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Role of microbial accumulation in biological sulphate reduction using lactate as electron donor in an inversed fluidized bed bioreactor: Operation and dynamic mathematical modelling

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ABSTRACT

This study evaluated the impact of substrate accumulation (sulphate and polyhydroxybutyrate (PHB)) on bioprocess control of a sulfate reducing inversed fluidized bed bioreactor. To investigate the impact of substrate accumulation, step feed changes were induced to an inversed fluidized bed bioreactor performing biological sulphate reduction. A first step feed change set both the chemical oxygen demand (COD) and sulphate influent concentration to zero. As hypothesised, sulphide was still being produced after 15 days of operation without electron donor and sulphate supply. This suggests that accumulated and/or sorbed COD and sulphate supported the continued biological sulphide production. PHB was indeed found present in the sludge and batch tests showed PHB can support the sulphate reduction. A second step feed change of adding solely COD (and no sulphate) to the bioreactor influent resulted in a continuous production of sulphide, suggesting that sulphate had accumulated in the inversed fluidized bed bioreactor sludge. A mathematical model that includes microbial growth, PHB and sulphate storage as well as metabolism of lactate oxidizing sulphate reducing bacteria was developed, calibrated and validated. The model was able to simulate the accumulation of both PHB and sulphate in the inversed fluidized bed bioreactor.

1. Introduction

Anaerobic reduction of sulphate by sulphate reducing bacteria (SRB) is successfully applied for the treatment of sulphate contaminated wastewater from many industries on a large scale, since it offers an efficient treatment with low operation costs using various organic carbon sources (Liamleam and Annachhatre, 2007). The end product is sulphide, making this technique also suitable for the treatment of wastewater that contains dissolved metals, which react with sulphide to form metal sulphide precipitates (Lewis, 2010). The inversed fluidized bioreactor (IFB) concept combines sulphate reduction with metal recovery in a single reactor unit (Celis et al., 2009; Villa-Gomez et al., 2011). The biomass in the IFB is attached to a floatable carrier material which is fluidized downwards.

Controlling the sulphide production in a sulphate reducing bioreactor is highly relevant to avoid overproduction of H_2S with all its negative economical and environmental effects, especially H_2S toxicity to anaerobic bacteria and supersaturation leading to very small (<20 μm) metal sulphide precipitates, also called fines, with poor settling properties (Villa-Gomez et al., 2014). To avoid overproduction of H_2S, the manipulation of the organic loading rate (OLR) or sulphate (electron or acceptor donor, respectively) as the control input is required. For the acquisition of the controller parameters the creation of step responses on these inputs is required (Cassidy et al., 2015 and references therein). Proportional-integral-derivative (PID) controllers have been successfully used in anaerobic bioreactors to control parameters such

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as pH, alkalinity, volatile fatty acid concentration and gas concentration flow rates (Cassidy et al., 2015 and references therein).

At the microbial level, these changes in OLR create feast/famine conditions, which induce different metabolic responses as compared to continuous feeding. For SRB, both sulphate and polyhydroxyalkanoate (PHA), mainly as polyhydroxybutyrate (PHB), can accumulate as storage compounds (Cypionka, 1989; Hai et al., 2004). This accumulation was hypothesized given the results obtained in a previous study on sulphate reducing bioreactors where sulphide was being produced even though no sulphate or carbon substrate were supplied (Villa-Gomez et al., 2014). Such an accumulation of storage products can result in response delays, leading to longer response times and a higher control gain (Villa-Gomez et al., 2014). But on the other hand, these storage compounds can protect the microbial community from external stress such as substrate and/or nutrient limitation (famine conditions) (Ni et al., 2015) and such storage can be used to optimize the sulphate reducing processes. For instance, although not yet tested for sulphate reduction, decoupled substrate addition has been applied for denitrifying microorganisms removing nitrogen from wastewater to reduce overall costs (Scherson et al., 2013). The denitrifying microorganisms accumulated PHB when using acetate as the substrate during an anaerobic period. PHB was consumed during the subsequent anoxic period for NO_2^- reduction. The biological nitrogen removal efficiency from the wastewater was 98% over more than 200 cycles. Thus, optimization of decoupled substrate addition for SRB processes could be an attractive way of reducing the overall costs of SRB bioreactor systems as less supply of external electron donor would be required.

