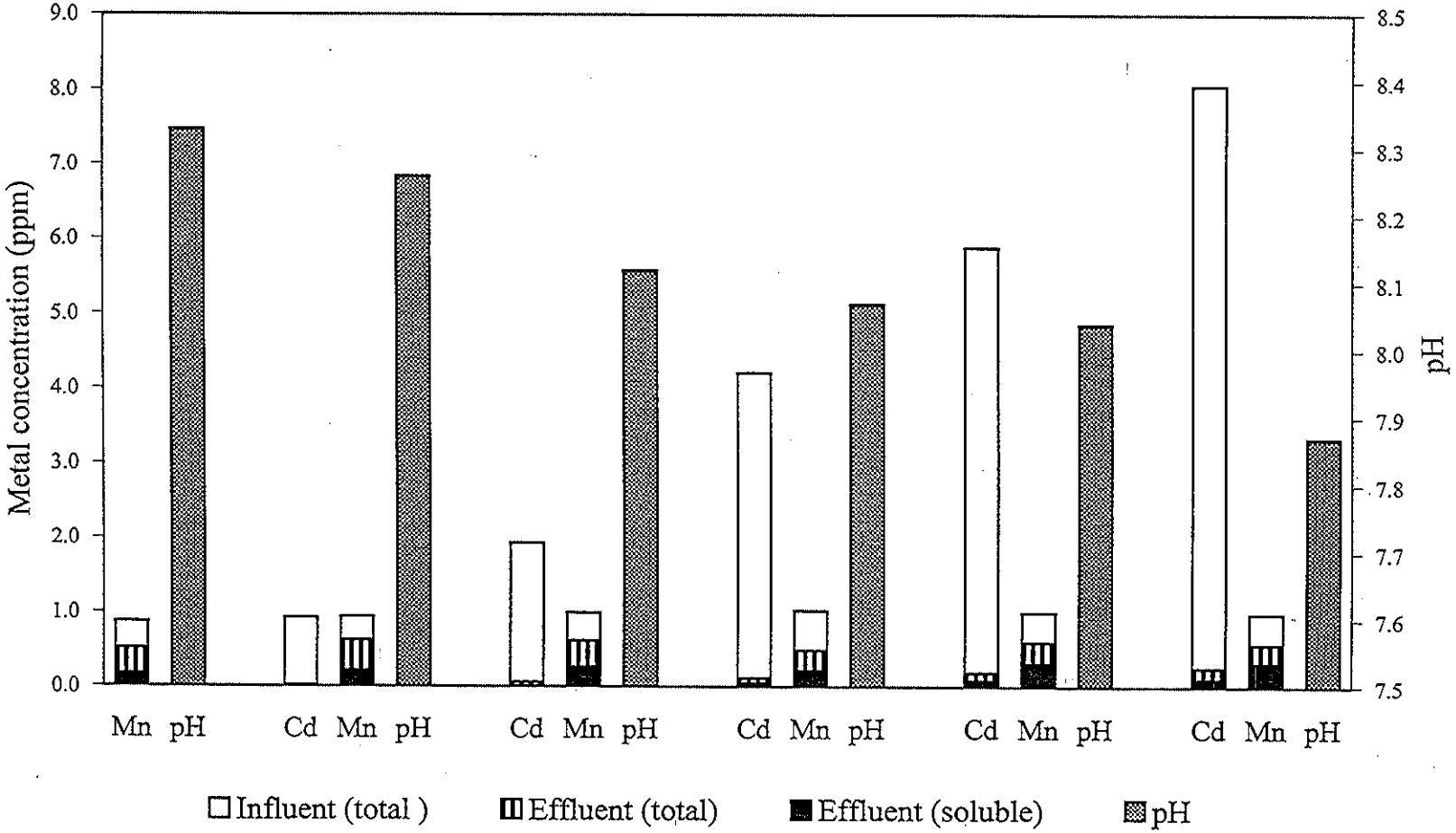


Fig. 18 Removal of Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB)

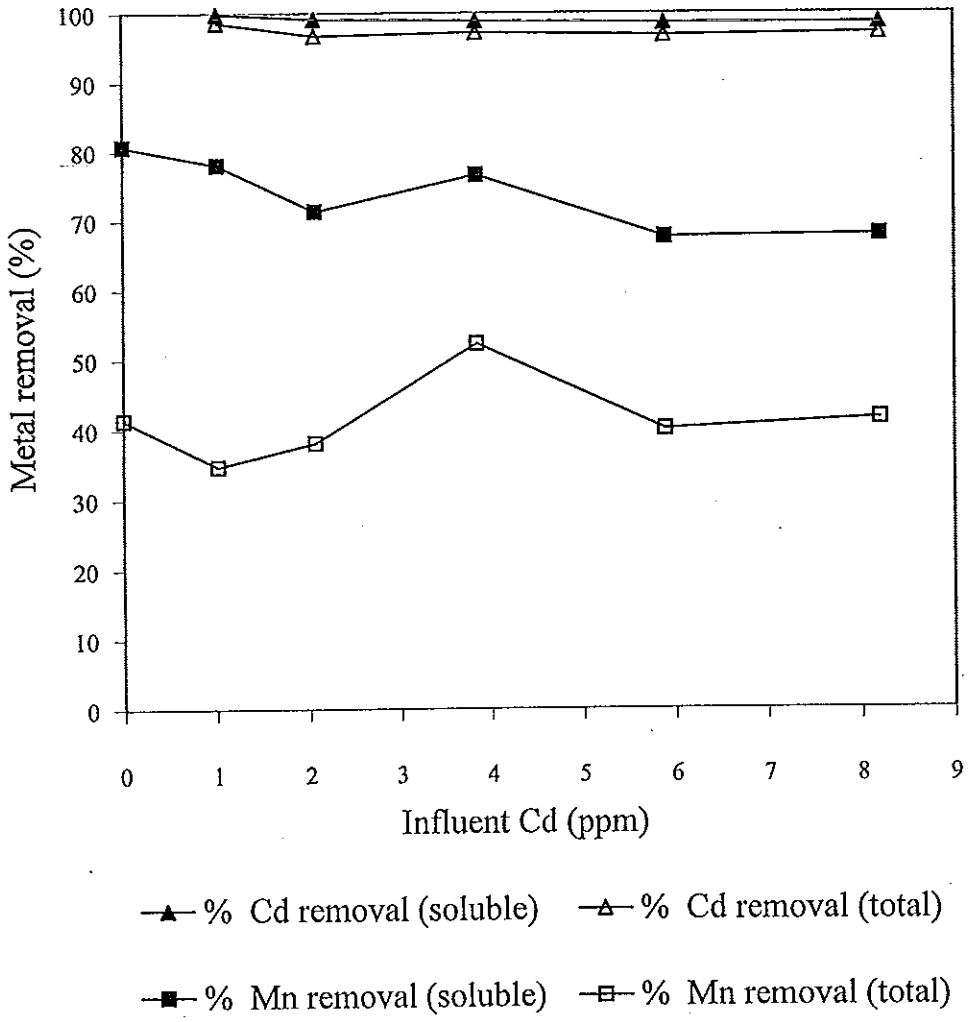


Fig. 19 The percentage of Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Cd concentration

MnO_2 increased in series: Ni < Co < Cd ≤ Zn < Mn. A similar finding was reported by Balistrieri and Murray (1986), which indicated that an increase in the solid Mn content enhanced the ability of the Panama Basin sediments to bind certain metals e.g. Zn, Pb, Cu, Co, Cd and Ba while the other metals e.g.Cs, Sn, Sc, Be, Fe and Ni was not as strongly affected by the Mn content. In addition, the adsorption of metal ions onto MnO_2 accompanied by a release of protons which results in the pH drift. For metal removal by the ECP, several studies have implicated carboxyl group as the main metal binding sites to form metal organic complexes (McLean and Beveridge, 1990). Geesey and Jang (1990) described the general interaction between metal ions and organic molecules as an acid-base reaction involving the interaction of a metal ion (M^{n+}), a proton (H^+) and organic ligand (L) as $M^{n+} + LH \rightarrow M^{n+}\text{-}L + H^+$. Thus, decreasing of pH when the ECP was exposed to various metals were likely due to in part of the liberation of protons from the carboxylate metal-binding sites (McLean *et al.*, 1990). For Ni and Cd removal by the ECP, Ni was bound to greatest extent than other metals and a binding series of the capsular anionic polysaccharides from *Klebsiella aerogenes* polymer was as following: Ni > Cu > Cd > Co > Mn > Ti (Rudd *et al.*, 1984). The capsular exopolymer isolated from a freshwater sediment bacterium can adsorb both Ni and Cd, however, Cd was adsorbed less than Ni (Mittelman and Geesey, 1985). In this current experiment, Ni and Cd were adsorbed by both MnO_2 and the ECP. According to all of the above pieces of evidence, MnO_2 seemed to more preferentially adsorb Cd than Ni, whereas, the ECP seemed to more preferentially adsorb Ni than Cd. The result of Ni and Cd removal when adding each metal individually also showed that the removal of Cd was higher than Ni when comparing at the same level of Ni and Cd in the influent medium. Thus, this reveals that MnO_2 mainly involved with Ni and Cd removal by *P. manganicum* immobilized in the CRFB. Similarly, *P. manganicum* ACM 3067 cells grown in Mn free medium did not adsorb Cu, Pb and Zn and it appeared, therefore, that these heavy metals

are adsorbed onto MnO_2 deposits in the ECP rather than directly on the ECP produced by the cell (McLean, 1994). In addition, it seemed to be possible that this deposition of MnO_2 may cause the reduction of the surface area of the ECP and consequently, result in the reduction of the area for metal adsorption. Moreover, hydrous MnO_2 was reported to have an enormous surface area of around 300 m^2/g. This indicates the effective binding ability of MnO_2 as well.

The result also showed that when the concentration of Cd in the influent medium increased in steps from 0 to about 8 ppm, the Mn concentration in the effluent increased slightly and when Cd was not added in the influent medium the concentration of Mn in the effluent decreased again. This may indicate that there was competition between Cd and Mn for the adsorption site. In addition, it was also possible due to the release of Mn ion that Cd was adsorbed onto the surface of MnO_2. The release of Mn ion when Zn was adsorbed onto MnO_2 surface was also demonstrated by Gray (1981). In contrast, adsorption of Ni seemed to be independent from Mn adsorption since when Ni concentration in the influent increased the Mn concentration in the effluent was not obviously changed.

4.2.2 Ni and Cd removal when adding metals in combination

As the concentration of metals in the influent increased in steps from 0 ppm of Ni and Cd to 3.336 ppm Ni and 4.732 ppm Cd, the concentrations of soluble and total Mn in the effluent increased noticeably, especially for the total Mn in the effluent (Fig. 20 and 21). The pH of the CRFB decreased from 7.90 (at 0.877 ppm Ni and 0.912 ppm Cd) to 7.85 (at 3.336 ppm Ni and 4.732 ppm Cd). The reason for this pH drop may be due to the release of protons as metal ions are adsorbed to the adsorption sites proposed to be MnO_2 (from the section 4.2.1). According to the result of individual metal removal, it can indicate that this pH drop possibly due to Cd adsorption more than Ni.

When the concentration of the influent Ni and Cd increased in steps from 0.877 ppm and 0.912 ppm to 3.336 ppm and 4.732 ppm, respectively, the

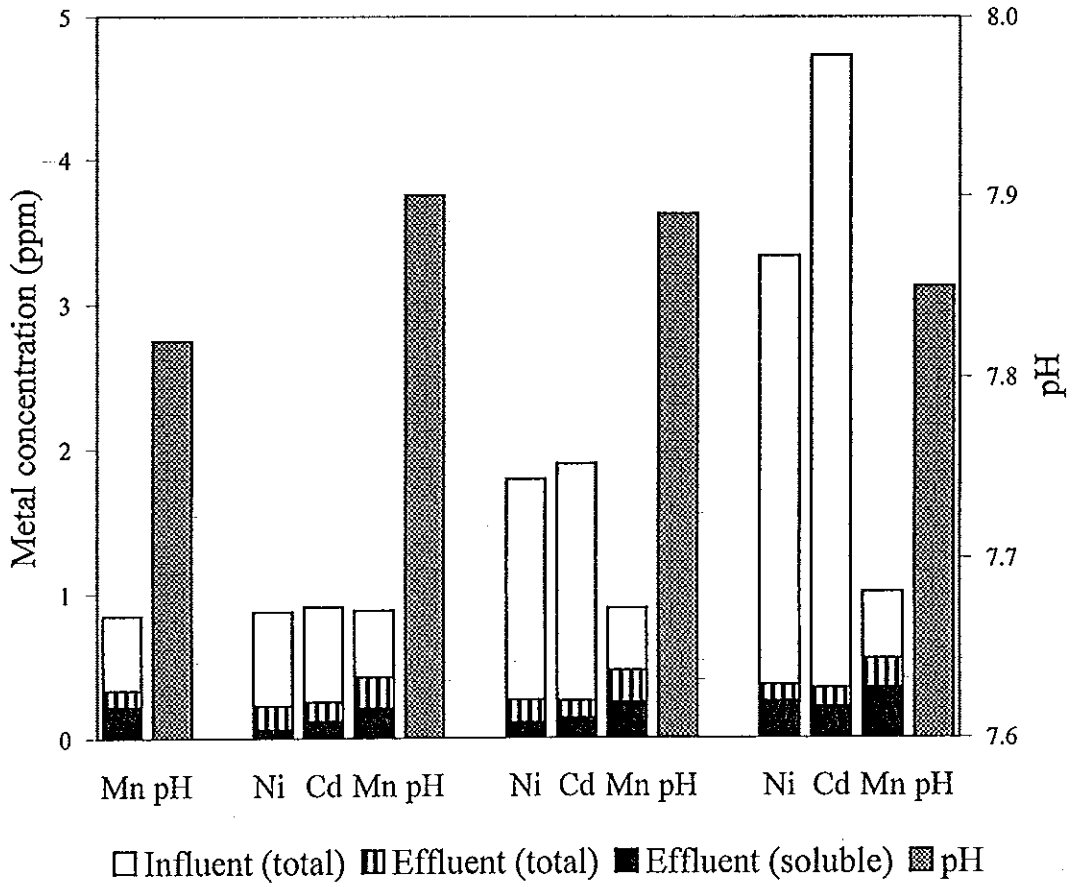


Fig. 20 Removal of Ni, Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB)

