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Effect of acetate to biomass ratio on simultaneous polyhydroxybutyrate generation and direct microbial growth in fast growing microbial culture

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HIGHLIGHTS

- Biomass adapts to acetate fluctuations by adjusting metabolic reaction rates.
- Lower acetate to biomass ratios divert a larger substrate fraction to storage.
- High acetate levels increases substrate fraction directly utilized for growth.
- High acetate increases growth rate whereas low acetate enhances storage rate.

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ABSTRACT

The study investigated the effect of variations in the acetate to biomass ratio on substrate storage potential, and the kinetics of substrate utilization. A series of batch experiments were conducted with biomass taken from the fill and draw reactor operated at a sludge age of 2 d. One of the batch reactors duplicated the substrate loading in the main reactor. The others were started with different initial acetate to biomass ratios both in lower and higher ranges. Increasing available acetate did not totally divert excess substrate to storage; the microbial culture adjusted the kinetics of the metabolic reactions to a higher growth rate so that more substrate could be utilized for direct growth at high acetate levels. Conversely, storage rate was increased, utilizing a higher substrate fraction for *polyhydroxybutyrate* generation when acetate concentration was lowered. The physiological and molecular bases of storage at low substrate levels were discussed.

1. Introduction

Storage of intracellular biopolymers is now recognized as a significant metabolic process during substrate utilization by microbial cultures (van Loosdrecht et al., 1997). Substrate is either directly introduced into the microbial growth mechanism, or it may be diverted to storage. This mechanism is mainly observed under transient feeding conditions, sustained as inherent characteristics of some of the biological treatment schemes such as *sequencing batch reactors, intermittent aeration*, etc (Insel et al., 2006). Dynamic conditions sustained in these systems induce a process of physiological adaptation for the microbial community, often leading to substrate storage. Disregarding storage in organic substrate removal, as generally adopted in traditional studies and earlier activated sludge models (Henze et al., 1987) involves a significant risk of eclipsing the correct mechanism and may distort results of kinetic evaluation. Therefore, the magnitude of overall storage and the composition of generated biopolymers need to be assessed for each case.

In essence, storage results from an imbalance between removal of available substrate and microbial growth potential; while substrate may be removed, limitations on the metabolic reactions leading to growth may prevent consumption of all energy and divert a fraction of the substrate for generating intracellular biopolymers (van Loosdrecht et al., 1997; Ciggin et al., 2012). In biological systems, substrate loading – or the food to microorganism (F/M) ratio – defines the stoichiometric balance between the growth rate and the amount of substrate that should be available for maintaining the selected growth conditions (Orhon et al.,

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2009a,b). This is obviously an average value, defining in theory the balance for substrate utilization at steady state. In real systems however, substrate feeding fluctuates with time around the average level, causing perturbations of the substrate/growth balance. These perturbations obviously affect substrate utilization dynamics and the storage mechanism. In other words, the amount of storage products generated would also exhibit a similar fluctuation along with the variable substrate feeding regime. This information is needed for an accurate understanding of system operation.

The storage mechanism is often studied with simple, readily biodegradable substrates like acetate or glucose, generating *polyhydroxybutyrate* (PHB) and glycogen as storage products. Reported results suggest that up to 70% of the simple substrate could be converted to storage products under pulse feeding. They also indicate that the magnitude of storage is likely to exhibit significant variations depending on the nature of substrate, the feeding regime and culture history – i.e. sludge age of the microbial culture (Beun et al., 2000; Ciggin et al., 2012). Among extensive research effort on substrate storage, only a few studies focused on the effect of F/M ratio: Carta et al., (2001) investigated simultaneous utilization of acetate and glucose in and SBR system sustained at state at a sludge age of 6.1 d, with pulse feeding within 10–13 m of each cycle. They observed a PHB/acetate range of 0.56–0.66 mg COD/mg COD within consecutive cycles; with five-higher acetate dosage the PHB/acetate ratio was increased to 0.76 mg COD/mg COD, a level which indicated full acetate storage. Similarly, Beun et al., (2002) indicated that a four-time higher dose of acetate did not appreciably affect the PHB acetate ratio of 0.70 mg COD/mg COD associated with the regular acetate level of 420 mg/L, while an eight-time higher acetate level increased this ratio to 0.76 mg COD/mg COD Recently, Insel et al., (2012) suggested that the heterotrophic biomass was able to increase its direct growth activity, while reducing its storage potential, when the cyclic availability of acetate, which was used as the organic carbon source in the experiments was reduced. These results, while useful, still require additional information for a clear understanding of the stoichiometry and kinetics of substrate storage sustained at different F/M ratios.

