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REVIEW PAPER

POTENTIAL BIOLOGICAL PROCESSES AVAILABLE FOR REMOVAL OF NITROGENOUS COMPOUNDS FROM METAL INDUSTRY WASTEWATER

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Suboptimal pH and high nitrogenous compounds content in metal industry wastewaters often make using traditional biological treatment methods problematic. It is too early to draw conclusions as to the best technology by comparing novel processes such as OLAND, CANON, SHARON, and ANAMMOX and so on, owing to the fact that most of these new processes are still to be fully tested at pilot and commercial scale. Therefore, it is worthwhile to review the novel nitrogenous compounds removal processes and to look at their potential for use in biological treatment of inorganic wastewaters with reference to the metal industry. The biochemical reactions and enzymes involved in each step of the nitrification and denitrification processes, microbiology of each process, different technologies such as OLAND, CANON, SHARON, ANAMMOX and their operational requirements in evaluating the application of the nitrification–denitrification process to metal industry wastewater are discussed in this review.

Keywords: nitrification; denitrification; metal industry wastewater; OLAND; CANON; SHARON; ANAMMOX.

INTRODUCTION

Usually metal refinery wastewater contains nitrogen as a result of the use of nitrogenous compounds during production processes (e.g., ammonium hydroxide as a precipitant and ammonium sulphate as an ion exchange resin eluent). Therefore metal industry wastewater must be treated properly prior to discharge to the environment, according to the minimum standards set by government monitoring and regulating agencies. Nitrogenous compounds lead to eutrophication of environmental waters if discharged without sufficient treatment and prevent the recovery of metals from the effluent, as some of the nitrogenous compounds (e.g., nitrates) are strong metal ligands. However, treatment of metal industry wastewaters is somewhat complicated by their inherent characteristics and variablility, as these inhibit the traditional biological treatment processes used in municipal wastewater treatment. Low pH and high ammonium and nitrate concentrations (Koren *et al.*, 2000) are typical characteristics of mineral and metal processing wastewaters. Further, high ammonium or nitrite concentrated metal industry wastewater inhibits the nitrification process in conventional municipal wastewater treatment (Carrera *et al.*, 2003).

Removing the nitrogenous compounds from wastewater usually involves a two-step biological process, namely nitrification–denitrification, by transforming the nitrogenous compounds to dinitrogen gas (Maier *et al.*, 2000). The nitrification process is mainly carried out by autotrophic aerobic bacteria, while denitrification is mainly carried out by anoxic heterotrophic bacteria. Therefore, the use of a two-step reactor system is common practice, which enables independent control of each process. As nitrification is carried out by autotrophic nitrifying bacteria, the presence or addition of organic matter inhibits the growth of nitrifying bacteria by allowing the heterotrophic bacteria which compete for other nutrients contained in the reactor to dominate, and ultimately nitrification is inhibited. As nitrification is an aerobic process and denitrification is anoxic, perhaps it is wise to operate the two processes independently.

In order to find an appropriate technique for treatment of metal industry wastewaters, factors to consider include the chemical (oxidation state, ionization energy), physical (precipitation, solubility) and biological (microbial response to different metal concentration, accumulation of metals by metabolically active biomass) properties of metals, characteristics and composition of the metal industry wastewater, seasonal composition variations, suitable species of microbes and their optimum environmental conditions, reactor types to be used and their operational characteristics. Therefore, in implementing technologies for treating nitrogenous metal industry wastewater, it is

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necessary to consider both the optimum operational environment and the operational cost of those technologies. This may achieve the dual objectives of meeting the regulating standards and the recovery of metals from the effluent, which would otherwise be wasted. Further, it would ensure the maximum recycling of treated wastewater, minimizing the demand for potable water. Therefore, the objectives of this paper are to present the process selection aspects of removal of nitrogenous compounds from metal industry wastewaters, to review the microbial types used, removal efficiency and reactor types in early biological treatment processes and to identify the critical parameters to be considered in selecting and tailoring a bioreactor type for removing nitrogenous compounds from metal industry wastewaters.

PROCESS MICROBIOLOGY

As previously mentioned, nitrification–denitrification is a two-step process. First, ammonium is converted to nitrite by ammonium oxidizing bacteria (AOB). Then, nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB). Conversion of ammonium into nitrate is known as nitrification. In the denitrification process, nitrate is converted to dinitrogen gas in two steps by denitrifying bacteria, first nitrate to nitrite and then nitrite to dinitrogen gas. The overall process can be summarized as shown in Figure 1.

