

Full Length Research Paper

# Degradation of morpholine by *Mycobacterium* sp. isolated from contaminated wastewater collected from Egypt

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Accepted 13 June, 2011

The biodegradation of morpholine has attracted much interest because morpholine causes environmental pollution. Ten species belonging to nine genera were tested for their abilities to degrade morpholine in mineral salts medium containing morpholine (1 g/l). *Mycobacterium* sp. isolated from polluted water sample collected from Abu Za'baal lakes, effectively utilized morpholine as carbon, nitrogen and energy source. The tested *Mycobacterium* was able to grow in high concentrations of morpholine but the rapidly increase in pH of the growth medium and accumulation of ammonia inhibited bacterial growth and complete mineralization of morpholine. The molar conversion ratio of morpholine to ammonia was 1:0.89. Growing of the selected bacterium in liquid medium with 1 g/l morpholine at 37°C and pH 6.5, enhanced morpholine degradation. Addition of metyrapone to the growth medium inhibited morpholine degradation. Immobilization of *Mycobacterium* cells in sodium alginate increased morpholine degradation compared with free cells. At high concentrations of morpholine (4 to 6 g/l), there was a decrease in both cell viability and respiration of *Mycobacterium* but no genotoxicity was found.

**Key words:** Morpholine, *Mycobacterium*, biodegradation, pollution, ammonia, cytochrome P450, metyrapone, immobilization.

## INTRODUCTION

The heterocyclic xenobiotic compound morpholine (1-oxa-4-azacyclohexane) is of great importance for different industrial purposes (Mijos, 1978). Morpholine (C<sub>4</sub>H<sub>9</sub>NO) is a colourless, oily, hygroscopic, volatile liquid with a characteristic amine smell and it is completely miscible with water. Because of its wide range of applications, morpholine occurs in the environment, detected in foods (Mohri, 1987) and water. Thus, the removal of morpholine from contaminated industrial waste waters is of environmental interest. Unfortunately, Calamari et al. (1980) and Tölgyessy et al. (1986) both reported the resistance of morpholine to biodegradation. In contrast, many authors have indicated that bacteria utilize morpholine as sole source of carbon, nitrogen and energy (Subrahmanyam et al., 1983). Knap et al. (1982) first discovered two strains of *Mycobacterium* (MorD and MorG) that were able to utilize morpholine as a sole source of carbon, nitrogen and energy. A few years later,

Cech et al. (1988) found a strain of *Mycobacterium aurum* MO1 that had morpholine degradation properties. Knapp's group studied other *Mycobacterium* strains isolated from activated sludges (Knapp and Whytell, 1990). Aly (2004) attributed the decrease in morpholine degradation to ammonia accumulation in the growth medium. The aims of this study were selection of the most active bacterium in morpholine degradation and studying factors affecting the degradation process. Growths of the selected bacterium using either intermediate or heterocyclic compounds similar to morpholine were also studied.

## MATERIALS AND METHODS

### Chemicals used

Morpholine, glycolic acid, ethanolamine, piperidine, pyrrolidine,