In this context, the study was planned and conducted with two main objectives: To investigate and evaluate the effect of variations in the acetate to biomass ratio (i) on substrate storage potential, and (ii) on the kinetics of substrate utilization associated with fast growing microbial culture sustained under pulse feeding.

For this purpose a fill and draw reactor was operated at steady state with daily pulse feeding at a sludge age of 2 d. A faster growing microbial community and a low sludge were selected to better evaluate the substrate requirements of the microbial culture for growth compared with simultaneous storage. Then, a series of batch experiments were conducted with biomass taken from the fill and draw reactor and therefore acclimated to fast growth conditions. One of the batch reactors duplicated the substrate loading in the main reactor. The others were started with a range of substrate loadings, i.e. different initial acetate to biomass ratios both in the lower and higher ranges with respect to the one representing the operating conditions in the fill and draw reactor.

Acetate was selected as the sole organic carbon source, mainly because it is a well known substrate for generating a typical storage product, namely *polyhydroxybutyrate,* (PHB), and also, to be able to compare the results with previous findings in similar studies (Carta, et al., 2001; Beun et al., 2002; Ciggin et al., 2012). Batch reactors were basically monitored for the fate of PHB and respirometric analyses yielding the corresponding oxygen uptake rate (OUR) profiles. The experimental data obtained were used for the calibration of an appropriate model, yielding values of model coefficients for process kinetics associated with different experimental conditions.

2. Methods

2.1. Experimental setup

The first part of the experimental study was the acclimation of the biomass taken from a wastewater treatment plant to acetate in two laboratory scale fill and draw reactors (parent reactors). No specific effort was devoted to enhance the microbial community towards PHB generation, other than the feeding conditions selected in accordance with the objectives of the study. The parent reactors with a volume of 4 L were operated at a sludge age of 2 days in a pH range 6–8 at 20 °C ± 1 °C under aerobic conditions. Acetate was daily fed with an average concentration of 250 mg COD/L and steady state conditions were reached in 30 days. Reactors were continuously aerated through air diffusers to keep the oxygen concentration above 2 mg/L and stirred via magnetic stirrer. The substrate feed was prepared by diluting a stock solution of sodium acetate trihydrate (CH_3COONa) in distilled water. Additionally for the nutrient requirements of activated sludge macro and micronutrients solutions, namely Solution A and Solution B (O'Connor, 1972) were added with a composition as given in Table 1 with an amount of 10 ml per 1000 mg COD/L.

Six batch experiments were conducted to observe the storage phenomena of microorganisms at different F/M ratios. Characteristics of these batch experiments are summarized in Table 2. The reactors were monitored with chemical oxygen demand (COD), acetate, polyhydroxybutyrate (PHB), suspended solids (SS) and volatile suspended solids (VSS) measurements. Polyhydroxyalkanoate (PHA) measurements mainly consisted of PHB since the formation of PHB from the central metabolite acetyl-CoA is the main storage polymer, and therefore the results of experiments in fact represent PHBs as storage compound.

2.2. Respirometric measurements and modeling

Respirometric measurements were conducted to observe the oxygen uptake rate profiles. Oxygen uptake rate (OUR) profiles were obtained using a Ra-Combo (Applitek Co., Nazareth, Belgium) continuous respirometer. The mixed liquor (activated sludge) from the respirometer chamber was circulated continuously through respiration vessel with a 0.75 L volume, where the dissolved oxygen at the inlet and outlet was measured and the sample was returned to the chamber. During each experiment, 1.04 g Allyl Thio Urea (ATU) (Formula 2533TM, Hach Company) was added to the OUR reactors as a nitrification inhibitor to prevent any possible interference induced by nitrification.

Modeling and simulation of the obtained OUR data was performed using the AQUASIM simulation program (Reichert et al., 1998). AQUASIM, which is a frequently used simulation program (Insel et al., 2006; Orhon et al., 2009a,b; Pala-Ozkok et al., 2012), was utilized for model calibration and evaluation based on experimental data. Model calibration was implemented by means of an iterative calibration protocol; involving manual calibration of model components in each iteration step and fitting all the model outputs on real time data (Vanrolleghem, 2002). The model outputs were found to be sensitive to all selected model coefficients.

2.3. Analytical procedures

For soluble COD determination, samples were filtered using 0.45 μm Millipore membrane filters. COD measurements were conducted according to the ISO 6060 procedure (1986). Suspended solids (SS) and volatile suspended solids (VSS) measurements were conducted as defined in Standard Methods (APHA, 2005). PHA sampling and analysis were conducted according to the method