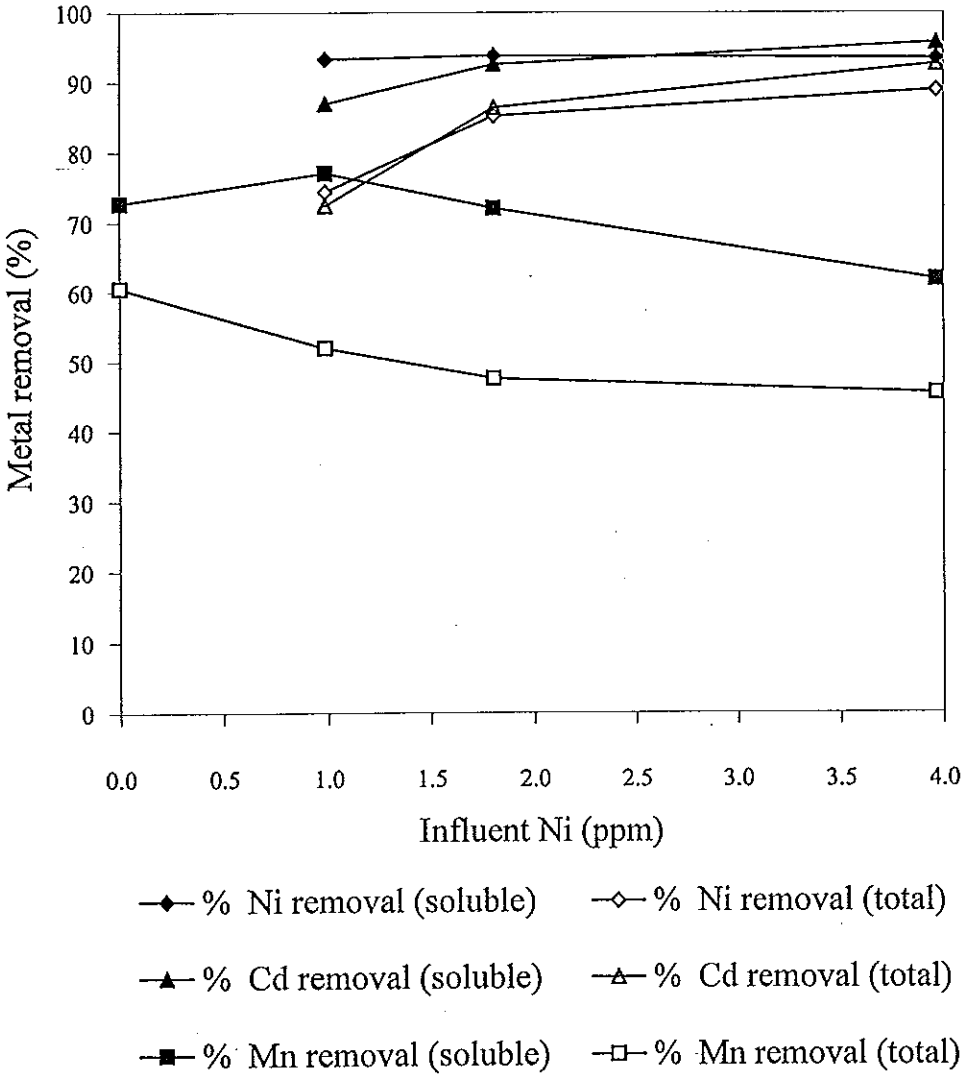


Fig. 21 The percentage of Ni, Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Ni concentration

concentration of soluble and total Ni in the effluent increased slightly from 0.060 ppm and 0.226 ppm to 0.256 ppm and 0.369 ppm, respectively. In the case of Cd, its soluble and total concentration in the effluent increased slightly from 0.119 ppm and 0.252 ppm to 0.219 ppm and 0.347 ppm, respectively. With respect to the efficiency of metals removal at 3.336 ppm Ni and 4.732 ppm Cd in the influent medium, the removal efficiency of soluble and total Cd (95% and 92%) was higher than Ni (93% and 88%). This indicates that Cd has the higher affinity for the adsorption sites which proposed to be MnO_2 and this was supported by previous research by Gray and Malati (1979), and Balistrieri and Murray (1986).

The decreasing of Mn removal when the concentration of Ni and Cd in the influent increased is proposed to involve the competitive adsorption for binding sites and the release of Mn ion from MnO_2 surface when metals are adsorbed. Comparison of each metal removal when metal was added individually and in combination showed that at the same level of each metal in the influent medium, the efficiency of these metals (Mn, Ni, Cd) removal when metals were added in combination was lower than when individually added. The decrease of each metal removal when metals were added in combination is apparently due to the competition for the adsorption sites for each metal which tend to be higher than when metal was added individually because the total metal concentration is double in the mixture.

5. Immobilization of *P. manganicum* ACM 3067 on GAC particles in flask

The possibility of using GAC as the supporting material for immobilization of *P. manganicum* was investigated. The surface of activated carbon was reported by many researchers to be excellent for colonization of microorganisms. Its adsorptive properties serve to enrich substrate and oxygen concentrations, the craggy surface provides recesses that are sheltered from fluid shear forces and its high surface area, its rough surface texture and the

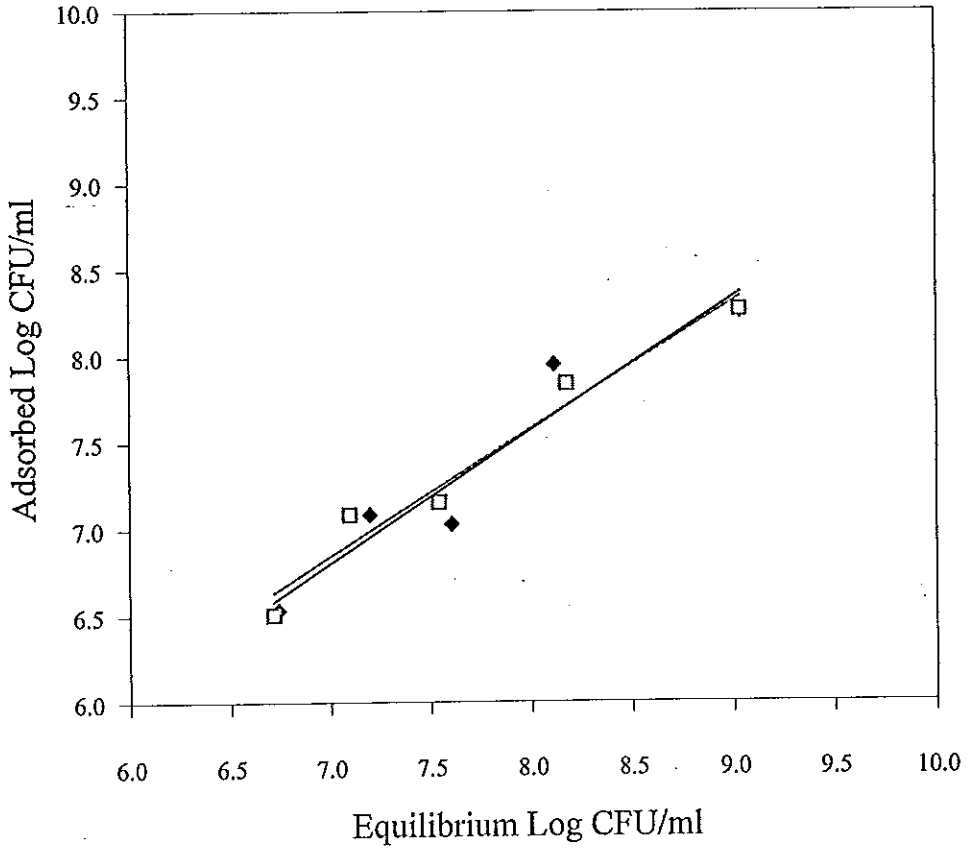
variety of functional groups on the surface can enhance attachment of microorganisms (Weber Jr. *et al.*, 1978). In addition, GAC could be used in both fixed bed and fluidized bed reactors and the GAC particles were also found to promote the metabolic activity of the immobilized microorganism (Grishin and Tuovinen, 1989). The activated carbon especially from coconut shell, is cheap and available in Thailand.

The adsorption of the strain to the GAC particles was studied in flasks prior to immobilization in the CRFB to obtain the best immobilization medium and particle size of GAC. In addition, time course of the adsorption and desorption of the strain were also studied.

5.1 Immobilization medium

Result (Fig. 22) showed that the isotherm curves of both suspending fluid and half strength PC medium with 1 ppm Mn were not significantly different. However, the correlation coefficient of half strength PC medium with 1 ppm Mn was higher than the other. MacRae and Evans (1983) have indicated that the adsorption of bacteria to magnetite was enhanced by using suspending fluid which is a microelectrophoresis medium, as the immobilization medium. However, in this experiment the suspending fluid did not clearly enhance the adsorption of the strain to the GAC particles. This may be due to a very low surface charge (ionic force) of the GAC compared to the magnetite particles and consequently, resulting in the low enhancement of cell adsorption as well. With respect to the desorption, the changes of component medium after immobilization may alter the surface bond condition of the cell or supporting material (Hassler, 1974) and thus, this may also affect the desorption process.

Therefore, the half strength PC medium with 1 ppm Mn was chosen as the immobilization medium for immobilization in the CRFB. Using nutrient medium as the immobilization medium for adsorption of mixed bacteria to the GAC particles was previously reported by Rao (1985).



◆ Suspending fluid (y = 0.7674x + 1.4324 ; R = 0.9135)

□ Half strength PC with 1 ppm Mn (y = 0.7352x + 1.3981 ; R = 0.961)

Fig. 22 Isotherms for the adsorption of *Pedomicrobium manganicum* ACM 3067 to 10 g granular activated carbon (1,000-1,800 µm) using suspending fluid and half strength PC medium with 1 ppm Mn as the immobilization medium

5.2 Particle size of GAC

The result in Fig. 23 showed that loading of the cells on both the smallest size (400-1,000 μM) and the medium size (1,000-1,400 μM) of GAC was not significantly different whereas the largest size of > 1,400 μM showed the lowest cell loading throughout this experiment. However, comparison between the 400-1,000 μM GAC and 1,000-1,400 μM GAC, it was found that at higher cell concentrations, the 400-1,000 μM GAC adsorbed the higher amount of cells than the other and the correlation coefficient of this size was higher than that of the 1,000-1,400 μM GAC. A similar result was reported by Shimp and Pfaender (1982) who revealed that bacterial growth on GAC was reduced by increasing the carbon particle size. Decreasing grain size of GAC resulted in the increase of the surface area per volume of GAC and so, exerted a small positive effect on the bacterial colonization (Shimp and Pfaender, 1982). Li and Digiano (1980 cited by Shimp and Pfaender, 1982) hypothesized that decreasing particle diameter decreases the diffusional pathlength of substrates from the internal pores of the carbon to the biofilm and hence increases the availability of adsorbed substrate to attached cells. Thus, the smallest size of 400-1,000 μM GAC was chosen to use for the immobilization in the CRFB.

5.3 Time course of the adsorption

The adsorption of cells on GAC particles was found to occur rapidly and the maximum adsorbed cells (8.17×10^7 CFU/ml) (≅ 32%) was obtained after 2 min of the adsorption process and the adsorbed cells slightly decreased thereafter (Fig. 24). The rapid adsorption of the cells to GAC was mainly involved with the physical adsorption by a weak Van der Waals force (Hassler,1974). However, the charge transfer complexes were proposed to occur simultaneously on the GAC surface (Yonge *et al.*, 1985). Hence, the decreasing of adsorbed cells throughout this experiment might be caused by the weak bond (reversible adsorption) between the cell surface and the GAC surface which is easily broken by the particle attrition during the shaking

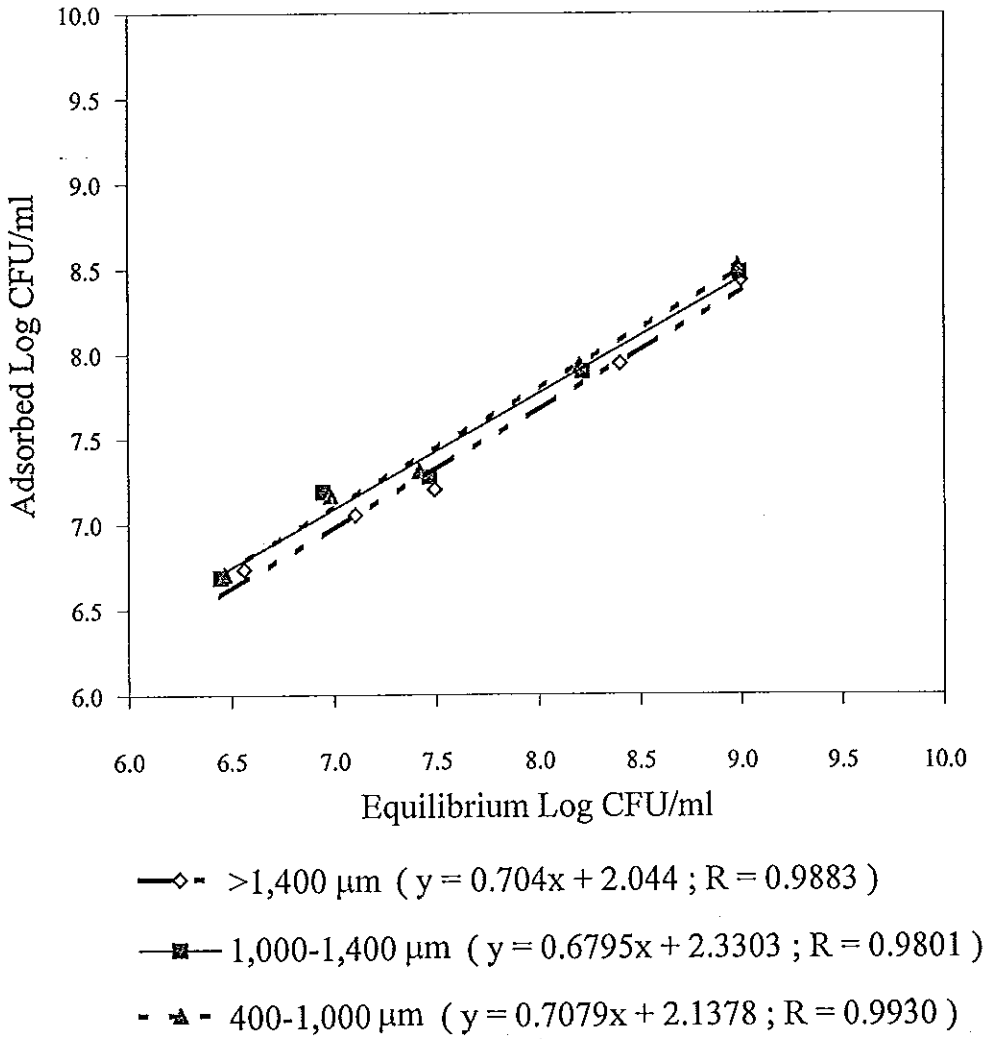


Fig. 23 Isotherms for the adsorption of *Pedomicrobium manganicum* ACM 3067 to 10 g granular activated carbon of various sizes in a half strength PC medium with 1 ppm Mn

