中文摘要

隨著工業的發展，重金屬污染物已然造成許多環境上的問題，其主要污染源包括製革業、冶金業、電鍍業、積體電路業及印刷電路板業等產業，就新竹科學園區某印刷電路板業而言，其廢水中含有五種重金屬如銅、鋅、鎳、鉻及鎘，其中以銅、鋅、鎳的濃度相對較高。而利用硫酸還原菌 (SRB) 之生物沈澱技術產生不溶性金屬硫化物，以去除水中及廢水中之有毒重金屬具有很高的生物復育潛能。因此，本研究主要目的在於利用硫酸還原菌之生物沈澱技術去除混合重金屬如：銅、鋅、鎳，藉由混合實驗設計法探討重金屬對生物沈澱效能的影響，並藉由分子生物技術—螢光原位雜交法 (FISH)、聚合酶鏈鎖反應 (PCR) 及變性梯度膠體電泳法 (DGGE) 等方法，找出微生物菌相變化。

此研究主題主要分成二部分，首先利用混合實驗設計法探討三種不同濃度重金屬 (銅、鋅、鎳) 對於硫酸還原菌進行生物沈澱程序之影響，其次藉由螢光原位雜交法計算出硫酸還原菌佔總菌之百分比，並透過變性梯度膠體電泳法探究反應過程中微生物族群之變化。

研究結果顯示，當溶液中不存在重金屬銅及鎳離子，且鋅離子濃度高達 180 mg/L 時，其最大硫酸還原效率可高達 37%，且當水溶液中含有鋅離子時，其硫酸還原率皆較含銅及鎳時為高，由此結果可間接證明為何實驗所得之重金屬鋅去除率較銅及鎳離子高，而鋅離子的平均去除率可達

97%，最大去除率則近乎 100%。由混合實驗所得之等高線圖得知三種重金屬對 SRB 之毒性順序依序為銅 > 鎳 > 鋅。而由 FISH 實驗結果發現，當分析樣品中含有重金屬鋅時，會對本實驗所採用的探針專一性產生干擾，而影響特殊菌種的計數；而單由控制組分析結果可之，反應槽中總 bacteria (EUB338) 佔總微生物 (DAPI) 百分比約為 80%，而 SRB 族群佔總微生物族群約介於 55-60%。而單由控制組分析其染上 DAPI 的微生物計數結果發現，反應槽中總微生物數量約為 10^9 cell/mL。在確認實驗控制組及實驗組的 DGGE 分析結果中更發現，控制組在反應 14 天內微生物菌相幾乎維持不變，而實驗組中的分析結果中則可明顯看出在反應時間為 20 小時及 92 小時處，分別有不同種類的微生物產生及銷匿，顯示重金屬鋅的添加會引起 SRB 菌相的改變，使在重金屬存在的環境下亦能生長之 SRB 菌種成為優勢菌種。

關鍵字：生物沈澱、混合實驗設計法、硫酸還原菌、螢光原位雜交法、變性梯度膠體電泳法。

Abstract

Heavy metal contamination is a serious problem from various industries such as leather manufacturing industry, metallurgy industry, electroplating industry, integrated circuit industry and printed circuit board industry, etc. Printed circuit board industry wastewater is contaminated with five heavy metals such as copper, zinc, nickel, chromium and cadmium. Among these heavy metals contamination, copper, zinc and nickel are relatively higher concentration than cadmium and chromium. The sulfate-reducing bacteria (SRB) have bioremediation potential to precipitate toxic heavy metals from water and wastewater as highly insoluble metal sulfides. Therefore, the present investigations are to optimize the bio-precipitation of copper, zinc and nickel using sulfate-reducing bacteria by mixture design and examine the bacterial population using FISH, DGGE and PCR.

The objective of this research consists of two parts. In the first part, the heavy metals such as copper, zinc and nickel will be taken to study the effect on SRB during bioprecipitation process using mixture design. In the second part, bacteria population will be examined thoroughly using molecular techniques such as FISH, DGGE and PCR.

The results demonstrated that the sulfate reduction ratio was 37% in the absence of copper and nickel when the concentration of zinc was 180 mg/L. This finding concluded that the removal of zinc was significantly higher because of the higher sulfate-reduction activity than nickel and copper. The experiment results confirmed that the average removal of Zn^{2+} was 97% by sulfate reducing bacteria (SRB), and the maximum removal ratio was 100%. The contour plot also indicated that the toxic order of the three heavy metals were: Cu^{2+} > Ni^{2+} > Zn^{2+}. By the experimental result of FISH, we can find that the ratio of EUB338/DAPI was about 80%. And the SRB/DAPI ratio was between 55% and 60%. From the calculation of DAPI, we can also find that the microorganism number of the reactor were 10^9 cells/mL. From the photograph of DGGE, we can also find that the bacterial population of control was almost constant during the period of reaction time. However in the experimental reactor, the bacterial population has altered at the 20 and 92 hour.

Key words: bioprecipitation, mixture design, SRB, FISH, DGGE.