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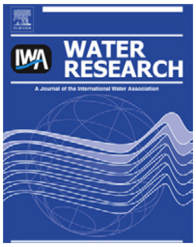

Biologically induced phosphorus precipitation in aerobic granular sludge process

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ABSTRACT

Aerobic granular sludge is a promising process for nutrient removal in wastewater treatment. In this work, for the first time, biologically induced precipitation of phosphorus as hydroxyl-apatite ($Ca_5(PO_4)_3(OH)$) in the core of granules is demonstrated by direct spectral and optical analysis: Raman spectroscopy, Energy dispersive X-ray (EDX) coupled with Scanning Electron Microscopy (SEM), and X-ray diffraction analysis are performed simultaneously on aerobic granules cultivated in a batch airlift reactor for 500 days. Results reveal the presence of mineral clusters in the core of granules, concentrating all the calcium and considerable amounts of phosphorus. Hydroxyapatite appears as the major mineral, whereas other minor minerals could be transiently produced but not appreciably accumulated. Biologically induced precipitation was responsible for 45% of the overall P removal in the operating conditions tested, with pH varying from 7.8 to 8.8. Major factors influencing this phenomenon (pH, anaerobic phosphate release, nitrification denitrification) need to be investigated as it is an interesting way to immobilize phosphorus in a stable and valuable product.

1. Introduction

Phosphorous is a key nutriment for the development of life, constituting one of the major nutrients necessary for agricultural activity. However, the quantities of mineral phosphorus resources (phosphate rock) are decreasing in the world, making phosphorus recovery necessary in the coming century. On the other hand, the high phosphorus and nitrogen content of wastewaters leads to serious problems of eutrophication in ponds, rivers and seas. Therefore, research is now focusing increasingly on combined processes that remove phosphorous from wastewaters and simultaneously recover it in the form of a valuable product, for example, struvite or hydroxyapatite (De-Bashan and Bashan, 2004; Shu et al., 2006; Suzuki et al., 2006). Phosphorous recovery techniques are particularly suited to high strength wastewaters produced by anaerobic sludge digestion (Demirel et al., 2005; Lemaire, 2007). Calcium or magnesium phosphates can be formed by crystallization and recovered in specific reactors via pH control and chemical dosing (Seckler et al., 1996; Katsuura et al., 1998; Münch and Barrm, 2001; Giesen, 1999; Baur et al., 2008). The spontaneous phenomenon has been reported to cause economic damage related to pipe clogging when it is not controlled (van Rensburg et al., 2003). In activated sludge systems, biologically induced

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Nomenclature

DO	dissolved oxygen mg/L
PAO	polyphosphate accumulating organisms
VFA	volatile fatty acids
EBPR	enhanced biological phosphate removal
SI	supersaturation Index Log Ω
STR	struvite $MgNH_4PO_4 \cdot 6H_2O$
HAP	hydroxyapatite $Ca_5(PO_4)_3(OH)$
DCPD	brushite $CaHPO_4 \cdot 2H_2O$
ACP	amorphous calcium phosphate $Ca_3(PO4)_2$
HDP	hydroxy dicalcium phosphate $Ca_2HPO_4(OH)_2$
Ω	supersaturation ratio
IAP	ionic activity product
K'	conditional solubility product
K_p	thermodynamic equilibrium of precipitation constant
TN	total nitrogen mgN-/L
MLSS	mixed liquor suspended solids g/L
MLVSS	mixed liquor volatile suspended solids g/L
CAL	calcite $CaCO_3$
MAG	magnesite $MgCO_3$
WHT	whitlockite $Ca_{18}Mg_2H_2(PO_4)_{14}$
OCP	octacalcium phosphate $Ca_8(HPO_4)_2(PO_4)_4$
Ü	raman shift (cm^{-1})
PCA	cold perchloric acid