Chemolitho-autotrophic AOB, which have the ability to utilise ammonia as a sole source of energy and carbon dioxide as the main source of carbon, are responsible for the rate-limiting step of nitrification. Generally, AOB are obligate aerobes, but there are some species that may be highly tolerant to low oxygen or anoxic environments (Kowalchuck and Stephen, 2001; van de Graaf *et al.*, 1995). Ammonia oxidizing bacteria were classified into different genera based on cell morphology, such as *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosovibrio* and *Nitrosolobus*. However, based on 16S rRNA sequence homology, *Nitrosospira, Nitrosovibrio* and *Nitrosolobus* have since been proposed to combine into one common genus *Nitrosospira* (Bothe *et al.*, 2000). Nitrifying bacteria (aerobic ammonia and nitrite oxidizers) belong to a very restricted group of autotrophs. *Nitrosomonas* and *Nitrosospira* are the best known ammonium oxidizers (Schramm *et al.*, 1998) while *Nitrobacter* (Burerell *et al.*, 1998) and *Nitrospira* are the most well known nitrite oxidisers (Sliekers *et al.*, 2002).

Egli *et al.* (2003) reported that the AOB subgroups, *Nitrosomonas eutropha* and *Nitrosomonas europea*, were observed only at pH 7.5 and 30°C. Further, they suggested that pH is more important than temperature in selecting *N. eutropha* and *N. europea*. AOB produce large quantities of extracellular polysaccharides (EPS) when growing in surface biofilm communities. High EPS bacteria have a greater tolerance to low pH (Hesselsøe and Sorensen, 1999). Ammonia oxidizing bacteria populations tend to reside in the more external regions of flocs or biofilms, where oxygen levels are typically high, whereas NOB typically reside internally and adjacent to AOB, where lower oxygen conditions are detected. Nitrification is mainly limited to the outer 100–150 μm of flocs or films (Schramm *et al.*, 1998; Kowalchuck and Stephen, 2001).

Denitrifiers belong to a diverse group of facultative anaerobic bacteria which have the ability to use nitrogen oxides (nitrate and nitrite) as electron acceptors, and produce N_2 as the end product (Etchebehere *et al.*, 2001). Denitrifying bacteria are spread in well diversified genera with a spectrum of heterotrophic and autotrophic metabolism (Szekeres *et al.*, 2002). The denitrifying bacteria most frequently isolated from soil belong to the genera *Alicaligenes* and *Pseudomonas*. However, predominant members isolated from activated sludge belong to the *Rubrivivax* subgroup in the β-purple subdivision, the *Rhodobacter* group in the α-purple subdivision, and the *Pseudomonas* subgroup in the γ-purple subdivision (Martienssen *et al.*, 1999; Etchebehere *et al.*, 2001).

The *Planctomycetales* effect anaerobic nitrification and denitrification processes. The order *Planctomycetales* includes four genera: *Planctomyces*, *Pirellula*, *Gemmata*, and *Isosphaera* (Schmidt *et al.*, 2002). Two species of *Planctomyces* are *Brocadia anammoxidans* and *Kuenenia stuttgartiensis* (Fujii *et al.*, 2002). Under oxygen-limited conditions, the AOB oxidise ammonium to nitrite and keep oxygen concentrations low, while *B. anammoxidans* convert the produced nitrite and the remaining ammonium to dinitrogen gas (Schmidt *et al.*, 2002). This sequence has been used in different reactors systems such as the oxygen limited autotrophic nitrification denitrification process—OLAND (Kuai and Verstraete, 1998) and completely autotrophic nitrogen removal over nitrite—CANON (Third *et al.*, 2001; Sliekers *et al.*, 2002).

Both mixed and pure cultures of *Nitrosomonas eutropha* are capable of denitrification under oxygen limited conditions. According to Jetten *et al.* (2001), in the presence of nitrogen dioxide (NO_2), the anaerobic activity of *N. eutropha* was boosted to 2.2 nmol/NH_4^+/min/mg protein. However, this is 50-fold slower than the obligate anaerobe, *B. anammoxidans* and 200 times slower than the aerobic activity of *N. eutropha* itself (Jetten *et al.*, 2001).

Table 1 presents a summary of bacteria and enzymes involved in nitrification and denitrification processes. Figure 2 illustrates nitrification–denitrification in terms of process steps, oxygen conditions, participating microorganisms, and their trophic level.

Metal toxicity to microbes plays an important role in metal industry wastewater treatment. The toxicity of

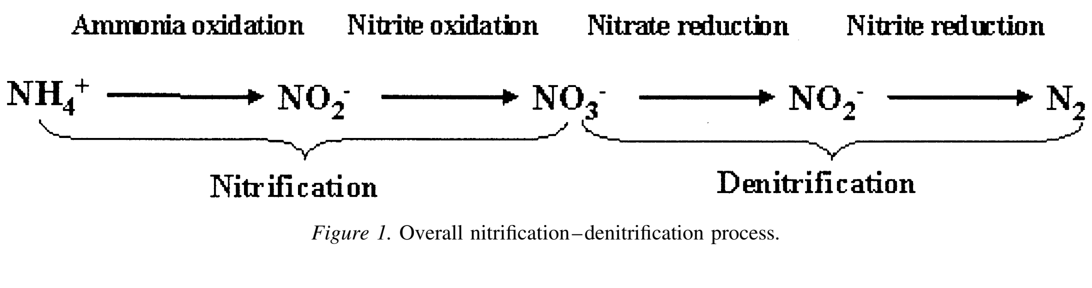

Figure 1. Overall nitrification–denitrification process.