Supplementary Material

Phosphorus recovery by re-dissolution from activated sludge – Effects of carbon source and supplementation level revisited

Annika Anders, Harald Weigand, Harald Platen

Competence Centre for Sustainable Engineering and Environmental Systems (ZEuUS), THM University of Applied Sciences, Wiesenstr. 14, D-35390 Giessen, Germany.

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Table S1: Best-fit parameters (I [0 min; 220 min]) of the modified Gompertz model used to describe the kinetics of P re-dissolution after supplementation of activated sludge with formate, acetate, propionate and butyrate.

	formate	**acetate**	**propionate**	**butyrate**
A_{max} [mmol P/L]	0.25	1.45	0.56	0.46
μ [mmol P/ (L*min)]	$1.41*10^{-3}$	$17.20*10^{-3}$	$4.29*10^{-3}$	$2.58*10^{-3}$
λ [min]	28.57	8.49	14.93	29.71
R^2	0.990	0.991	0.991	0.977

Calculation of indicators of the P re-dissolution stoichiometry

$P_{release}/C_{uptake}$ ratio:

$P_{release}/C_{uptake}$ ratios were calculated for the time t_μ of the maximum P release rate μ as predicted from the modified Gompertz model (Equation (1)). For each of the different VFA supplementations t_μ was located by equating the second derivative of the modified Gompertz model (Equation (2)) to zero. Employing the best-fit parameters summarized in Table 1A t_μ values were 40 min, 63 min, 94 min and 96 min for acetate, propionate, formate and butyrate, respectively. Equation (1) was solved for $P_{i,rel}$ (t_μ) to obtain the numerator of the $P_{release}/C_{uptake}$ ratio. Its denominator was found from the corresponding VFA time profile (Figure 1B). Therefore, linear regression lines fit to an appropriate time interval were evaluated for the t_μ values. C_{uptake} was calculated from obtained aqueous phase VFA concentration converted in C-mol.

Modified Gompertz Model:

$$P_{i,rel}(t) = A_{max} \cdot \exp\left\{-\exp\left[\frac{\mu \cdot \exp(1)}{A_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

Second derivative:

$$P_{i,rel}''(t) = \frac{\mu}{A_{max}} \cdot \left\{ \exp\left[\frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}} \right] - 1 \right\} \cdot \exp\left\{ \frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}} + 3 - \exp\left[\frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}} + 1 \right] \right\} \quad (2)$$

$$P_{i,rel}''(t) = 0 \quad \text{with} \quad t = \frac{A_{max}}{\mu \cdot \exp(1)} + \lambda$$

P_{yield}/VFA_{spike} and $P_{yield}/VFA_{consumed}$ ratio:

P_{yield}/VFA_{spike} and $P_{yield}/VFA_{consumed}$ were calculated with Equation (3) and (4), respectively. The term $P_{i,rel,t=220\ min}$ [mmol P] is the aqueous P concentration at the end of the experiment. $VFA_{spike,t=0min}$ [mmol VFA] is the initial concentration of the supplemented VFA. $VFA_{t=220min}$ [mmol VFA] is the aqueous VFA concentration at the end of the experiment.

$$\frac{P_{Yield}}{VFA_{spike}} = \frac{P_{i,rel,t=220min}}{VFA_{spike,t=0min}} \quad (3)$$

$$\frac{P_{Yield}}{VFA_{consumed}} = \frac{P_{i,rel,t=220min}}{VFA_{spike,t=0min} - VFA_{t=220min}} \quad (4)$$

Table S2: Key enzymes and transporter involved in polyP hydrolysis, glycogen degradation and PHB synthesis.

No.	EC number[a]	name
1	--	low affinity inorganic phosphate transporter (pit)
2	--	acetate permease, acetate:H+ symporter (ActP)
3	3.6.1.1	inorganic diphosphatase
4	6.2.1.1	acetate-CoA ligase
5	2.7.4.3	adenylate kinase
6	2.3.1.9	acetyl-CoA C-acetyltransferase
7	1.1.1.35	3-hydroxyacyl-CoA dehydrogenase
8	2.3.1.304	poly((R)-3-hydroxyalkanoate) polymerase
9	2.7.4.1	polyphosphate kinase 1
10	2.4.1.1	glycogen phosphorylase
11	5.4.2.6	beta-phosphoglucomutase
12	5.3.1.9	glucose-6-phosphate isomerase
13	2.7.1.11	6-phosphofructokinase
14	4.1.2.13	fructose-bisphosphate aldolase
15	5.3.1.1	triose-phosphate isomerase
16	1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)
17	2.7.2.3	phosphoglycerate kinase
18	5.4.2.11/5.4.2.12	phosphoglycerate mutase
19	4.2.1.11	phosphopyruvate hydratase
20	2.7.1.40	pyruvate kinase
21	1.2.1.104	pyruvate dehydrogenase system
22	7.1.2.2	H^+ transporting ATPase
23	--	Mg^{2+} transporter

[a] obtained using the MetaCyc database[S1]

Additional information on Figure 3: PolyP composition

Please note that intracellular polyP is stabilized by metal counter ions such as Mg^{2+}, K^{+}, Na^{2+} or Ca^{2+}. The contribution of individual ions in polyP granula has been suggested to depend on EBPR conditions and influent composition.[S2] Li et al. (2019)[S2] found an approximate relation of P:Mg:Na:K in the stoichiometric ratio of 1 : 0.3 : 0.17 : 0.17 for polyP granules in EBPR sludge.

For simplification, in Figure 3, we assumed that polyP has Mg^{2+} as a counter ion, only. The stoichiometric ratio of P to Mg^{2+} of 2:1, was derived from the molecular formula $(NaPO_3)_n$ (Graham's salt). Therefore, during anaerobic P re-dissolution and with hydrolysis of polyP 1 mol P will be released together with 0.5 mol Mg^{2+}.

References

S1 R. Caspi, R. Billington, I. M. Keseler, A. Kothari, M. Krummenacker, P. E. Midford, W. K. Ong, S. Paley, P. Subhraveti and P. D. Karp, The MetaCyc database of metabolic pathways and enzymes-a 2019 update, *Nucleic Acids Res.*, 2020, **48**, D455–D453.

S2 Y. Li, S. M. Rahman, G. Li, W. Fowle, P. H. Nielsen and A. Z. Gu, The Composition and Implications of Polyphosphate-Metal in Enhanced Biological Phosphorus Removal Systems, *Environ. Sci. Technol.*, 2019, **53**, 1536–1544.