phosphate precipitation has also been reported but less investigated (Maurer et al., 1999; Pambrun, 2005; De Kreuk et al., 2005). Calcium phosphate precipitation is thought to contribute to P removal in Enhanced Biological Phosphorous Removal processes (EBPR) and it is considered to enhance biological P removal efficiency (Maurer et al., 1999). Local precipitation is naturally induced when the pH and ion concentrations lead to mineral supersaturation. In the case of calcium or magnesium phosphate, their formation can be caused by phosphate release due to Polyphosphate Accumulating Organisms (PAO) during the anaerobic phase, but also clearly depends on pH. Bioreactions (e.g. nitrification and denitrification) or aeration (CO_2 stripping) lead to pH gradients which can be responsible for mineral precipitation in biological sludge (Pambrun, 2005; Bogaert et al., 1997; Saidou et al., 2009; Zhu et al., 2007). These processes still need to be clarified in granular sludge systems.

The aerobic granular sludge process is a promising technology for wastewater treatment because of its small footprint and capacity to treat high organic loading rates and its simultaneous nutrient removal through nitrification, denitrification and BioP accumulating processes (Morgenroth et al., 1997; Etterer and Wilderer, 2001; De Kreuk et al., 2005; Lemaire, 2007). The dense-spherical structure of granules leads to transfer limitations (Liu and Tay, 2004; Adav et al., 2008), promoting not only DO gradients but also local pH gradients coming from biological reactions, especially in the case of enhanced denitrification (Wan and Sperandio, 2009; Wan et al., 2009). As phosphate accumulating bacteria can also be present inside the granules (Lemaire, 2007), anaerobic phosphate release can encourage P precipitation within the core of the microorganisms, where subsequent solubilization of the crystals would be more difficult than in the bulk. Phosphate precipitation in a granular sludge process was assumed (but not directly demonstrated) by Yilmaz et al. (2007), De Kreuk et al. (2005) and De Kreuk and van Loosdrech (2007). By estimating its supersaturation index, Yilmaz et al. (2007) suggested that struvite could be transiently formed during the anaerobic phase of the SBR cycle. The contribution of this process to overall P removal was estimated to be less than 10 percent on the basis of a perchloric acid extraction method (Haas et al., 1988; Daumer et al., 2008). Similarly, experimental results by De Kreuk et al. (2005) suggest that P-removal occurs partly by biologically induced precipitation in granular sludge. Extraction techniques indicated that 2.6% of the sludge mass was due to precipitates (P/VSS), but the whole contribution of this process compared to biological P removal processes was not quantified. For simplicity, precipitation was not included when modelling the process but De Kreuk and van Loosdrech (2007) proposed to increase the maximum fraction of polyphosphate in PAO from 0.35 (Hu et al., 2002) to 0.65 assuming that about 46% of the P removal could be due to P precipitation and 54% due to polyphosphate accumulating bacteria. Recently, Maurer et al. (1999) have proposed a model for naturally induced P precipitation in activated sludge, which is based on the assumption that hydroxyapatite (HAP) and hydroxydicalcium phosphate (HDP) are formed. The model can predict calcium and phosphate concentrations at different pH. However, in all these studies, phosphate minerals formed in biological granules or flocs have never been directly characterized, and the nature of the phosphate precipitate is not demonstrated but only indirectly deduced from stoichiometry of soluble species.

The characterization of precipitates inside aerobic granules is still a relatively unexplored field. Minerals involved in phosphorus immobilization have been poorly qualified in biological sludge because traditional techniques (like X-ray diffraction) are difficult to apply directly in such organic matrices (Cloete and Oosthuizen, 2001). SEM-EDX analysis has recently been applied to determine calcite formations in granules and in nacre shells (Ren et al., 2008). However, calcium or magnesium phosphates have not been quantified in previous studies of aerobic granules (Wang et al., 2006; Ren et al., 2008).

Therefore, the aim of this study is to reveal the nature of P minerals which can accumulate in EBPR granular sludge systems. In an attempt to determine the chemical composition of precipitates in granules, RAMAN spectroscopy, EDX (Energy Dispersive X-ray) technique coupled with Scanning Electron Microscopy (SEM), and X-ray Diffraction analysis (XRD) are evaluated.

2. Materials and methods

2.1. *Reactor operating conditions*

Aerobic granules were cultivated in a Sequencing Airlift Batch Reactor (SBAR), with a working volume of 17 L, consisting of an