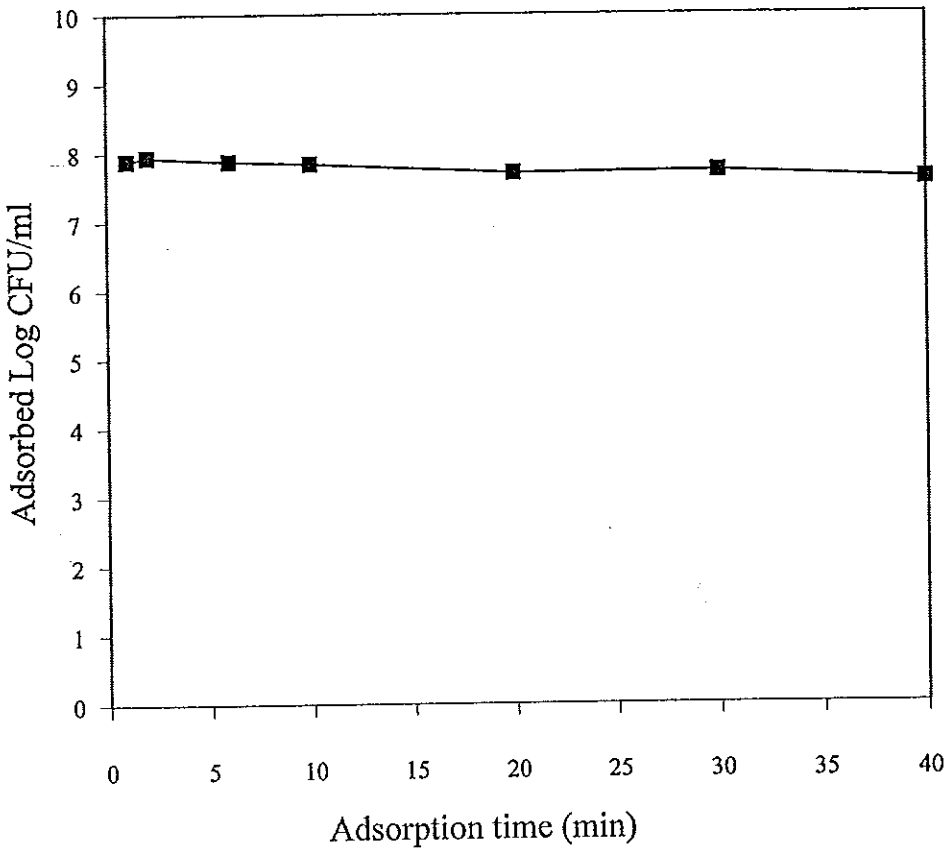


Fig. 24 Time course on the adsorption of *Pedomicrobium manganicum* ACM 3067 to 10 g granular activated carbon (400-1,000 μm) in a half strength PC medium with 1 ppm Mn

Remark : The inoculum concentration was 2.585×10^8 CFU/ml

period. In addition, the other possible cause of the reduced adsorption was the nature of the *P. manganicum* growth which occurs as a network of hyphae and budding cells (Sly *et al.*,1990). Thus, taking more time on shaking may increase the degree of cell clumps breaking and result in higher cell counts or reducing the adsorption efficiency (Sly *et al.*, 1990).

5.4 Time course of the desorption

Result in Fig. 25 revealed that the desorption occurred rapidly as in the previous experiment of the adsorption. The efficiency of the desorption was approximately 33% and 34% after 1 and 2 min of the desorption period, respectively, and tended to be stable thereafter. This indicated that the loss of cells was more related to the weak bond between the cell surface and the particles surface than the detachment of the cells by particle attrition. Comparison with the result of the desorption experiment by Sly *et al.* (1990), it was found that the desorption efficiency of this strain from the GAC was higher than that from the magnetite which is approximately 26%. This indicated that the physical attachment of the cell on the surface of GAC particle (Rao, 1985) which proposed to be the Van der Waals force (Hassler, 1974) was weaker than the charge transfer complexes on the magnetite particle (MacRae and Evans, 1983).

6. Removal of heavy metals by the CRFB using GAC as the supporting material

6.1 Immobilization of *P. manganicum* cells in the CRFB

Result (Fig. 26) showed that the cell immobilization in the CRFB reached the equilibrium within 10 and 20 min as indicated by viable cell count and the absorbance reading at 540 nm. After 60 min of the immobilization period, the adsorption efficiency was 50.2% which was lower than the adsorption efficiency (about 73%) in the experiment with magnetite particles by Sly *et al.* (1993). However, the concentration of the supporting material in

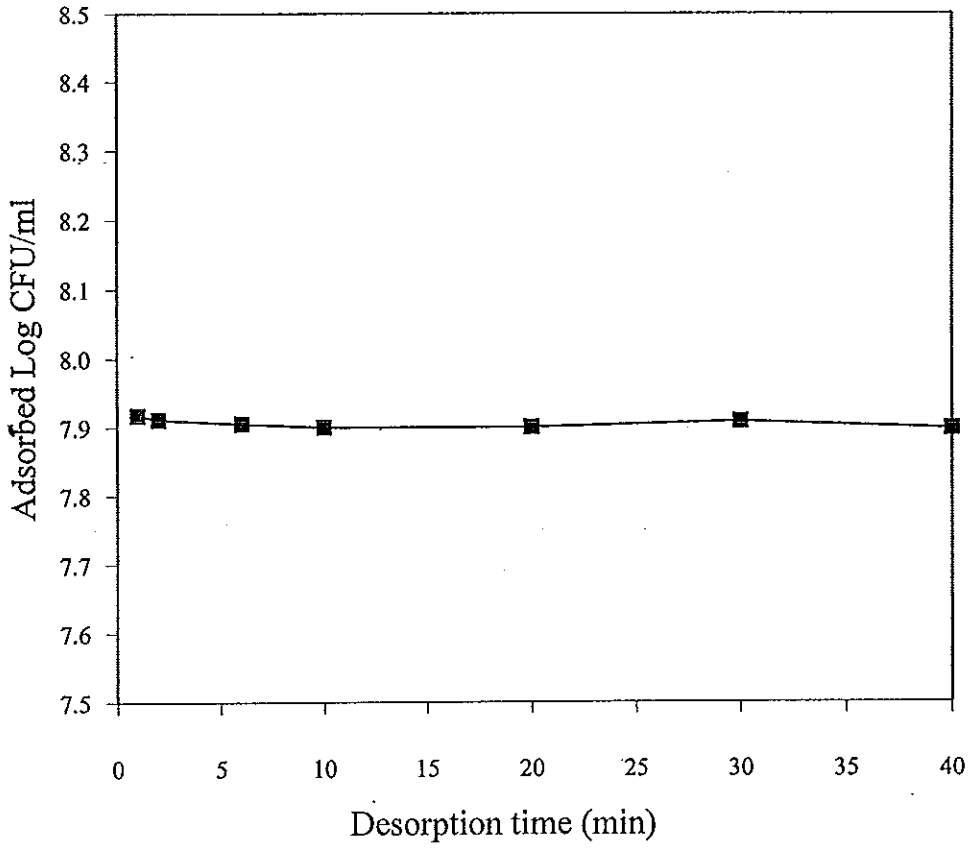


Fig. 25 Time course on the desorption of *Pedomicrobium manganicum* ACM 3067 from 10 g granular activated carbon (400-1,000 μm) in a half strength PC medium with 1 ppm Mn

Remark : The original adsorbed cells were 1.233×10^8 CFU/ml

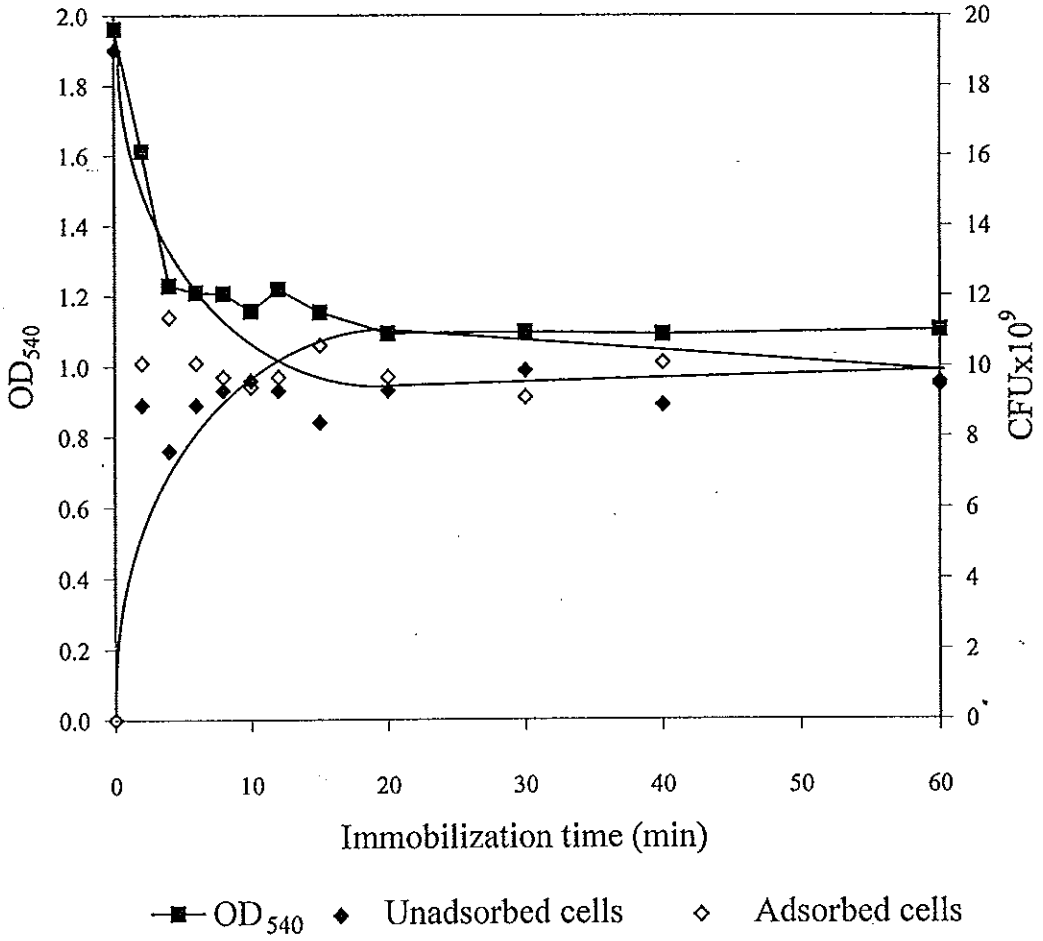


Fig. 26 Immobilization of *Pedomicrobium manganicum* ACM 3067 on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB)

this experiment (31%) was lower than the previous work (36%) by Sly *et al.*(1993). This indicated a low number of the adsorption sites and the weak adsorptive bonding of GAC surface. In addition, the immobilization of the strain to magnetite took a shorter time (5 min) to complete the adsorption than to GAC particle.

The adsorbed cells per gram GAC in the CRFB was 2.03×10^{7} CFU (Table 9) which was lower than the immobilization value of 2.52×10^{9} cells mixed bacterial culture per gram GAC reported by Rao (1985). The reduction of cell loading in this experiment may be caused by using a high recirculation flow rate (0.6-0.8 L/min) to obtain the expanded bed of 50%. Table 9 also showed that the number of immobilized cells in the CRFB was lower than the predicted value in the flask. This is because of the difference in the immobilization condition in flask and in the CRFB such as mixing method, speed of shaker or flow rate in the CRFB, the actual volume of GAC and the occurrence of particle attrition and shear force.

After 10 days of bioreactor startup, the GAC particles from the top and the middle of the column were sampled to determine the attached *P. manganicum* cells using SEM (Fig. 27 and 28). These figures showed that in both the top and middle GAC particles the growing cells were mainly observed in the crevices, craggy areas and in the macro pores since these areas can protect the cells from shear force (between flowing medium and particle) and particle attrition (between each particle). It was also found that the larger number of the growing cells were found in the particles from the top of the column than that of the middle one as well as in the section 4.1. This is because at the top of the column, the density of GAC particles was lower than that in the middle and the bottom of the column and this resulted in the low shear force and particle attrition so, allowed better growth of the immobilized culture. However, comparison the cell growth on magnetite and GAC particles from the middle of the column (Fig. 14 and 15), it was found that the cell growth on

Table 9 Comparison on the immobilization of *Pedomicrobium manganicum* ACM 3067 on granular activated carbon particles in shaken flasks and in the continuous recycle fluidized bioreactor (CRFB)

	Shaken flask	CRFB
GAC concentration	31%	31%
Inoculum (CFU)	1.54×10^{9}	1.90×10^{10}
Adsorbed cells (CFU)	6.15×10^{8}	9.54×10^{9}
% Adsorbed cells	39.9	50.2
Adsorbed cells/ml GAC	1.98×10^{7}	9.54×10^{6}
Adsorbed cells/g GAC	4.21×10^{7}	2.03×10^{7}
Adsorbed cells/m^2 GAC	3.66×10^{4}	1.76×10^{4}

Remark : 20 ml GAC = 9.42 g dry weight

1 g GAC has a surface area of 1,150 m^2

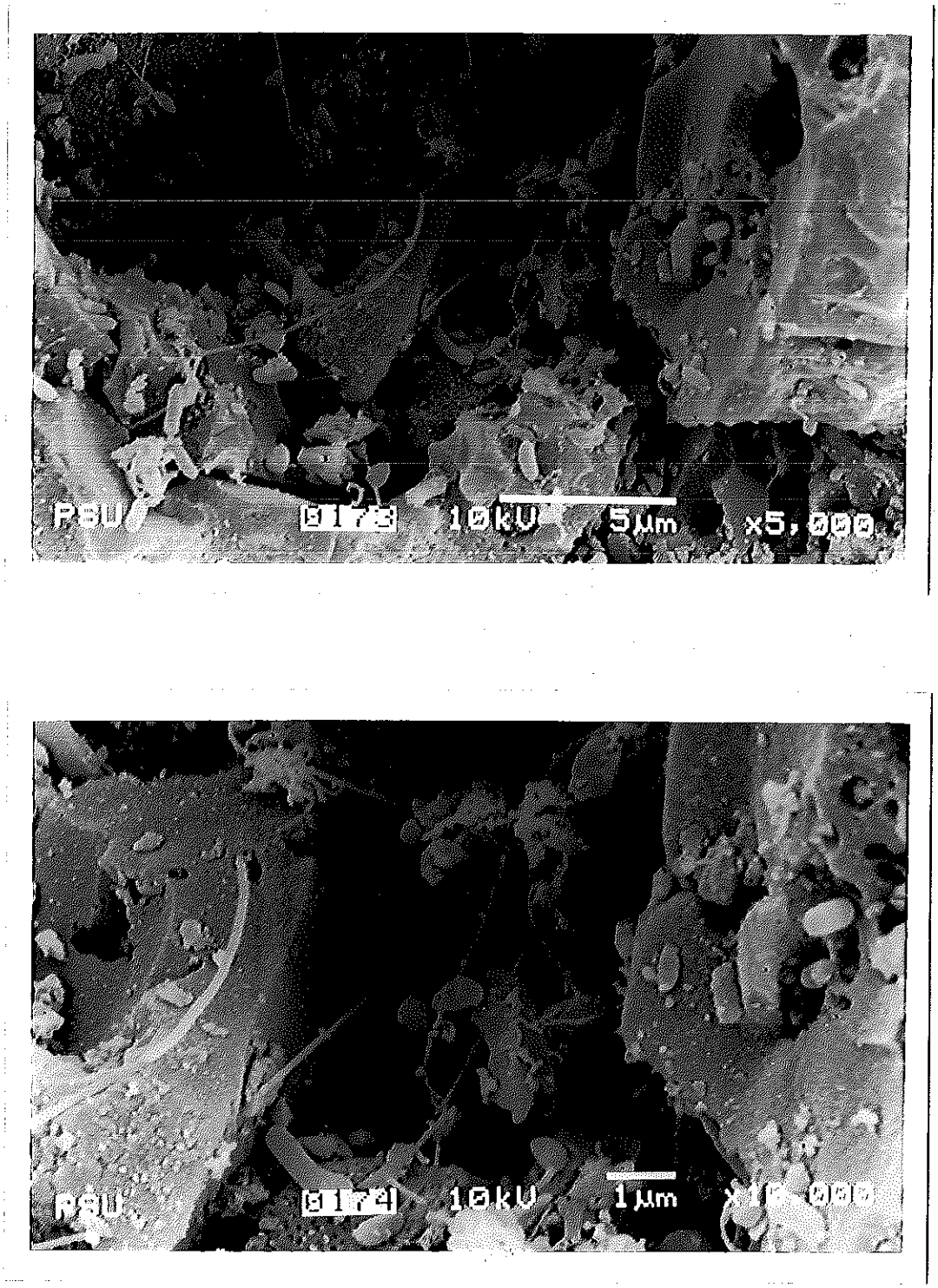


Fig. 27 SEM micrographs of *Pedomicrobium manganicum* cells immobilized on granular activated carbon particles taken from the top of the fluidized granular activated carbon bed in the continuous recycle fluidized bioreactor (CRFB)

