Denitrification by heterotrophic bacteria during activated sludge treatment

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Abstract

It is generally accepted that *Pseudomonas* spp. are the predominant heterotrophic bacteria involved in denitrification during activated sludge treatment. However, uncertainty still exists regarding other bacteria involved. This study therefore aimed to determine which heterotrophic bacteria present in mixed liquor samples from a biological nutrient removal process are responsible for denitrification as well as to establish the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. Heterotrophic bacteria were isolated, using plating techniques, from the anoxic zone of the Darvill activated sludge process and assessed for nitrate and nitrite reduction under anoxic conditions. Results show a significant involvement of *Pseudomonas* spp. in nitrate and nitrite reduction. It was also found that many other heterotrophic bacteria are involved to some extent in denitrification, most of which were found to be incomplete denitrifiers only capable of reducing nitrates to nitrites with no further reduction of the nitrites produced. Furthermore, results demonstrated varying strengths of nitrate and nitrite reduction amongst the isolated heterotrophic bacteria, possible simultaneous oxygen and nitrate respiration by many incomplete denitrifiers as well as involvement of gram-positive rods and gram-negative cocci.

Introduction

The activated sludge process is the most widely applied biological wastewater treatment process in the world. Originally the process was designed as a single aerobic reactor for the removal of organic matter from wastewater but it has since been significantly developed to enhance its nutrient removal capabilities (Lu and Leslie Grady (Jr), 1988; Gray, 1990; Ekama et al., 1992; Wentzel et al., 1992). These improvements were induced by modifying the process from a single aerobic reactor to multi-reactor processes consisting of anaerobic, anoxic and aerobic zones with inter-reactor recycles thus enabling the process to progressively include nitrification, denitrification and phosphorus removal (Wentzel et al., 1992). These nitrification/denitrification/biological excess phosphorus removal processes are referred to as biological nutrient removal (BNR) processes and are currently being designed and implemented worldwide using established mathematical models and related software (Gujer and Kappler, 1992). These models provide very accurate information regarding process design and performance and can result in the development or simulation of effective BNR processes. However, according to Henze (1992) and Kristensen et al. (1992), these activated sludge models fall short in that they do not take into consideration the structure of biomass present in the process. Success of an activated sludge process is ultimately dependent on the functions of the constituent micro-organisms as well as the related process parameters (e.g. anaerobiosis, anoxia, aerobiosis) affecting microbial growth and activity (Simpkin, 1988; Bux et al., 1994). It is therefore believed that inadequate control of the micro-organisms in the activated sludge process is responsible for many variations in process performance. This is due to a lack of understanding of the ecological, physiological and biochemical activities of these micro-organisms which is resulting in growing movement towards a better understanding in order to gain optimal control of the process (Lu and Leslie Grady Jr, 1988; Davelaar, 1989; Wagner et al., 1993; Jansen et al., 1994; Hu et al., 1996; Satoh et al., 1996; Hippen et al., 1997).

Denitrification by heterotrophic bacteria in activated sludge treatment is of particular interest in that nitrates and nitrites are eutrophic (Gray, 1990), hazardous to human health (Terblanche, 1991; Kempster et al., 1997) as well as inhibit phosphorus removal during activated sludge treatment (Gruenebaum and Dorgeloh, 1992; Kuba et al., 1996). Furthermore, denitrifying heterotrophic bacteria are often implicated in enhanced biological phosphorus removal (EBPR) both under aerobic as well as anoxic conditions (Osborn et al., 1989; Kuba et al., 1993; Kavanaugh and Randall, 1994; Jørgensen and Pauli, 1995; Kuba et al., 1997;). In a BNR process denitrification is achieved in the anoxic zone/s of the process. Under anoxic conditions certain heterotrophic bacteria are stimulated into utilising nitrates and nitrites as final electron acceptors for cellular respiration in place of oxygen (Ketchum, 1988; Cappuccino and Sherman, 1992). This results in oxidation of organic matter as well as reduction of the nitrates and nitrites into nitrous oxides and nitrogen gas (Wanner and Grau, 1988).

In the wastewater industry uncertainty exists regarding the bacteria involved in denitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. It is generally presumed that *Pseudomonas* spp., as well as being involved in EBPR (Osborn et al., 1989; Kavanaugh and Randall, 1994; Jørgensen and Pauli, 1995), are the predominant micro-organisms through which denitrification is achieved (Janda et al., 1988; Gray, 1990; Lazarova et al., 1992). According to Otlanabo (1993) various species of *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Hyphomicrobium*, *Pseudomonas*, *Vibrio* and others are responsible for denitrification in soil. It therefore seems unlikely that only *Pseudomonas* spp. are responsible for denitrification occurring in such an incredibly diverse microbial consortia as that of activated sludge.

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