Activity of PAO Bacteria in Mesophilic Digesters and Their Significance for P Recovery

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INTRODUCTION

Phosphorus (P) is essential for all life on earth. Growing world population causes increasing demand for this nutrient while the availability of the P rock reserves is quickly depleting (Elser & Bennett, 2011; Cordell *et al*., 2009). In order to secure P sufficiency, researchers have turned their focus towards the optimization of P recovery methods. Wastewater is a potential source, from which P can be efficiently recovered by biological means (Yuan *et al*., 2012). It requires the Enhanced Biological Phosphorus Removal process to be implemented in the wastewater treatment plants (WWTPs) where Polyphosphate Accumulating Organisms (PAO), such as *Accumulibacter* and *Tetrasphaera* (Mielczarek *et al*., 2013; Nguyen *et al*., 2011), take up excess amounts of P and store it as intracellular poly-P thus removing it from the wastewater (Mino *et al*., 1998; Seviour *et al*., 2003). Subsequently, in the anaerobic digester, the P is released to the bulk liquid, from which it can be precipitated in the form of e.g. struvite that can further be used as a fertilizer (Kumar and Pal, 2015). An increasing number of modern WWTPs has started to recover P from anaerobic digesters, however, the process requires further optimization since our knowledge of it with the emphasis on P release potential and microorganisms involved is limited.

The aim of this study was to investigate factors that influence the P-release from surplus activated sludge after it enters anaerobic digesters. The P-release potential and the influence of temperature as well as high salt and ammonia concentrations on anaerobic P release activity were investigated.

METHODS

Sludge collection

Activated sludge was collected from Aalborg West WWTP and the experiments were run immediately upon the arrival to the laboratory. *Accumulibacter*-enriched sludge was produced in sequencing batch reactor, where the sludge was exposed to anaerobic and aerobic periods and fed with synthetic wastewater and acetate (Lisbon, Portugal). The enriched sludge was collected and shipped one day prior to experiments. Digester sludge was collected from the anaerobic digester at Bruunshåb WWTP, Denmark, operating at mesophilic conditions. The digester sludge was mixed with equal part of *Accumulibacter*-enriched sludge, which hereafter is referred to as mixed sludge.

Mimicking digester conditions

In order to measure P release under digester conditions, an artificial digester fluid was prepared with the following composition: ammonium bicarbonate 4.8 g/L, sodium chloride 0.2 g/L, calcium carbonate 62 mg/L, magnesium sulphate 54 mg/L, potassium sulphate 85 mg/L, SL-11 Trace Element Solution (DSMZ)1 ml/L; pH 7.8. Phosphate was not included in order to minimize background P content normally present in the digester. Acetate was added separately before the experiment. Prior to the experiment, activated sludge was gently centrifuged (2000 rpm, 2 min) and

the water phase was replaced with the artificial digester fluid.

Ortho-P measurement

Prior to the experiments, the sludge was aerated for 30 min in order to remove ortho-P from the water phase. Next, oxygen was removed by N_2 purging, sludge was heated to the desired temperature and acetate (final concentration 500 mg/L) was added to the chosen incubations. The sludge was continuously mixed and samples were taken for the ortho-P measurement over the chosen time period. Samples collected for ortho-P determination were stored in 0.04M sulfuric acid (final concentration) at 4 °C and the measurements were performed according to Danish Standard DS 291.

Determination of PAO abundance

In order to determine the abundance of *Tetrasphaera* and *Accumulibacter* PAO in the sludge samples, 16S rRNA amplicon sequencing was performed. The whole procedure was performed and described by Albertsen *et al.* (2015).

RESULTS AND DISCUSSION

A series of experiments were performed to test the influence of elevated temperature on the P-release (Fig. 1). For this purpose, activated sludge from Aalborg West WWTP was incubated with/without the addition of acetate at 20, 27 and 35°C. The results are presented in Fig. 1 and show the average and standard deviation of three biological replicates.

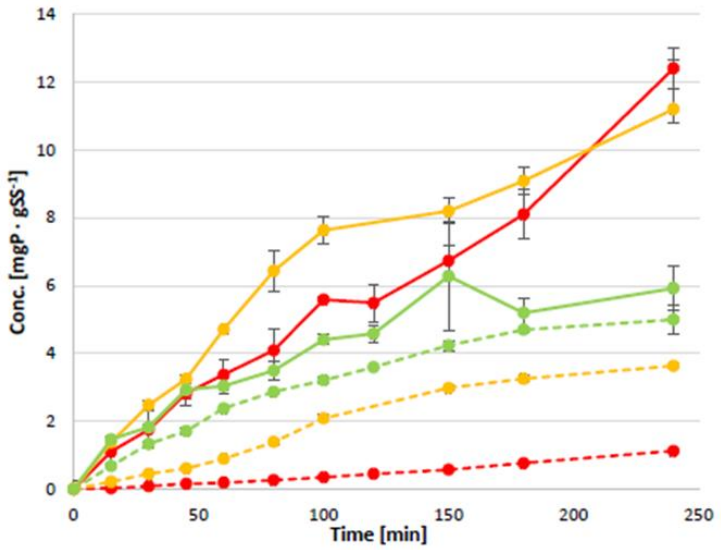

Figure 1. Measurement of P released from activated sludge under different temperature conditions. Solid line represents incubations with the addition of acetate; dashed line represents control incubations without the substrate addition. Red: 20°C; yellow: 27°C; green: 35°C.