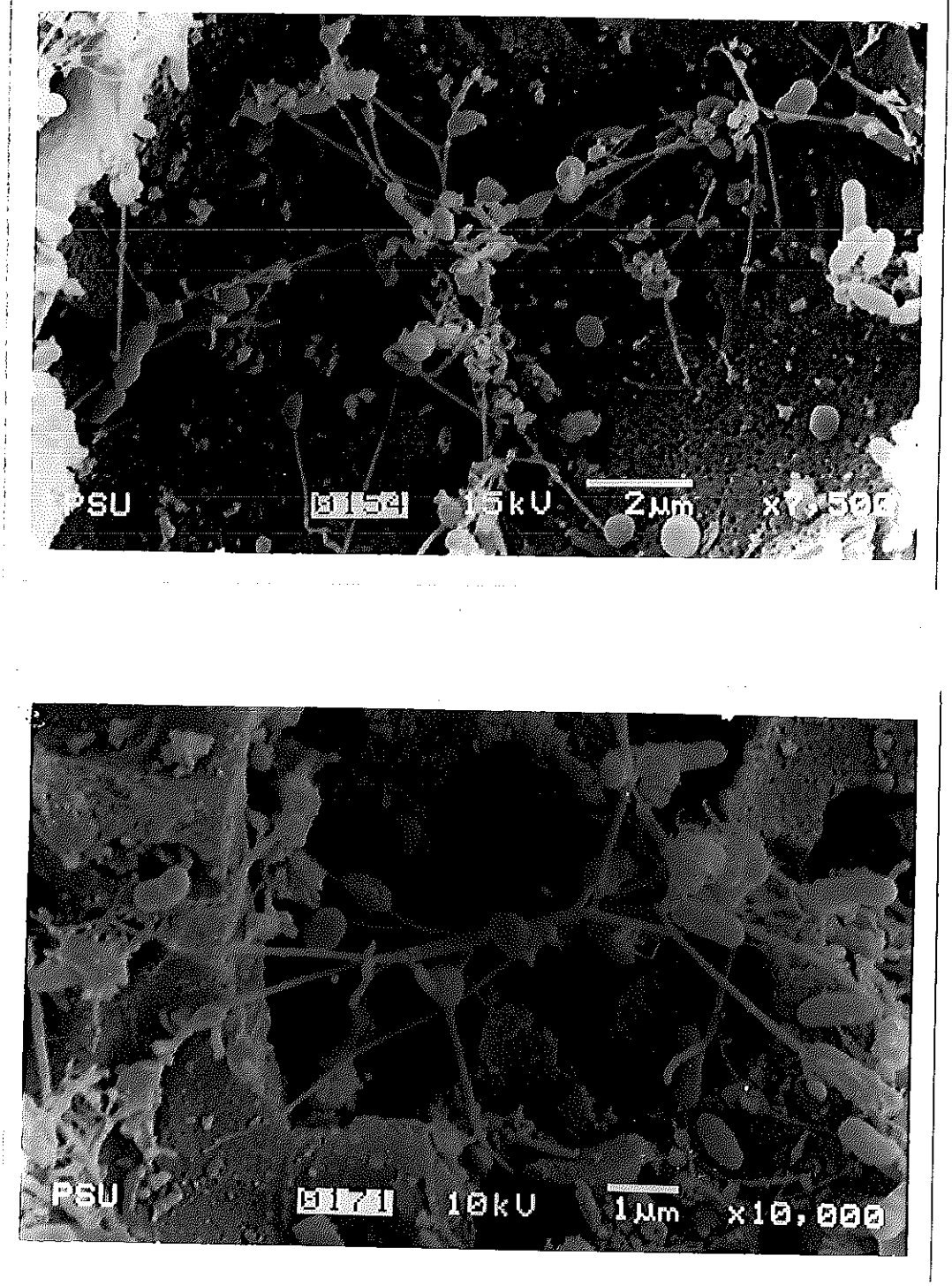


Fig. 28 SEM micrographs of *Pedomicrobium manganicum* cells immobilized on granular activated carbon particles taken from the middle of the fluidized granular activated carbon bed in the continuous recycle fluidized bioreactor (CRFB)

GAC was larger than on magnetite since the GAC particle has a lower specific density than magnetite. Consequently, the distribution and expansion of GAC particles in the column were higher than those of the magnetite in which the expanded particles were found mainly at the top of the column.

6.2 Removal of 1 ppm Mn by the CRFB operated in batch and continuous mode after cell immobilization

After cell immobilization in the CRFB, the CRFB was filled with a half strength PC medium containing 1 ppm Mn and then, operated in batch mode for 4 days to allow the culture growth and to monitor the Mn oxidizing ability of the strain immobilized on GAC. During this batch mode operation, pH of the CRFB was controlled at 8 to promote growth of the culture and to control the growth of possible contaminant microorganisms. The result from Fig. 29 revealed that the growth of the strain and the Mn oxidization by the strain were observed as indicated by the rapid decrease of the residual Mn in the recirculation medium after 10 h of the operation period and the soluble Mn fell to zero after 50 h of the operation period. Although the soluble Mn in the medium fell to zero, the insoluble Mn still remained stable at approximately 0.1 ppm. The occurrence of the insoluble Mn in the medium may due to the over flow of the fine carbon particles from the column to the mixing vessel at the startup period. Moreover, some detached cells from GAC could oxidize Mn and remained in the medium. The total and soluble Mn removal after 4 days operation were approximately 90% and 100%, respectively.

After 4 days of batch mode operation, the CRFB was fed with a half strength PC medium containing 1 ppm Mn again and operated in the continuous mode for 12 days to allow the CRFB to reach a steady state of Mn removal. During this time, pH of the CRFB was still controlled at about 8. The result in Fig. 30 showed that in the first 6 days of operation, the soluble Mn in the effluent increased from zero after 4 days of batch mode operation to approximately 0.20 ppm, then the curve dropped slightly to approximately 0.15

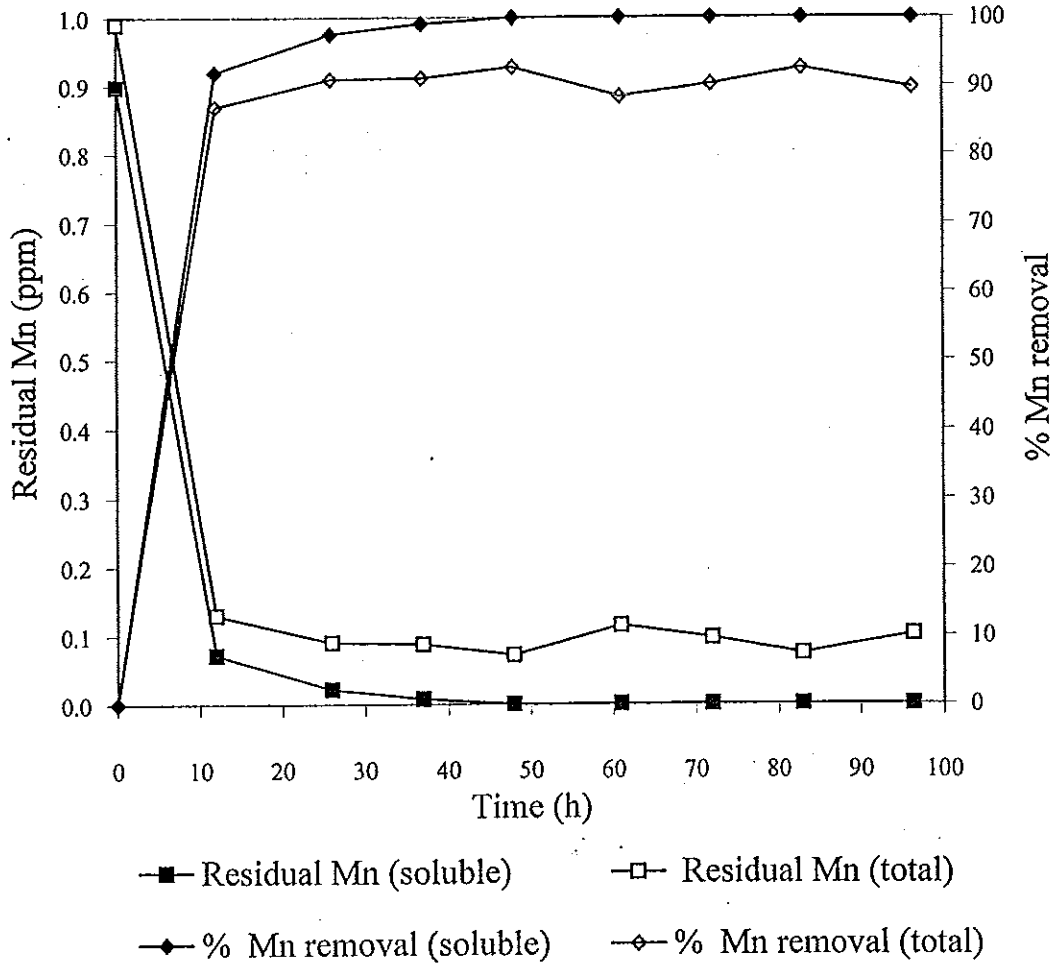


Fig. 29 Removal of 1 ppm Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB) operated in a batch mode for 4 days after cell immobilization

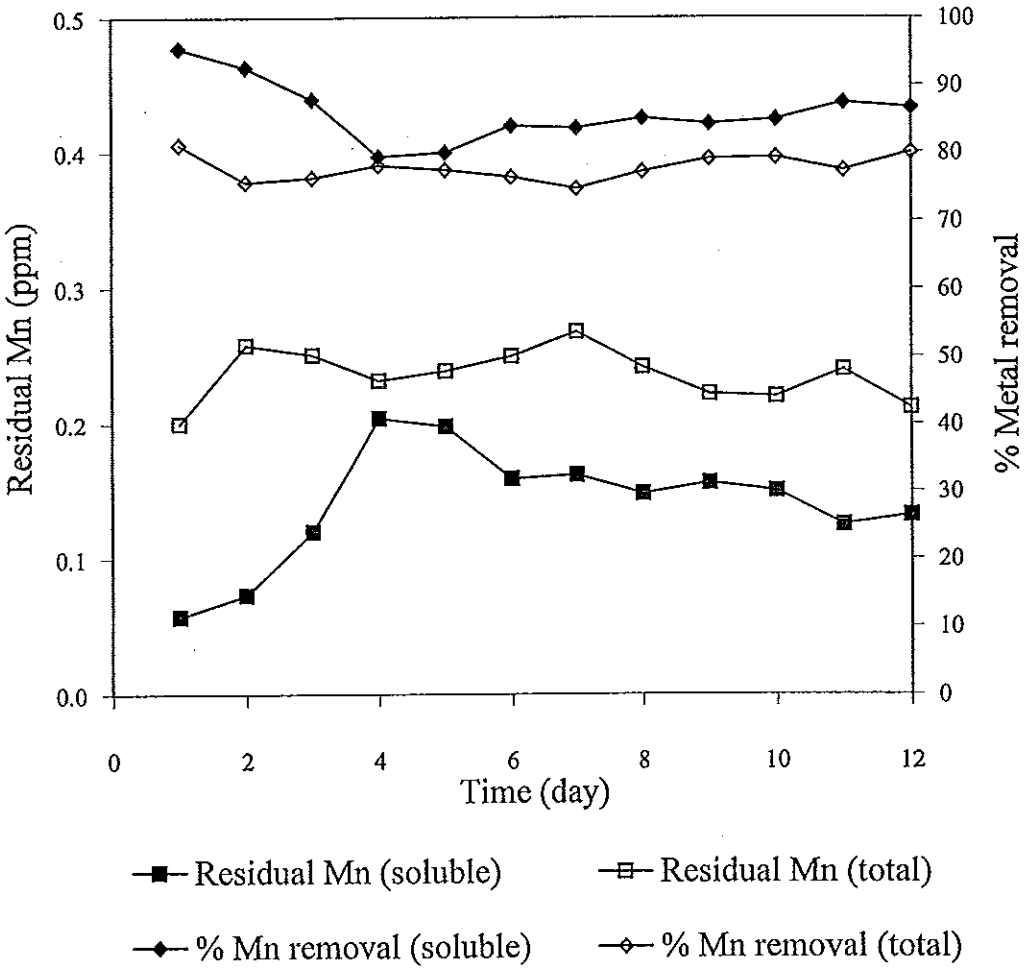


Fig. 30 Removal of 1 ppm by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB) operated in a continuous mode for 12 days after cell immobilization

ppm and tended to be stable thereafter. In contrast, the curve of total Mn in the effluent showed only a little fluctuation between 0.20 to 0.27 ppm during the period of continuous mode. The total and soluble Mn removal were 80% and 87%, respectively after 12 days operation. Comparison to the work by Sly *et al.* (1993), it was found that the percentage of Mn removal obtained in this experiment was lower than the previously reported by Sly *et al.* (1993) in which the Mn removal by the CRFB using magnetite as supporting material at steady state was about 93% or 71 μg/l residual. However, it should be noted that the number of immobilized cells in the experiments by Sly *et al.* (1993) was higher at 1.6×10^{11} cells on the bed.

6.3 Removal of heavy metals

6.3.1 Ni and Cd removal when adding each metal individually

The concentration of Ni and Cd used in this study was varied from 0 ppm to about 8 ppm as in the previous experiment of Ni and Cd removal by the CRFB using magnetite as the supporting material. The pH of the CRFB was not controlled in this experiment to possibly observe the relationship between the changes of pH and the adsorption of metals.

