The effect of media on evaluating the phosphate uptake capacity of activated sludge bacterial isolates

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Abstract

Since the identification of *Acinetobacter calcoaceticus* as the primary agent responsible for biological phosphorus removal, much research has been conducted to either confirm or refute this claim. The majority of these studies was conducted under defined laboratory conditions using various artificial media; as a result, many conflicting reports exist regarding the role of *A. calcoaceticus* and other activated sludge isolates in their possible roles as true polyphosphate-accumulating bacteria. This study was therefore conducted to determine the effect of media type on biological phosphate removal when performed in the laboratory under defined conditions. Five isolates were obtained from a full-scale biological nutrient removal activated sludge system and a previously identified *A. calcoaceticus* isolate was inoculated into three different media types. The inoculated media were subjected to anaerobic and aerobic conditions to stimulate the phosphate removal mechanism. The test micro-organisms released phosphate, under anaerobic conditions, in the three test media. Synthetic wastewater was found to promote the release of higher concentrations of phosphate by the micro-organisms than in the other two media. During the aerobic phase, phosphate uptake was highest in synthetic wastewater and settled sewage. Due to inconsistencies obtained in the readily biodegradable chemical oxygen demand, total chemical oxygen demand and soluble oxygen demand tests, it was concluded that the medium formulation may skew qualitative or quantitative data regarding biological phosphate removal. It can be concluded that the media used for P uptake studies are of importance as it was observed that the organisms' removal/uptake capabilities were enhanced or reduced depending on the media used.

Introduction

Enhanced biological phosphorus removal (EBPR), in an activated sludge system, is a process used to remove phosphorus from wastewater before being discharged into natural water bodies. Removal of phosphate (P) is of significance in preventing eutrophication (excessive algal growth and related negative consequences) of inland water systems. EBPR is achieved by mixing activated sludge, containing phosphate-accumulating organisms (PAOs) with the wastewater, and subjecting the activated sludge to alternating anaerobic and aerobic conditions (Fuhs and Chen, 1975).

Preconditioning of bacteria for P uptake in excess of what is deemed as normal metabolic requirements under aerobic conditions, occurs as activated sludge passes through the anaerobic zone situated at the head of the biological wastewater treatment systems. (Muyima et al., 1997). During anaerobiosis, PAOs take up short-chain fatty acids and store them intracellularly as polyhydroxy alkonates (PHA). Hydrolysis of polyphosphate (polyP) to orthophosphate, which is subsequently released to the external environment, and simultaneous degradation of intracellular glycogen are thought to provide the required energy for this process (Smolders et al., 1994; Nielsen et al., 1999).

The aerobic zone provides an environment in which P previously released in the anaerobic zone and P entering the plant in the feed sewage can be removed biologically from the biomass solution (Pitman, 1984). PAOs utilise stored PHA to take up soluble P to restore polyP and glycogen pools, during the aerobic phase (Nielsen et al., 1999). In most bacteria, polyP serves as a phosphorus pool during periods of P starvation. In some bacterial strains, polyP also acts as an energy source similar to organic storage products (PHB and glycogen). The role of polyP as intracellular energy storage material is decisive for the mechanism of EBPR, as well as for the metabolic selection of bacterial species in activated sludge systems within the anaerobic zone (Muyima et al., 1997).

Members of the *Acinetobacter* spp. were first proposed as the bacteria responsible for EBPR (Fuhs and Chen, 1975). Subsequently, many researchers reported the predominance of these organisms in EBPR processes based on culture-dependent identification methods (Lötter, 1985; Wentzel et al., 1986).

Isolation of PAOs has been reported to be problematic due to their slow growth rates. In environments with diverse microbial populations and limited organic resources, it is possible that PAOs are often out-competed by organisms capable of utilising available nutrients more rapidly. Another limiting factor in the isolation of PAO species from activated sludge is the lack of suitable media that would support the growth of all viable nutritional types (Muyima et al., 1997). It has been reported that isolated PAOs do not show the same characteristics in their P uptake capabilities under laboratory conditions, compared with full-scale *in situ* studies (Van Loosdrecht et al., 1997). A possible explanation could be that in their natural environment the organisms are continuously exposed to changes in critical factors such as the nature and concentration of substrates. However, laboratory conditions imposed on the organism may differ vastly from conditions in a full-scale EBPR plant. Therefore, micro-organisms may behave differently in the laboratory environment when compared to those in a full-scale EBPR activated sludge plant (Muyima and Cloete, 1995).

Many full-scale EBPR process plants have demonstrated the ability to reduce P concentrations to very low levels. However, studies done under defined and controlled laboratory conditions were unsuccessful (Nielsen et al., 1999; Cech and Hartman, 1990). In most laboratory experiments, batch tests are normally done to

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