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Short-term and long-term effects on carbon storage of pulse feeding on acclimated or unacclimated activated sludge

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ABSTRACT

This study was aimed to investigate the effect of different feeding patterns on the physiological state of the activated sludge and related microbial composition in an SBR (SRT of 2 days, acetate as the sole carbon source, aerobic conditions). The activated sludge was acclimated to two subsequent feeding patterns, namely to continuous feeding throughout the reaction phase and then to pulse feeding. FISH and microscopy staining procedure (Nile blue) were used to investigate the microbial composition, in combination with quantitative determination of storage. At steady state, storage was significant even under continuous feeding whereas pulse feeding brought a strong increase of both rate and yield of storage. Short-term and long-term effects were clearly distinguishable: the immediate adaptation of biomass coming from continuous feeding to a single spike accounted for a significant portion of the overall increase of both rate and yield of polymer storage that was obtained after long acclimation to pulse feeding. On the contrary, after either type of feeding, both cultures were mainly constituted from the members of *Thauera/Azoarcus* group. Thus, the same dominant group preferably consumed the acetate via storage or growth depending on acclimation conditions. Our study clearly showed that a progressive increase of storage capacity is not necessarily due to a shift of microbial composition.

1. Introduction

Extensive researches have been conducted for the mechanistic description of biological processes occurring in activated sludge systems, in order to obtain best practices for their design and operation. This effort led to recognize the storage phenomena as an important mechanism for the carbon source removal, particularly when the activated sludge experiences highly dynamic conditions (aeration tanks with plug-flow configuration, selectors for bulking control, contact-stabilization processes, and sequencing batch reactors), so called the feast and famine conditions (van Loosdrecht et al., 1997).

Storage is explained as a way that the microorganism can use for face against the highly dynamic environment in activated sludge systems, while maintaining favourable growth conditions. Indeed, biomass growth is unbalanced due to the gradients in the substrate availability. Thus, cells are not able to instantaneously adapt their growth rate to the changing substrate concentration because it would require also for

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Nomenclature

COD	chemical oxygen demand
DO	dissolved oxygen
FISH	fluorescence *in situ* hybridization
F/M	food to microorganism ratio
HRT	hydraulic retention time (d)
OLR	organic loading rate (mgCOD/L·d)
PHA	polyhydroxyalkanoate
Q_f	feed flow rate (L/d)
Q_{eff}	supernatant flow rate (L/d)
Q_w	mixed liquor withdrawal flow rate (L/d)
q_p	PHA production rate (mgCOD/mgCOD·h)
$-q_p$	PHA consumption rate (mgCOD/mgCOD·h)
$q_p/-q_s$	Substrate fraction used for storage (adimensional)
$-q_s$	specific substrate removal rate (mgCOD/mgCOD·h)
S	effluent COD (mgCOD/L)
S_0	feed COD (mgCOD/L)
SBR	sequencing batch reactor
SRT	sludge retention time
SS	suspended solid
SVI	sludge volume index (mL/g)
V	SBR liquid volume during the reaction phase (L)
VSS	volatile suspended solid (mgCOD/L)
X	biomass concentration in the stirred mixed liquor (gVSS/L)
X_e	biomass concentration in the supernatant after the settling phase (gVSS/L)
Y_{obs}	average observed yield (gCOD/gCOD)
Y_{STO}	average storage yield of PHA on acetate (gCOD/gCOD)
θ_C (SRT)	sludge retention time (d)

maintaining the relative synthesis rate of all cellular components. Therefore, under transient conditions, microorganisms can more quickly activate other mechanisms of substrate uptake like storage of substrate into specialized internal polymers, usually polyhydroxyalkanoate (PHA), especially with volatile fatty acids as substrates.

Initially, the role of storage on population dynamics in activated sludge has been studied in detail for filamentous bulking control, where selection of floc-formers against filaments is exerted by selectors or other process configurations that introduce a substrate concentration gradient. Because the storage response is faster than growth response (less adaptation is required), the more the microorganisms are able to store substrates during imposed transients and subsequently reuse them for growth, the more they would have a competitive advantage and the resulting enriched activated sludge should have a higher storage response (Majone et al., 1996).

Generally, storage has been reported as the dominant mechanism when the length of feast phase became a lower fraction of the overall reaction length (Dionisi et al., 2001). However, there is no agreement whether a lower storage capacity is truly a general feature of filamentous microorganisms and bulking sludge with respect to floc-formers and well-settling sludge, respectively (Martins et al., 2004). Beccari et al. (1998) observed that feast and famine conditions created a bulking sludge with a high storage capacity. Martins et al. (2003) also found that there were no differences in PHB storage rates or yields of bulking and well-settling sludge and observed that filamentous bacteria are usually no more than 20% of the bacterial population in bulking sludge and therefore small kinetic differences may be undistinguishable in mixed activated sludge populations. Moreover, when dealing with mixed cultures, the type and extent of biomass response to the transient conditions can depend not only on microbial composition of the consortium but also on the physiological state of any microorganisms in the consortium, which is also affected by the operating conditions (e.g. sludge residence time).

A similar short-term effect was also observed for mixed cultures; van Loosdrecht et al. (1997) tested pulse feeding of acetate on the system previously operated at steady state with continuous feeding and observed immediate PHA formation. Similarly, Martins et al. (2004) has operated a continuously fed SBR under anoxic conditions and observed PHA storage when pulse feeding was applied in a single cycle. In addition, pure culture studies have clearly shown that the role of storage (yield and rate) is also depending on operating conditions (organic load rate and culture residence time), even with well-adapted microorganism (Dionisi et al., 2005; Majone et al., 2007).

The identification of microorganisms responsible for the storage gained increasing interest in recent years, mainly in the frame of PHA production from modified activated sludge processes (i.e. under highly dynamic conditions to favour as much storage as possible), *Thauera spp*. and *Azoarcus* genus within *Betaproteobacteria* phylum were often dominant in the PHA storing biomass (Dionisi et al., 2005; Lemos et al., 2008; Serafim et al., 2006). Because the main objective was to evaluate the microbial composition in the presence of the storage, the identification of the microbial composition under less dynamic conditions, i.e. in the absence of storage, received minor interest.

Thus, the aim of this paper is to better understand the mechanism by which the presence of more or less dynamic conditions can affect the mechanism of substrate removal (i.e. the relative role of storage and growth) in acclimated or unacclimated mixed cultures. In particular, the experimental activity was designed to understand whether the increase of storage under dynamic conditions is induced either by changes on microbial composition or by physiological adaptation of the given microbial composition to the dynamic conditions. Moreover, the latter mechanism has been further investigated to understand how much the physiological adaptation is a short-term effect or it requires longer acclimation to dynamic feeding. Therefore, the effect of a shift from continuous to dynamic conditions has been investigated by two subsequent runs in a sequencing batch reactor (SBR, aerobic conditions) with two different feeding patterns, namely by continuous feeding throughout the reaction phase and then by pulse feeding. Moreover, single disturbance of usual feeding conditions was imposed to each acclimated biomass to understand short-term effects and distinguish