Result from Fig. 31 and 32 showed that when the concentration of Ni in the influent medium was increased in steps from 0.857 ppm to 7.467 ppm, the concentration of soluble and total Ni in the effluent also increased from 0.020 ppm and 0.074 ppm to 0.303 ppm and 0.376 ppm, respectively. In addition, the concentration of soluble and total Mn in the effluent increased slightly from 0.112 ppm and 0.168 ppm (when no Ni was added) to 0.190 ppm and 0.251 ppm (at 7.467 ppm Ni), respectively however, the removal efficiency of soluble and total Mn were still higher than 80%. The increase of Ni and Mn concentrations in the effluent when the Ni concentration in the influent increased was also found in the previous experiment using magnetite as the supporting material (Fig. 16). The result also showed that pH of the medium slightly decreased from 8.05 to 7.79 when Ni concentration in the influent

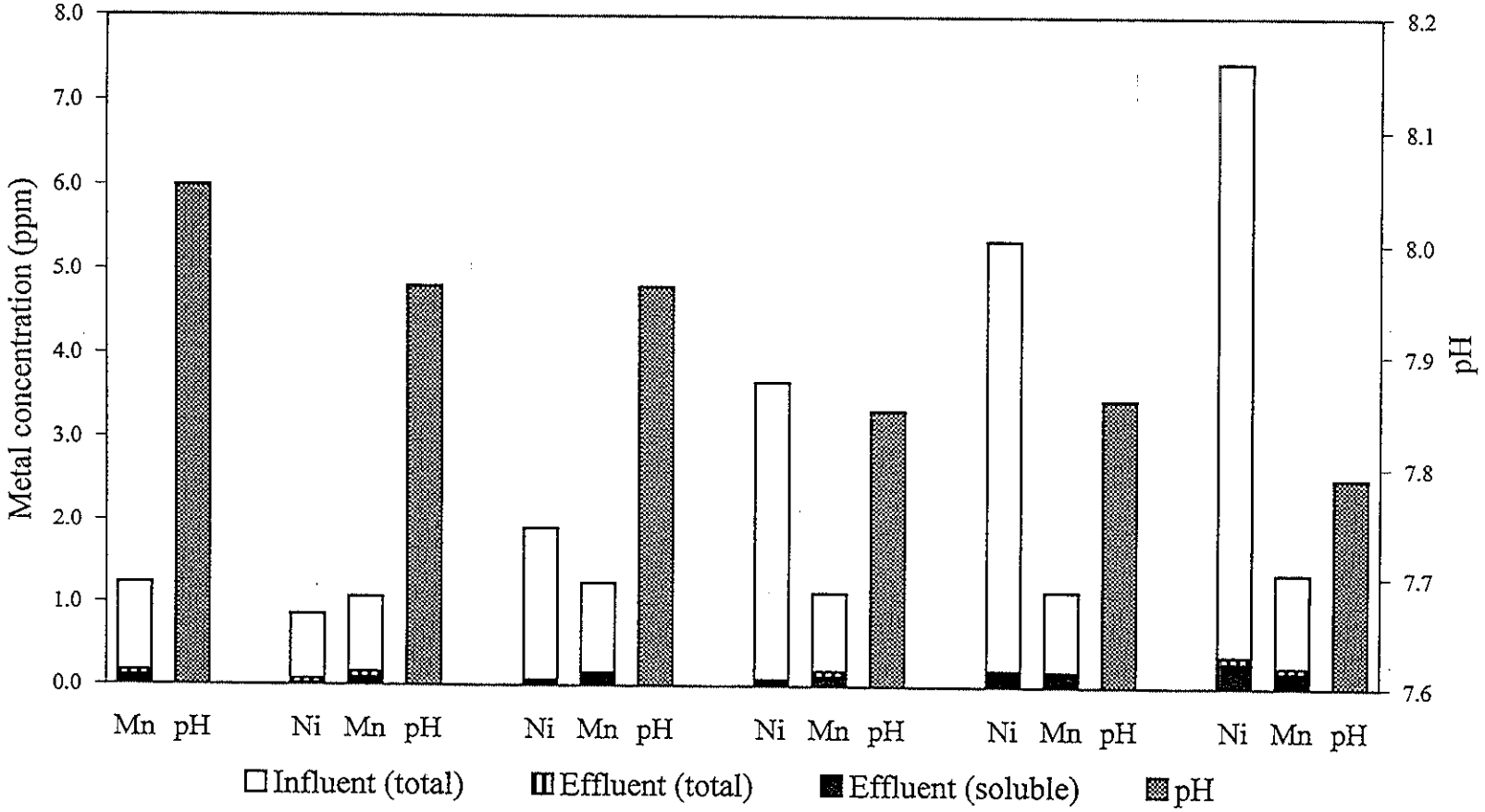


Fig. 31 Removal of Ni and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB)

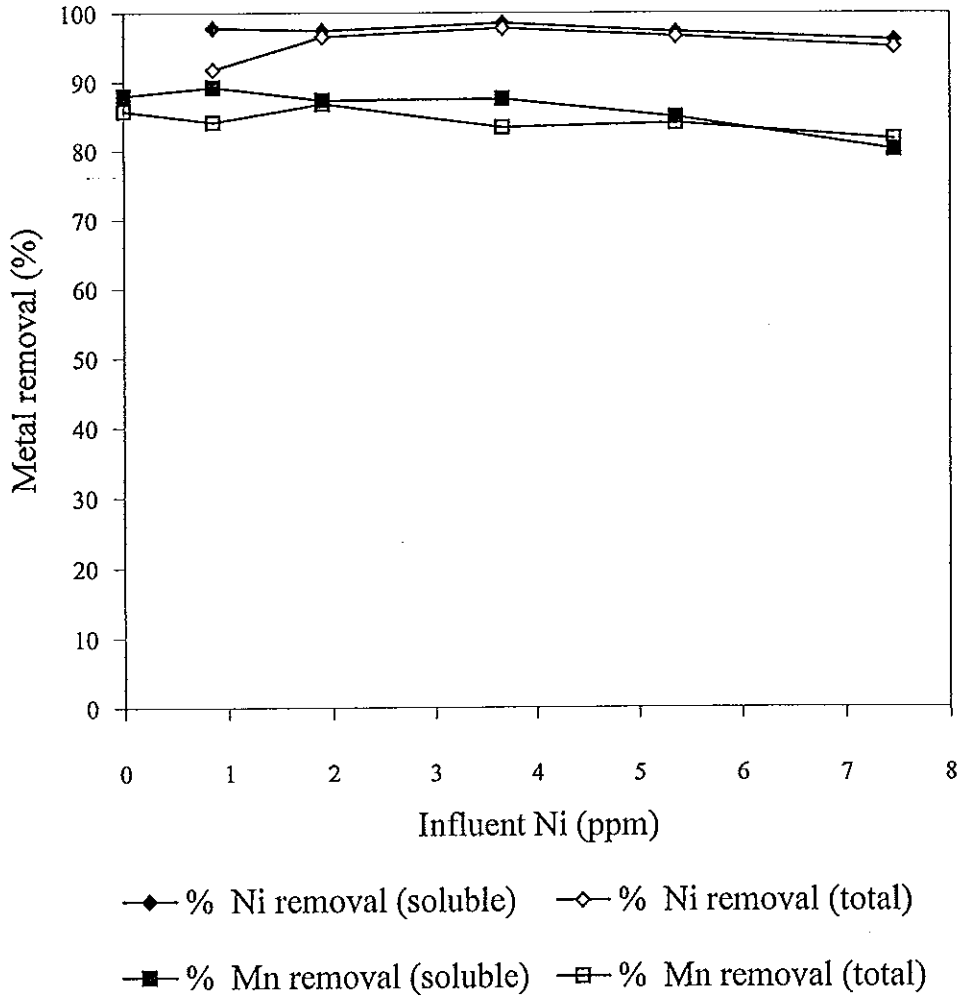


Fig. 32 The percentage of Ni and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Ni concentration

increased in steps from 0 ppm to 7.467 ppm and this is contrast to the previous experiment using magnetite as the supporting material in which the pH of the medium increased when the concentration of Ni in the influent increased. The decrease of pH in this experiment revealed that the adsorption of Ni by *P. manganicum* resulted in the release of proton as H^+ from the adsorption site. The ECP and MnO_2 have been reported to be the effective adsorbent for Ni ion and in addition, both of them were proposed to release H^+ during adsorption of metal cation as well (Gray and Malati, 1979; McLean *et al.*, 1990). Thus, this indicates the possible involvement of the ECP and MnO_2 in the Ni adsorption by *P. manganicum* in this experiment.

From Fig. 33 and 34, it was found that when the concentration of Cd in the influent medium was increased in steps from 0.952 ppm to 7.816 ppm the Cd concentration in the effluent slightly increased from 0 ppm to 0.069 ppm for the soluble and from 0.063 ppm to 0.113 ppm for the total. The Mn concentration in the effluent obviously increased from 0.150 ppm to 0.402 ppm for the soluble and from 0.258 ppm to 0.445 ppm for the total when the influent Cd concentration increased in steps from 0 ppm to 7.816 ppm. In addition, pH of the medium drastically decrease from 7.89 to 7.52. The slight increase of Cd in the effluent and the higher than 94% of both total and soluble Cd removal throughout this experiment indicates that *P. manganicum* has a high efficiency for Cd adsorption.

The obvious increase of Mn in the effluent when the concentration of Cd in the influent medium increased was also observed in the previous experiment using magnetite as the supporting material. This indicated that the adsorption of Cd by *P. manganicum* tends to relate closely with the Mn removal such as Cd ions compete with Mn ions for the adsorption sites which is proposed to be the ECP and MnO_2. Besides, it was possible that Mn ions were released from MnO_2 surface when Cd was adsorbed on the surface of MnO_2.

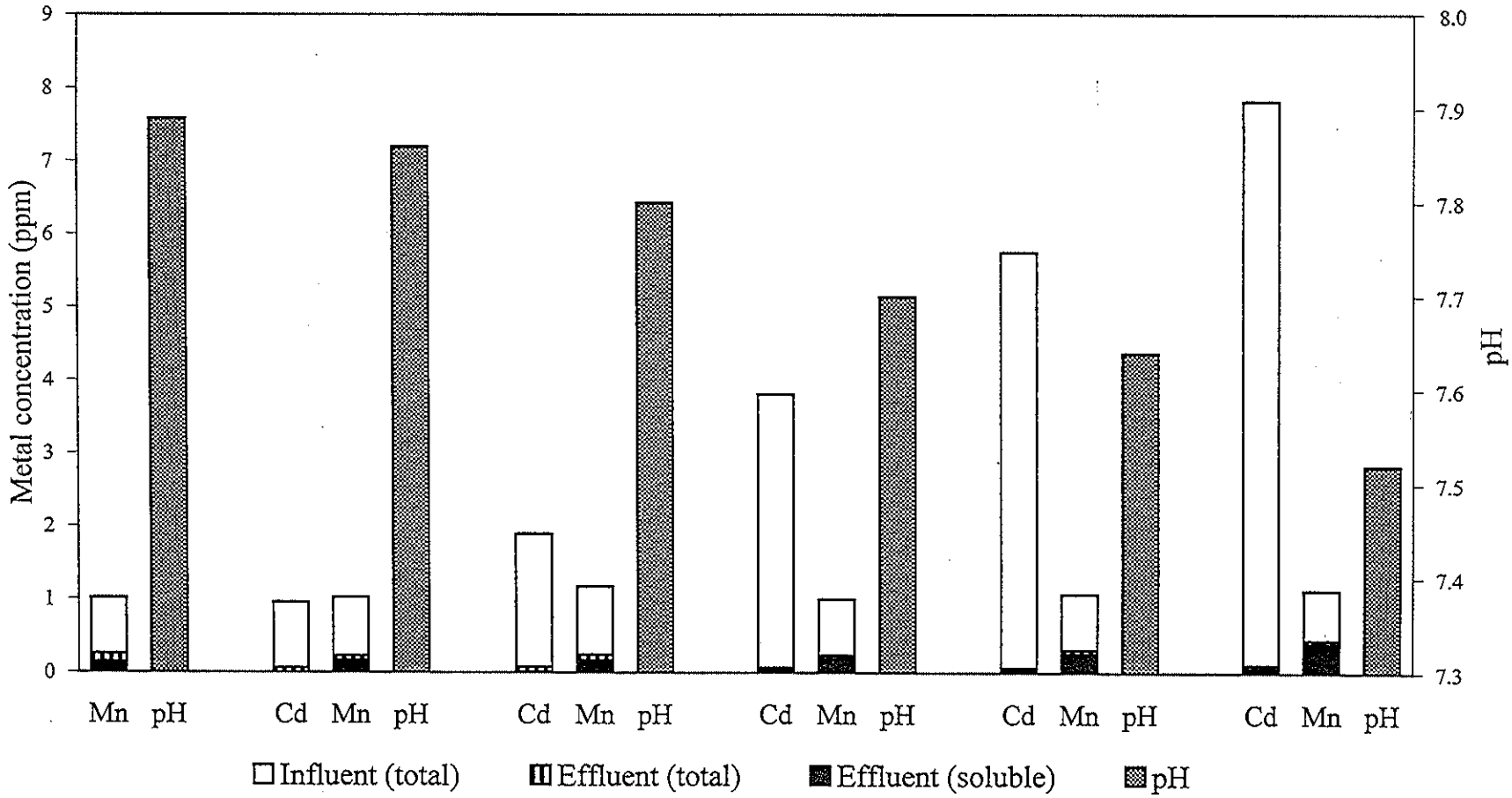


Fig. 33 Removal of Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB)

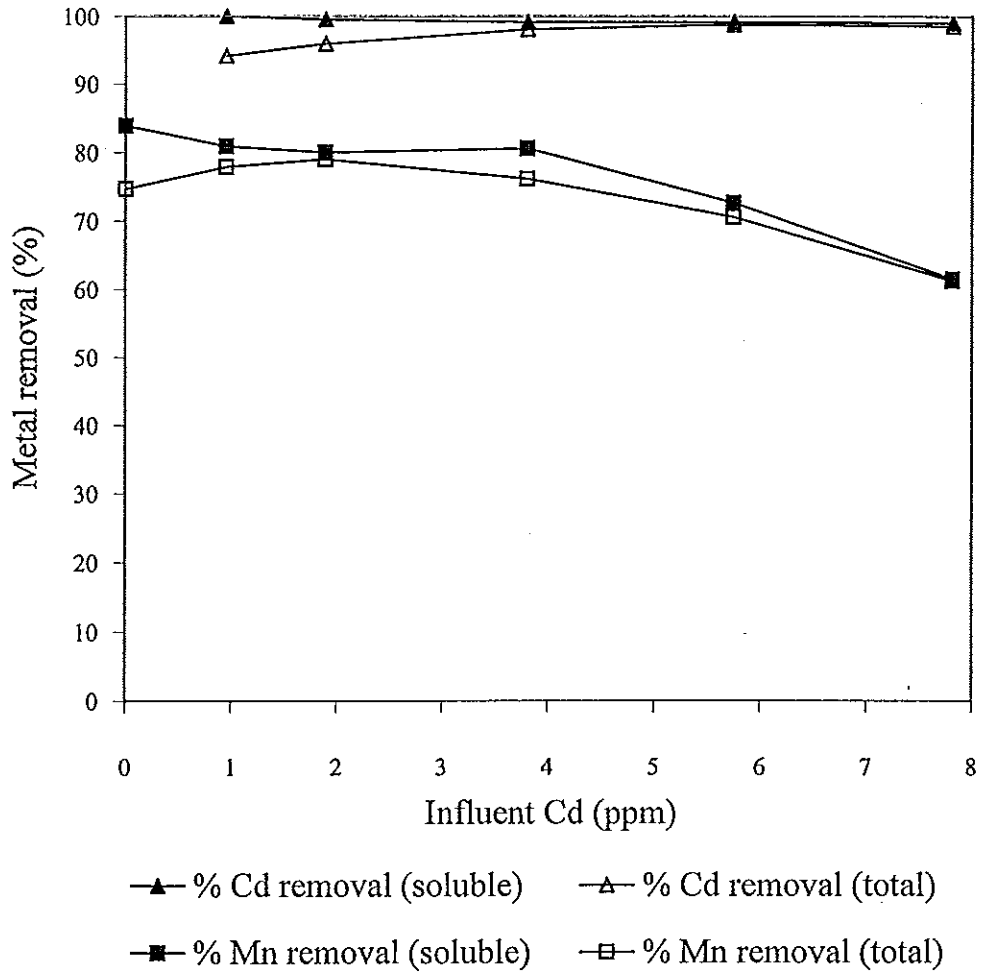


Fig. 34 The percentage of Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Cd concentration

The decrease of pH during Cd adsorption was also found in the previous experiment using magnetite as the supporting material. This reveals that the adsorption of Cd by the strain is involved with the release of H^+ from the adsorption sites. The low pH of 7.52 which was lower than in the experiment of Ni removal may be because more metal is adsorbed to the MnO_2. The low pH of 7.52 when 7.816 ppm Cd was added in the influent medium may also indicate the toxicity effect of Cd on *P. manganicum* cells since the metabolic activity of the living cell of the strain normally maintained pH of the medium at about 7.8 (Sly *et al.*, 1993). However, when the influent medium in which Cd was not added was run after the Cd removal experiment, the slight increase of pH of the medium to 7.69 and the increase of Mn removal were observed. This indicates that the pH drift may be caused by the effect of Cd adsorption, but it is also possible that cells recovered their metabolic activity when the toxic Cd was removed.