The aim of this study was to evaluate the impact of substrate accumulation in microorganisms on the design of a control strategy of a continuous sulphate reducing IFB. For this purpose, step feed changes were applied to an IFB bioreactor that had been operated previously for 2 years in feast/famine conditions (Villa-Gomez et al., 2014) and the biological response was monitored to determine to what extent the accumulation products affected the control of the sulphide production. Additionally, a mathematical model describing the processes of microbial accumulation of sulphate and PHB in the IFB bioreactor was developed, calibrated and validated. This model can be used as a tool to predict the response of sulphate reducing systems to dynamic conditions and assist in finding optimal operational conditions, such as the addition of electron donor.

2. Materials and methods

2.1. IFB bioreactor operation and step feed changes

Sulphate reduction, using a sulphide ion selective electrode (pS) for sulphide monitoring, was performed in an IFB that had been in operation for 858 days with varying conditions (Villa-Gomez et al., 2014). The IFB was operated for another 60 days (days 859–920) in this study.

Lactate was used as the electron donor and carbon source. When added, sulphate was supplied as Na_2SO_4. The hydraulic retention time (HRT) was kept constant at 1 day.

To determine the relevance of substrate accumulation, step changes in the OLR were applied to create a response in the sulphide production by changing the COD concentration in the influent: the OLR was changed from 1 to 0 $gCOD.L^{-1}$ at time zero (Experiment A). COD, volatile fatty acids, sulphate, sulphide and PHB concentrations were monitored daily. Once the sulphide concentration reached zero, a dynamic COD loading step was induced without sulphate addition (Experiment B). In a first phase of the COD step change experiment (B), a pulse of 0.3 $gCOD.L^{-1}$ for 1 day was given and when the sulphide concentration reached zero, the COD load was increased back to 0.3 $gCOD.L^{-1}$ in a second phase of experiment B for the remaining 9 days.

2.2. Batch tests

2.2.1. Activity tests procedure

Activity tests were performed at 30 (±2) °C and a constant rotation of 125 rpm for 21 days to evaluate sulphate accumulation in the biomass and the feasibility of PHB as an electron donor for sulphate reduction. Serum bottles (117 mL) were used and a COD/SO_4^{2-} ratio of 0.67 was maintained. The pH was adjusted to 7.0 (±0.2) with NaOH.

Each bottle contained 5 mL of IFB carrier material (sampled on day 855) or 2.5 mL of anaerobic granular sludge from a methanogenic reactor (Industriewater Eerbeek, Eerbeek, the Netherlands, sampled January 2013) submerged in 15 mL of mineral medium (same composition as used for the IFB bioreactor). The serum bottles were closed with butyl rubber stoppers and flushed with N_2 gas for 3 min to achieve anaerobic conditions and to remove any remaining H_2S.

2.2.2. Sulphate accumulation

To evaluate sulphate accumulation in the biomass, incubations were carried out in triplicate using as substrates: 1) lactate; 2) lactate and sulphate, 3) lactate, sulphate and FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone) as an uncoupler agent to inhibit sulphate accumulation (Warthmann and Cypionka, 1990). Both biomass sources, from the IFB bioreactor or methanogenic reactor in Eerbeek, were subjected to a 20 days starvation period prior to the start of the accumulation test to ensure complete consumption of endogenous substrate. Initial and final sulphate and sulphide concentrations were measured.

To determine the amount of sulphate accumulated, biomass samples were washed three times with phosphate buffer by centrifugation at 19 000 rpm at 4 °C for 20 min to remove any remaining sulphate sorbed to the biomass. The supernatant was then removed, 5 mL of phosphate buffer solution was added and the samples were sonicated under anaerobic conditions at high frequency (40 kHz) for 15 min to lyse the cells and release the intracellular sulphate present. The accumulated sulphate concentration was then measured in the supernatant.

2.2.3. PHB as electron donor

To evaluate the feasibility of PHB as an electron donor for sulphate reduction (SR), PHB (Sigma Alrich, natural origin) and/or with lactate were used as the substrates, always maintaining 0.5 $gCOD.L^{-1}$. The incubations (PHB, PHB + lactate, lactate, no COD) were done in triplicate. Sulphate, sulphide, PHB, COD and methane concentrations were determined throughout the experiment.

2.3. Model development

2.3.1. Numeric integration

The numerical integration of the mass balances with corresponding kinetics, describing the relevant processes in the IFB bioreactor, was performed using the MATLAB® built-in function *ode15s*, which is a multi-step, variable-order differential equation solver.

2.3.2. Model calibration and validation

A sensitivity analysis (SA) was performed to determine the parameters that affected the model output results the most. These parameters were subsequently calibrated from the