The highest release of P was observed at lower temperatures (20 and 27°C) with acetate present. Further increase of the temperature to 35°C hindered P release in the incubation where acetate was added. At the same time, temperature increase caused higher P release in control incubations indicating that elevated temperature had a negative effect on biological P release activity, while the amount of P released as a result of other processes (e.g. cell lysis and hydrolysis) increased. The read abundance of PAO microorganisms in the above incubations was determined to be 4.2% for *Tetrasphaera* and 0.2% for *Accumulibacter*.

In order to investigate how *Accumulibacter* PAO alone reacted on the conditions present in

anaerobic digesters *Accumulibacter*-enriched sludge was used with the fraction of *Accumulibacter* reaching 42.5% and very few other PAOs present. The sludge water was substituted by artificial fluid mimicking digester conditions (Fig. 2, yellow data points) as well as by mixing *Accumulibacter*-enriched sludge with fresh digester sludge in 1:1 volume ratio (Fig. 2, green data points). As control we measured the amount of P released in *Accumulibacter*-enriched sludge with and without acetate addition (red and blue data points, respectively). After completion of standard, short-term experiment (4 hours), the incubations were continued for 3 weeks in order to monitor P release for the time period corresponding to the typical solid retention time in the digester. All incubations were run at 35°C. The results presented in Fig. 2 show the average and standard deviation of three biological replicates.

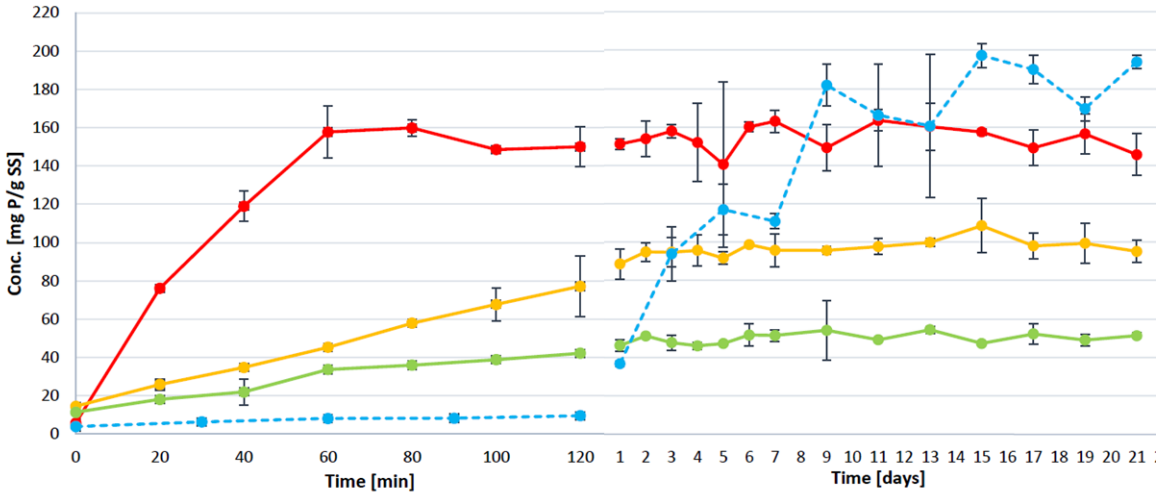

Figure 2. Short- and long-term measurement (left and right side, respectively) of P released from activated sludge enriched with *Accummulibacter* under following conditions: blue: control incubation containing only activated sludge; red: activated sludge with the addition of acetate; yellow: activated sludge incubated with artificial digester fluid with the addition of acetate; green: activated sludge incubated with artificial digester fluid with the addition of acetate mixed with digester sludge.

The observed P release was highest and fastest in control incubations with the addition of acetate suggesting that high salt and ammonia concentration present in digester had a large negative effect on the P-release. Only around half of the P was released in the incubation with digester fluid (yellow data points) when compared to the control incubation (red data points). The amount of P released in the incubation containing fresh digester sludge (green data points) is shown to be two times lower than in the incubation with artificial digester fluid (yellow data points). However, since the *Accumulibacter*-enriched sludge was diluted with fresh digester sludge in the ratio 1:1, the true P release in these incubations was comparable.

For all the incubations (excluding control without the acetate), the maximum amount of P was released within the first 24 hours of the experiment indicating cessation of the biological P release activity. Gradual release of ortho-P from the control sample without the substrate indicated that, as in case of the elevated temperature, other processes lead to an increase in ortho-P concentration in bulk liquid.

The amount of P released in *Accumulibacter*-enriched sludge was much higher than the amount released from activated sludge from Aalborg West WWTP (150 and 12 mgP/gSS in control incubations with acetate added, respectively, see Fig. 1 and 2). This was mainly caused by the 10

times higher *Accumulibacter* abundance (42.5% and 0.2%, respectively) and the fact that the P release curve did not reach steady state in the Aalborg West sludge incubations (shown in Fig. 1).

CONCLUSIONS

The conditions encountered in mesophilic anaerobic digesters at WWTPs seem to hinder biological phosphorus release from PAO bacteria resulting in slower release rate and potentially lower final P concentration in the bulk digester liquid. More studies are needed to confirm the results for other PAOs. However, these observations have an important implications for the P recovery from the digesters, suggesting potentially higher P recovery yield if the activated sludge is treated in e.g. anaerobic storage tank before entering the digester.

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