It should be noted that in both results of Ni and Cd removal, the concentration of the insoluble Mn in the effluent in this experiment was lower than that in the previous experiment using magnetite as the supporting material. However, when the concentration of Ni and Cd in the influent medium were increased in steps from 0 ppm to about 8 ppm, the increasing values of the insoluble Mn concentrations was not significantly different from the previous experiment.

At the same concentrations of both Ni and Cd in the influent medium, removal efficiency of Ni was lower than that of Cd for all concentrations of these metals. This was similar with the previous experiment using magnetite as the supporting material, therefore, the adsorption of Ni and Cd by *P. manganicum* immobilized on GAC in the CRFB might be mainly involved with the MnO_2 deposited on the ECP as well as would be expected.

6.3.2 Ni and Cd removal when adding metals in combination

In this experiment the concentration of Ni and Cd used was varied from 0 ppm to about 4 ppm as in the previous experiment using magnetite as the supporting material.

When Ni and Cd concentrations in the influent medium increased in steps from 0.781 ppm to 3.891 ppm Ni and from 0.920 ppm to 4.046 ppm Cd, the total concentration of both metals in the effluent slightly increased from 0.063 ppm to 0.104 ppm Ni and from 0.073 ppm to 0.152 ppm Cd. The soluble and total concentration of Mn in the effluent also increased from 0.300 ppm and 0.389 ppm (when no metals were added) to 0.486 ppm and 0.590 ppm (at 3.891 ppm Ni and 4.046 ppm Cd), respectively (Fig. 35). In addition, pH of the medium decreased slightly from 7.69 to 7.52.

The result indicated that adsorption of Ni and Cd by this strain tended to be independent from each other; that is, the concentrations of Ni and Cd in the effluent at the same concentration of each metal in the medium when added individually and in combination were not significantly different (Fig. 31, 33 and 35). In contrast, removal of both Ni and Cd by *P. manganicum* tended to relate with Mn removal. When Ni and Cd concentration in the influent medium increased in steps from 0 ppm to about 4 ppm for both the efficiency of Mn removal by the CRFB decreased obviously from 67.75% to 50.83% for the soluble and from 65.85% to 46.70% for the total (Fig. 36). This indicated the competition for the adsorption site. The other possible reason is that Mn ion was released from MnO_2 surface when metals were adsorbed on its surface. In addition, it should be noted that pH of the medium tended to decrease throughout this experiment. The decrease of pH may have been cause by the release of protons when metals were adsorbed on the adsorption sites and the toxicity effect of metals to *P. manganicum* cells as well as the previous experiment of Cd removal. However, when metals were not added in the

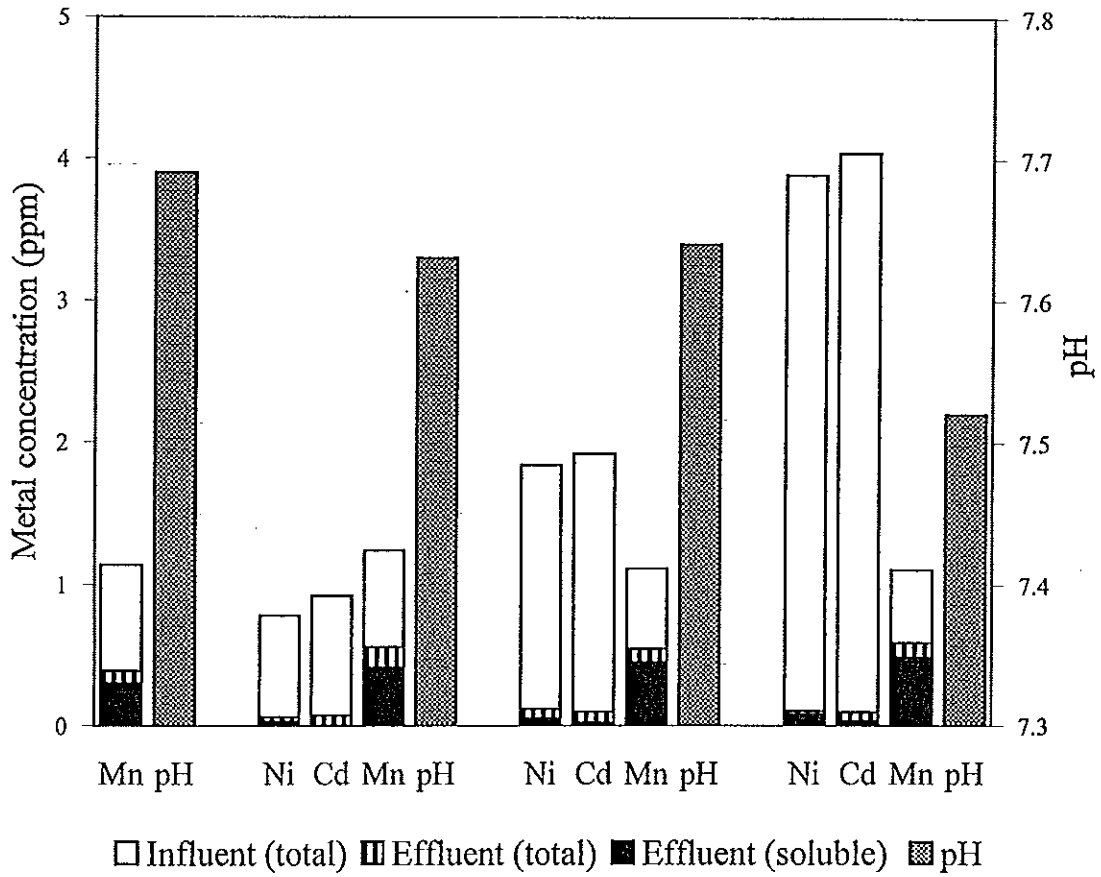


Fig. 35 Removal of Ni, Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB)

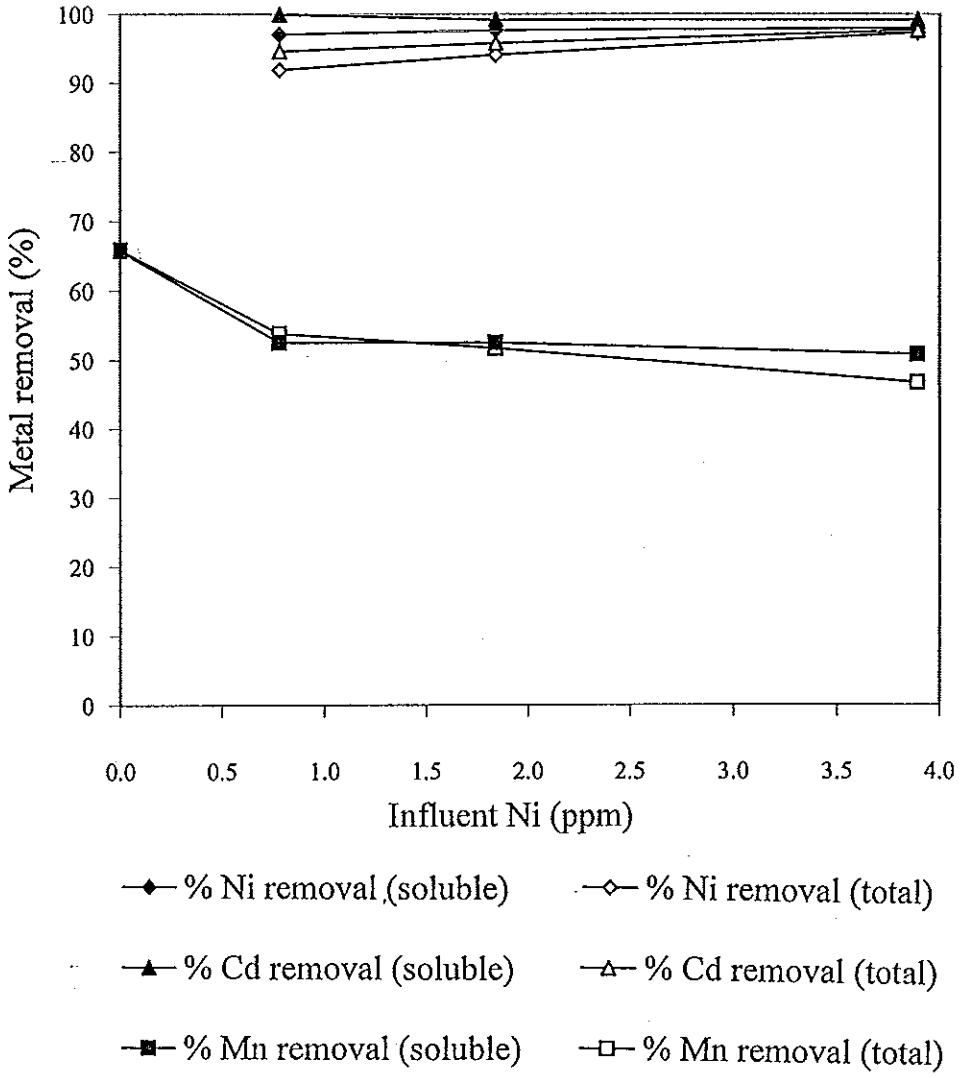


Fig. 36 The percentage of Ni, Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon particles in the continuous recycle fluidized bioreactor (CRFB) related to the influent Ni concentration

influent medium the Mn removal slightly increased again but was still lower than that at the time of bioreactor startup.

Ni and Cd removal throughout this experiment were higher than 91% and 94%, respectively for the total metals removal and higher than 97% and 99%, respectively for the soluble metals removal. This still showed the higher efficiency in Cd adsorption than Ni as well as the experiment of Ni and Cd removal when metal was added individually.

Chapter 4

Conclusions

1. The maximum growth of *Pedomicrobium manganicum* ACM 3067 in PSM medium was obtained after 4 days cultivation on a rotary shaker at 150 rpm and at room temperature (30±2°C).

2. The presence of high concentration of Mn (20 and 50 ppm) in the PSM and PC media exhibited an inhibitory effect on the growth of *P. manganicum* ACM 3067, however, survival of the culture was prolonged.

3. *P. manganicum* ACM 3067 can grow and oxidize Mn^{2+} to MnO_2 in the presence of 0, 2, 6, 12, 20 and 50 ppm Mn in PSM and PC media. The highest growth of the strain was obtained in both media with the addition of 2 ppm Mn. The complete oxidation of Mn^{2+} to MnO_2 was found only in the presence of 2 ppm Mn in both media.

4. Increasing of pH during growth of *P. manganicum* ACM 3067 in the PSM and PC media may result in the autooxidation of the medium with high concentrations of Mn.

5. The MIC value indicated that *P. manganicum* ACM 3067 can tolerate Cd higher than Ni and Ni+Cd, respectively. The MIC value of the combination of Cd and Ni depended on the toxicity of Ni more than Cd.

6. The presence of Mn in the medium provided a protective effect on the toxicity of Ni and Cd on *P. manganicum* ACM 3067.

7. Immobilization of 4.20×10^9 CFU of *P. manganicum* ACM 3067 on magnetite in the CRFB was completed in 3-5 min with the adsorption efficiency of 60.7% and the number of immobilized cells was 2.55×10^9 CFU.

8. The removal efficiency of Ni and Cd by *P. manganicum* ACM 3067 immobilized on magnetite in the CRFB was more than 95% and 96%, respectively when each metal was added individually and more than 72% and 74%, respectively when Ni and Cd were added in the combination.

9. The best immobilization medium and particle size of GAC for the immobilization of *P. manganicum* ACM 3067 were a half strength PC medium with 1 ppm Mn and 400-1,000 μM GAC, respectively. The adsorption of cells on GAC occurred rapidly and reached maximum after 2 min with the adsorption efficiency of 32%. The desorption of cells from GAC also occurred rapidly within 1-2 min and the efficiency of cells desorption was 33-34%.

10. The immobilization of 1.90×10^{10} CFU of *P. manganicum* ACM 3067 on GAC particles in the CRFB was completed within 10-20 min with an adsorption efficiency of 50.2% and the adsorption capacity of GAC for the *P. manganicum* was 2.03×10^{7} CFU/g GAC. At the end of the immobilization, the number of adsorbed cells in the CRFB was about 9.54×10^{9} CFU.

11. The removal of Ni and Cd by *P. manganicum* ACM 3067 immobilized on GAC in the CRFB was more than 91% and 94%, respectively when each metal was added individually and more than 91% and 94%, respectively when Ni and Cd were added in the combination.

12. It was anticipated that Ni and Cd adsorb onto MnO_2 deposits in the ECP of *P. manganicum* cells especially for Cd for which the adsorption is accompanied by the decrease of Mn removal and pH of the medium.

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Appendices

Appendix 1

Media

1. PSM medium (Gebers, 1981)

Sodium acetate	1.3608	g
Yeast extract	0.5	g
Metals 44 solution	1.0	ml
Vitamin solution	1.0	ml
Deionized water	1.0	L
Adjust to pH 9 with KOH		

Metals 44 solution

EDTA salts	250.0	mg
$ZnSO_4 \cdot 7H_2O$	1095.1	mg
$FeSO_4 \cdot 7H_2O$	500.0	mg
$MnSO_4 \cdot H_2O$	154.0	mg
$CuSO_4 \cdot 5H_2O$	39.8	mg
$Co(NO_3)_2 \cdot 10H_2O$	24.8	mg
$Na_2B_4O_7 \cdot 10H_2O$	17.7	mg
Deionized water	100.0	ml

Vitamin solution

Biotin	2.0	mg
Folic acid	2.0	mg
Pyridoxine HCl	10.0	mg
Riboflavine	5.0	mg

Thiamine HCl	5.0	mg
Nicotinamide	5.0	mg
Calcium pantothenate	5.0	mg
B_{12} (cyanocobalamin)	0.1	mg
P-aminobenzoic acid	5.0	mg
Deionized water	100	ml

PSM medium without added metals 44 solution and vitamin solution was sterilised by autoclaving at 121°C for 15 min. Metals 44 solution and vitamin solution were filter steriled by using 0.45 µm cellulose acetate membrane filter and then, added aseptically to the autoclaved medium.

2. PC medium (Tyler and Marshall, 1967)

Yeast extract	0.05	g
$MnSO_4 \cdot H_2O$	0.02	g
Deionized water	1.0	L

Adjust to pH 7 with KOH

PC medium without added $MnSO_4 \cdot H_2O$ was sterilised by autoclaving at 121°C for 15 min. $MnSO_4 \cdot H_2O$ was prepared as a stock solution of 1,000 ppm and filter sterilised by using 0.45 µm cellulose acetate membrane filter. After that, it was aseptically added to the the autoclaved PC medium.

3. Suspending fluid (MacRae and Evans, 1983)

$CaCl_2$	30.81	mg
$MgCl_2 \cdot 6H_2O$	80.84	mg
$NaHCO_3$	122.10	mg
NaCl	88.80	mg
Deionized water	1.0	L

Adjust to pH 7 with 1 M HCl

Suspending fluid was sterilised by autoclaving at 121°C for 15 min.

Appendix 2

Supporting materials

1. Magnetite (Sly *et al.*, 1990)

Crude ore obtained from commercial minerals was processed by sieving to the required size of 212-300 μm diameter. The magnetite particles were treated by eight alternating magnetic field cycles (8AMF) using the method of MacRae and Evans (1983). This treatment was carried out by mixing 1 volume of untreated magnetite with 4 volumes of 0.1 M NaOH for 10 min followed by washings with distilled water using decantation and then final readjustment to pH 4 with 1 M H_2SO_4. Decantation was facilitated by the use of a magnet to hold the magnetite in the base of the beaker. The magnetite was then passed through a demagnetising field (Eclipse AD960, England) to disperse the particles. This treatment process was repeated 8 times.

2. Granular activated carbon (GAC) (Hassler, 1974)

GAC from Eurocarb product Ltd., grade M-1100, was used in the immobilization experiment. Several characteristics of the GAC are shown in Table 1-A. Prior to use, the GAC was sieved to yield three diameter sizes of >1,400 μm, 1,000-1,400 μm and 400-1,000 μm. After that, each particle size was washed with diluted HCl to remove acid soluble ash. When this dissolving action was complete, the carbon was thoroughly washed with deionized water. The carbon was then brought to a neutral pH with dilute sodium carbonate and then, given a final wash with deionized water. After that, each particle size was dried at 105°C and stored in a sealed container.

Table 1-A Characteristics of granular activated carbon, Grade M-1100, from Eurocarb Product Ltd., England.

Characteristics	GAC (Grade M-1100)
Description	Coconut shell based activated carbon produced by high temperature steam activation of specially selected coconut shell char feedstock
Particle size	> 2.36 mm (5%) 2.36-1.70 mm (40-60%) 1.70-0.60 mm (30-50%) < 0.60 mm (5%)
Carbon tetrachloride adsorption	55-60%
Surface area (Bet Method N_2)	1,150 m^2/g
Apparent density	500-520 kg/m^3
Backwashed density	470-490 kg/m^3
Ash	3%
Iodine number (AWWA B604)	1100
Moisture content	8%
Hardness number	97

Appendix 3

Analytical methods

1. Preparation of stock colloidal manganese dioxide suspension (Johnson, 1991)

Chemicals

Potassium permanganate

Sodium hydroxide

Manganous chloride tetrahydrate

Method

A 0.0045 g amountof potassium permanganate was added to a 2 L Erlenmeyer flask. An equivalent amount of sodium hydroxide was added to maintain a basic pH throughout the synthesis. Approximately one litre of deionized water was added and 0.014 g manganous chloride tetrahydrate in one litre of water added dropwise to the potassium permanganate solution with stirring using a separatory funnel. The final concentration was approximately 2 ppm of manganese dioxide. At this concentration and pH, the suspension was stable for more than two years with no evidence of flocculation. Periodic standardisation confirmed this.

2. Standardisation of manganese dioxide by iodometric method (Johnson, 1991)

Chemicals

5×10^{-3} M Sodium thiosulfate : 1.24 g of sodium thiosulfate was dissolved in one litre deionized water.

3.341×10^{-4} M Potassium iodate : 7.14976 g potassium iodate, previously dried in an oven at 120°C, was dissolved in one litre deionized water. A 10 ml volume of this solution was then further diluted to one litre.

Method

The 2 ppm colloidal manganese dioxide suspension was standardised against thiosulfate solution which had previously been standardised against potassium iodate. A 100 ml aliquot of the colloid was used. An excess of potassium iodide was added to the manganese dioxide, followed by 5 ml of 0.1 M sulfuric acid. The liberated iodine was then determined by titration against thiosulfate. The end-point determination was aided by the addition of starch late in the titration.

3. Spectrophotometric determination of oxidized manganese by leuco crystal violet (LCV) method (Kessick *et al.*, 1972 cited by Johnson, 1991).

Chemicals

LCV reagent : 0.10 g leuco crystal violet was dissolved in 30 ml deionized water and 0.80 ml concentrated perchloric acid and then diluted to 100 ml. The solution was stable in light for up to one month.

Acetate buffer : 250 ml glacial acetic acid was diluted to one litre and titrated to pH 4 with 5 M sodium hydroxide.

1×10^{-3} M Sodium bicarbonate : 0.084 g sodium bicarbonate was dissolved in 50 ml deionized water and diluted to one litre.

Method

Suitable aliquots of stock manganese dioxide colloidal suspension and 5 ml of unknown samples were added to approximately 12.5 ml aliquots of the sodium bicarbonate in 25 ml volumetric flasks. To each sample 0.50 ml LCV reagent was then added followed by 1.0 ml buffer solution, The solution was then mixed and left to stand for at least five minutes. The resulting violet coloured solutions were made up to the mark with deionized water and the 1 cm absorbance was determined at 590 nm against a reagent blank.

4. Scanning electron microscopy

Chemicals

3% Glutaraldehyde

0.1 M Cacodylate buffer

1% Osmium tetroxide (OsO_4)

Method

The adsorption and adhesion of *P. manganicum* cells to the surface of magnetite and GAC particles from the CRFB were examined by scanning electron microscopy (SEM). Both magnetite and GAC particles from the top and middle of the column were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer and stored at 4°C for several days. This was followed by washing with 0.1 M cacodylate buffer 3 times for 10 min each, and postfixation in 1% OsO_4 in 0.1 M cacodylate buffer for 2 h at room temperature, then washing with cacodylate buffer 3 times for 10 min each, before storing overnight at 4°C. The preparations were then dehydrated in graded ethanol series, critical point dried and sputter coated with gold.

5. Analysis of metals (McLean, 1994)

Chemical

Aqua regia ($HCl : HNO_3$; 3 : 1)

Method

Samples were taken directly from the influent medium and effluent samples were taken twice daily (or every 12 h) from the mixing vessel. From each sample aliqouts were taken for total and dissolved metal determination. Metal concentrations from the CRFB using magnetite and the CRFB using GAC as the supporting material were determined by Flame Atomic Absorption Spectrophotometry (AAS) using a Varian AA875 spectrophotometer and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) using Emission Spectroscopy Plasma-1000, respectively.

For total metal analysis using the AAS and ICP-AES, the samples were acid digested by adding 1 ml of aqua regia (HCL : HNO_3 , 3:1) to 9 ml of sample. The mixture was then left overnight at ambient temperature before analysis. The samples analysed for dissolved metal using the AAS and ICP-AES were passed through a 0.2 μm sterile filter and stored at 4°C until analysed.

For determination of total metal ($Metal_T$) concentration, the reading data were adjusted using the following formula.

$$(A-C)d = (Metal_T)$$

where A = AAS or ICP reading

C = AAS or ICP reading for control of 1 ml aqua regia in 9 ml deionized water

d = Dilution factor due to aqua regia i.e. 10/9

Appendix 4

Calibration curves

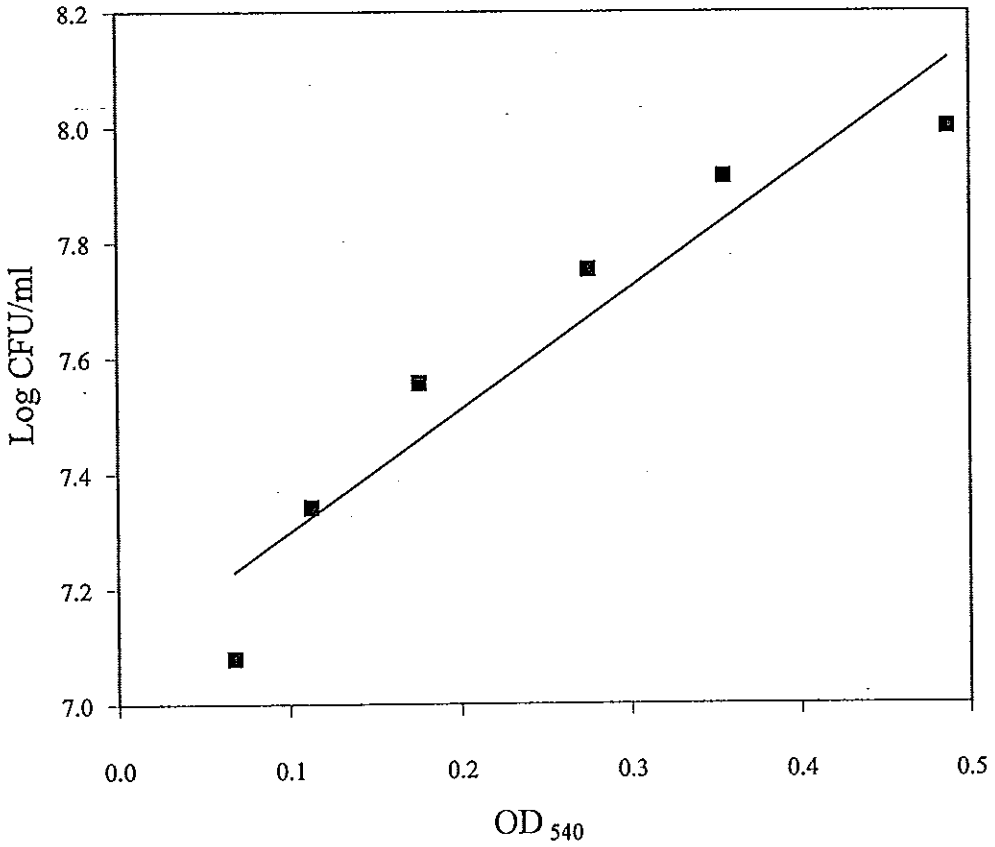

Fig. 1-A Calibration curve for *Pedomicrobium manganicum* ACM 3067 cell concentration (CFU/ml) grown in PSM medium shaken at 150 rpm at room temperature (30±2°C) for 4 days

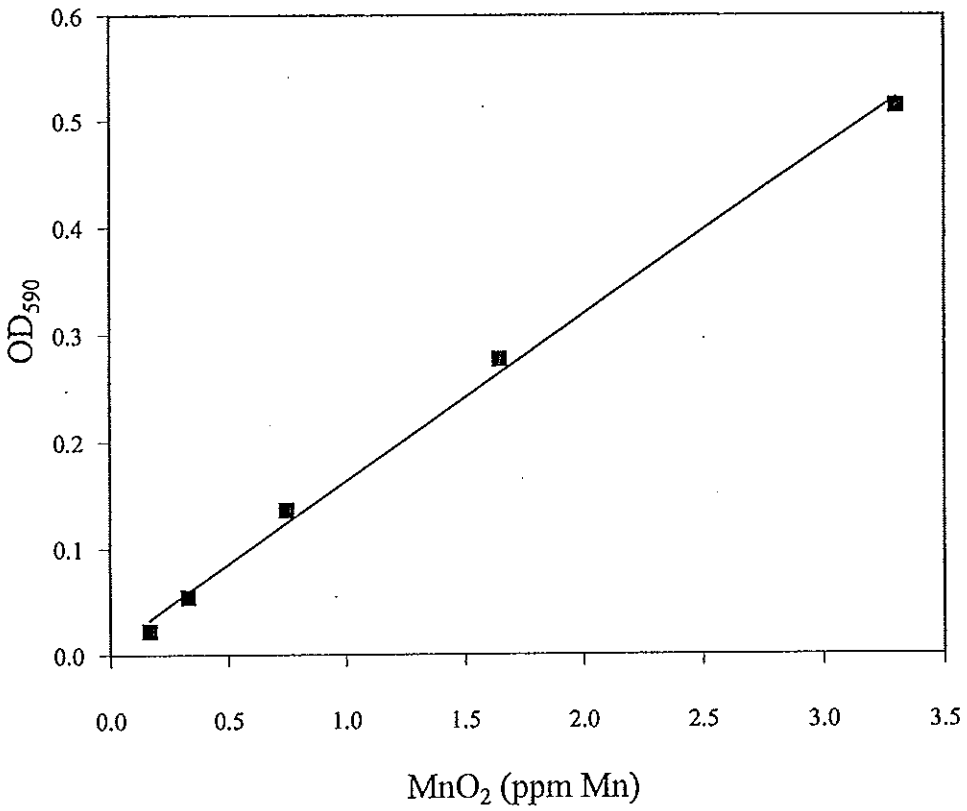

Fig. 2-A Calibration curve for MnO_2 produced by *Pedomicrobium manganicum* ACM 3067

Appendix 5

Results data

Table 2-A Immobilization of *Pedomicrobium manganicum* ACM 3067 on magnetite particles in the continuous recycle fluidized bioreactor (CRFB)

Time (min)	Absorbance (OD_{540})	Number of bacteria in the recirculation medium (CFU)	Number of immobilized bacteria (CFU)
Start-up	0.960	4.20×10^{9}	0
0.5	0.850	1.65×10^{9}	2.55×10^{9}
1.0	0.674	3.30×10^{9}	0.90×10^{9}
2.0	0.592	2.74×10^{9}	1.46×10^{9}
4.0	0.496	3.30×10^{9}	0.90×10^{9}
5.0	0.502	1.65×10^{9}	2.55×10^{9}
7.5	0.474	3.30×10^{9}	0.90×10^{9}
10.0	0.472	1.65×10^{9}	2.55×10^{9}
15.0	0.516	3.30×10^{9}	0.90×10^{9}

Table 3-A Immobilization of *Pedomicrobium manganicum* ACM 3067 on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB)

Time (min)	Absorbance (OD_{540})	Number of bacteria in the recirculation medium (CFU)	Number of immobilized bacteria (CFU)
Startup	1.962	1.90×10^{10}	-
2	1.612	8.90×10^{9}	1.01×10^{10}
4	1.229	7.60×10^{9}	1.14×10^{10}
6	1.209	8.90×10^{9}	1.01×10^{10}
8	1.206	9.31×10^{9}	9.69×10^{9}
10	1.157	9.58×10^{9}	9.42×10^{9}
12	1.218	9.31×10^{9}	9.69×10^{9}
15	1.153	8.40×10^{9}	1.06×10^{10}
20	1.093	9.31×10^{9}	9.69×10^{9}
30	1.097	9.88×10^{9}	9.12×10^{9}
40	1.090	8.90×10^{9}	1.01×10^{10}
60	1.102	9.46×10^{9}	9.54×10^{9}

Table 4-A Removal of Ni and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni

Mn (ppm)								Ni (ppm)								pH	SD
INF				EFF				INF				EFF					
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD		
0.885	0.021	0.933	0.057	0.093	0.013	0.326	0.032	No Ni added		No Ni added		-	-	-	-	8.06	0.02
0.981	-	0.994	-	0.125	0.003	0.307	0.044	0.980	-	0.986	-	0.016	0.008	0.037	0.024	8.03	0.01
0.969	0.036	0.944	0.093	0.094	0.010	0.288	0.008	1.936	0.115	1.888	0.022	0.069	0.003	0.105	0.035	8.05	0.02
1.038	-	0.995	-	0.119	0.010	0.346	0.033	3.869	-	3.687	-	0.098	0.013	0.135	0.022	8.05	0.02
0.995	0.036	1.098	0.018	0.103	0.019	0.327	0.017	5.776	0.166	5.468	0.248	0.071	0.017	0.114	0.025	8.05	0.03
0.997	0.008	0.819	0.009	0.131	0.014	0.433	0.030	7.792	0.159	7.328	0.124	0.156	0.015	0.322	0.054	8.32	0.01

Table 5-A Percentage of Ni and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni

Mn (ppm)								Ni (ppm)							
INF				% Removal				INF				% Removal			
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD
0.885	0.021	0.933	0.057	89.51	1.52	65.06	3.41	No Ni added		No Ni added		-	-	-	-
0.981	-	0.994	-	87.36	0.35	69.08	4.46	0.980	-	0.986	-	98.63	0.97	96.69	2.34
0.969	0.036	0.944	0.093	90.34	1.08	69.43	0.91	1.936	0.115	1.888	0.022	96.43	0.20	95.11	1.00
1.038	-	0.995	-	88.54	1.04	65.21	3.39	3.869	-	3.687	-	97.46	0.35	96.34	0.62
0.995	0.036	1.098	0.018	89.89	1.92	69.82	1.64	5.776	0.166	5.468	0.248	98.77	0.30	97.92	0.45
0.997	0.008	0.819	0.009	86.83	1.50	47.08	2.72	7.792	0.159	7.328	0.124	98.00	0.20	95.29	1.00

Table 6-A Removal of Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Cd

Mn (ppm)								Cd (ppm)								pH	SD
INF				EFF				INF				EFF					
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD		
0.950	0.040	0.873	0.004	0.171	0.033	0.512	0.057	No Cd added		No Cd added		-	-	-	-	8.33	0.01
0.978	-	0.943	-	0.213	0.013	0.625	0.029	1.020	-	0.927	-	0.001	0.002	0.013	0.008	8.26	0.05
0.918	-	1.000	-	0.263	0.029	0.619	0.006	2.070	-	1.928	-	0.009	0.004	0.058	0.018	8.12	0.06
0.926	0.017	1.025	0.038	0.216	0.037	0.489	0.062	3.844	0.227	4.206	0.291	0.051	0.021	0.118	0.018	8.07	0.01
0.964	0.091	1.002	0.029	0.312	0.028	0.600	0.028	5.894	0.056	5.882	0.028	0.083	0.005	0.191	0.028	8.04	0.03
0.997	-	0.985	-	0.319	0.022	0.575	0.044	8.201	-	8.050	-	0.099	0.030	0.256	0.015	7.87	0.09

Table 7-A Percentage of Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Cd

Mn (ppm)								Cd (ppm)							
INF				% Removal				INF				%Removal			
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD
0.950	0.040	0.873	0.004	80.72	4.47	41.37	6.62	No Cd added		No Cd added		-	-	-	-
0.978	-	0.943	-	78.08	0.32	34.76	2.35	1.020	-	0.927	-	99.88	0.21	98.62	0.86
0.918	-	1.000	-	71.41	3.22	38.10	0.69	2.070	-	1.928	-	99.11	1.03	96.71	1.06
0.926	0.017	1.025	0.038	76.62	3.99	52.29	6.06	3.844	0.227	4.206	0.291	98.86	0.65	97.19	0.44
0.964	0.091	1.002	0.029	67.65	2.99	40.10	2.88	5.894	0.056	5.882	0.028	98.59	0.09	96.75	0.49
0.997	-	0.985	-	67.99	2.24	41.64	4.55	8.201	-	8.050	-	98.62	0.52	97.12	0.78

Table 8-A Removal of Ni, Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni and Cd

Mn (ppm)				Ni (ppm)				Cd (ppm)				pH
INF		EFF		INF		EFF		INF		EFF		
Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	(SD)
0.804	0.847	0.219	0.334	No Ni	No Ni	-	-	No Cd	No Cd	-	-	7.82
(-)	(-)	(0.025)	(0.044)	added	added	(-)	(-)	added	added	(-)	(-)	(0.03)
0.943	0.886	0.209	0.425	0.898	0.877	0.060	0.226	0.887	0.912	0.119	0.252	7.90
(-)	(-)	(0.021)	(0.036)	(-)	(-)	(0.016)	(0.018)	(-)	(-)	(0.010)	(0.015)	(0.02)
0.855	0.901	0.248	0.472	1.804	1.792	0.108	0.265	1.882	1.898	0.139	0.260	7.89
(0.048)	(0.003)	(0.031)	(0.023)	(0.013)	(0.002)	(0.014)	(0.011)	(0.014)	(0.038)	(0.014)	(0.010)	(0.02)
0.916	1.015	0.349	0.553	3.964	3.336	0.256	0.369	5.130	4.732	0.219	0.347	7.85
(-)	(-)	(0.035)	(0.021)	(-)	(-)	(0.060)	(0.045)	(-)	(-)	(0.035)	(0.033)	(0.02)

Table 9-A Percentage of Ni, Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on magnetite particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni and Cd

Mn (ppm)				Ni (ppm)				Cd (ppm)			
INF		% Removal		INF		% Removal		INF		% Removal	
Soluble (S.D.)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)
0.804	0.847	72.71	60.51	No Ni	No Ni	-	-	No Cd	No Cd	-	-
(-)	(-)	(3.14)	(5.27)	added	added	(-)	(-)	added	added	(-)	(-)
0.943	0.886	77.05	51.96	0.898	0.877	93.27	74.38	0.887	0.912	85.96	72.42
(-)	(-)	(1.72)	(4.10)	(-)	(-)	(1.75)	(2.19)	(-)	(-)	(1.65)	(1.75)
0.855	0.901	72.05	47.58	1.804	1.792	93.78	85.20	1.882	1.898	92.57	86.38
(0.048)	(0.003)	(3.05)	(2.59)	(0.013)	(0.002)	(0.91)	(0.62)	(0.014)	(0.038)	(0.78)	(0.54)
0.916	1.015	61.90	45.54	3.964	3.336	93.45	88.93	5.130	4.732	95.74	92.66
(-)	(-)	(3.85)	(2.05)	(-)	(-)	(1.37)	(1.36)	(-)	(-)	(0.68)	(0.70)

Table 10-A Removal of Ni and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni

Mn (ppm)								Ni (ppm)								pH	SD
INF				EFF				INF				EFF					
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD		
0.998	0.026	1.233	0.016	0.112	0.006	0.168	0.007	No Ni added		No Ni added		-	-	-	-	8.05	0.056
0.893	0.018	1.068	0.153	0.092	0.008	0.162	0.013	0.943	0.118	0.857	0.177	0.020	0.006	0.074	0.007	7.96	0.014
1.010	0.156	1.239	0.088	0.128	0.011	0.163	0.007	2.016	0.054	1.908	0.078	0.052	0.014	0.071	0.008	7.96	0.030
0.951	0.051	1.128	0.045	0.125	0.019	0.187	0.021	3.831	0.030	3.665	0.020	0.050	0.011	0.081	0.007	7.85	0.026
0.986	0.006	1.150	0.033	0.148	0.008	0.184	0.011	5.702	0.308	5.362	0.229	0.169	0.018	0.197	0.038	7.86	0.018
0.960	0.044	1.371	0.201	0.190	0.024	0.251	0.011	7.725	0.249	7.467	0.030	0.303	0.025	0.376	0.013	7.79	0.017

Table 11-A Percentage of Ni and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni

Mn (ppm)								Ni (ppm)							
INF				% Removal				INF				% Removal			
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD
0.998	0.026	1.233	0.016	87.98	0.525	85.77	0.582	No Ni added		No Ni added		-	-	-	-
0.893	0.018	1.068	0.153	89.21	0.849	84.13	1.257	0.943	0.118	0.857	0.177	97.80	0.721	91.78	0.855
1.010	0.156	1.239	0.088	87.33	1.052	86.82	0.609	2.016	0.054	1.908	0.078	97.42	0.689	96.54	0.649
0.951	0.051	1.128	0.045	87.57	1.153	83.41	1.852	3.831	0.030	3.665	0.020	98.53	0.427	97.79	0.327
0.986	0.006	1.150	0.033	84.94	0.784	84.03	0.923	5.702	0.308	5.362	0.229	97.27	0.592	96.63	0.919
0.960	0.044	1.371	0.201	80.17	2.489	81.68	0.829	7.725	0.249	7.467	0.030	96.07	0.326	95.06	0.144

Table 12-A Removal of Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Cd

Mn (ppm)								Cd (ppm)								pH	SD
INF				EFF				INF				EFF					
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD		
0.933	0.058	1.016	0.121	0.150	0.011	0.258	0.023	No Cd added		No Cd added		-	-	-	-	7.89	0.032
0.912	0.042	1.024	0.145	0.174	0.019	0.226	0.019	1.114	0.211	0.952	0.076	0.000	-	0.063	0.007	7.86	0.015
0.865	0.038	1.169	0.048	0.173	0.013	0.245	0.008	2.049	0.122	1.892	0.127	0.012	0.004	0.077	0.005	7.80	0.018
1.008	0.029	1.001	0.084	0.195	0.009	0.238	0.022	3.986	0.136	3.807	0.134	0.034	0.012	0.073	0.006	7.70	0.063
0.973	0.075	1.067	0.177	0.265	0.034	0.313	0.042	6.067	0.076	5.749	0.198	0.047	0.011	0.069	0.001	7.64	0.059
1.028	0.043	1.122	0.113	0.402	0.023	0.445	0.019	8.070	0.210	7.816	0.235	0.069	0.014	0.113	0.020	7.52	0.044

Table 13-A Percentage of Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Cd

Mn (ppm)								Cd (ppm)							
INF				% Removal				INF				% Removal			
Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD	Soluble	SD	Total	SD
0.933	0.058	1.016	0.121	83.92	1.143	74.66	2.228	No Cd added		No Cd added		-	-	-	-
0.912	0.042	1.024	0.145	80.92	2.109	77.91	1.845	1.114	0.211	0.952	0.076	100.00	-	94.16	0.833
0.865	0.038	1.169	0.048	80.03	1.526	79.04	0.765	2.049	0.122	1.892	0.127	99.51	0.285	95.93	0.284
1.008	0.029	1.001	0.084	80.65	0.915	76.22	1.702	3.986	0.136	3.807	0.134	99.16	0.297	98.09	0.090
0.973	0.075	1.067	0.177	72.76	3.501	70.66	3.924	6.067	0.076	5.749	0.198	99.21	0.185	98.79	0.033
1.028	0.043	1.122	0.113	61.62	1.938	61.36	2.716	8.070	0.210	7.816	0.235	98.99	0.366	98.55	0.258

Table 14-A Removal of Ni, Cd and Mn by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni and Cd

Mn (ppm)				Ni (ppm)				Cd (ppm)				
INF		EFF		INF		EFF		INF		EFF		
Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	pH (SD)
0.929	1.139	0.300	0.389	No Ni	No Ni	-	-	No Cd	No Cd	-	-	7.69
(0.062)	(0.104)	(0.022)	(0.009)	added	added	(-)	(-)	added	added	(-)	(-)	(0.041)
0.893	1.242	0.412	0.558	1.006	0.781	0.029	0.063	1.132	0.920	0.000	0.073	7.63
(0.007)	(0.009)	(0.033)	(0.041)	(0.074)	(0.160)	(0.007)	(0.017)	(0.075)	(0.130)	(0.001)	(0.007)	(0.039)
0.950	1.114	0.451	0.544	2.008	1.841	0.050	0.117	2.119	1.922	0.020	0.099	7.64
(0.033)	(0.099)	(0.012)	(0.025)	(0.094)	(0.103)	(0.008)	(0.023)	(0.086)	(0.093)	(0.004)	(0.002)	(0.036)
0.989	1.107	0.486	0.590	4.245	3.891	0.080	0.104	4.350	4.046	0.035	0.152	7.52
(0.062)	(0.137)	(0.004)	(0.006)	(0.013)	(0.012)	(0.010)	(0.007)	(0.209)	(0.076)	(0.008)	(0.058)	(0.060)

Table 15-A Percentage of Ni, Cd and Mn removal by *Pedomicrobium manganicum* ACM 3067 immobilized on granular activated carbon (GAC) particles in the continuous recycle fluidized bioreactor (CRFB) when influent medium contained various concentrations of Ni and Cd

Mn (ppm)				Ni (ppm)				Cd (ppm)			
INF		%Removal		INF		%Removal		INF		%Removal	
Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)	Soluble (SD)	Total (SD)
0.929	1.139	67.75	65.85	No Ni	No Ni	-	-	No Cd	No Cd	-	-
(0.062)	(0.104)	(2.414)	(1.673)	added	added	(-)	(-)	added	added	(-)	(-)
0.893	1.242	52.55	53.78	1.006	0.781	97.06	91.91	1.132	0.920	99.95	94.59
(0.007)	(0.009)	(2.515)	(1.492)	(0.074)	(0.160)	(0.739)	(2.227)	(0.075)	(0.130)	(0.080)	(0.719)
0.950	1.114	52.50	51.69	2.008	1.841	97.62	94.06	2.119	1.922	99.16	95.75
(0.033)	(0.099)	(1.364)	(1.562)	(0.094)	(0.103)	(0.453)	(1.394)	(0.086)	(0.093)	(0.306)	(1.032)
0.989	1.107	50.83	46.70	4.245	3.891	97.93	97.29	4.350	4.046	99.19	96.23

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Vitae

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