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BIOLOGICAL DEPHOSPHATATION ACTIVITY OF AEROBIC GRANULAR SLUDGE INFLUENCED BY DIFFERENT SUBSTRATES

MASTER THESIS

David Scheibler

supervised by

Professor Christof Holliger, EPFL Lausanne
Professor Eberhard Morgenroth, ETH Zürich

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Summary

The aerobic granular sludge (AGS) technology including enhanced biological phosphorus removal (EBPR) offers the possibility to build compact wastewater treatment plants with reduced sludge production. A joint research project between EPFL and EAWAG investigates the impact of particulate matter on granulation and the nutrient removal efficiency.

Two AGS sequencing batch reactors (AGS-SBRs) were operated with synthetic wastewaters including different carbon sources. Reactor RA was fed with acetate and propionate, whereas the wastewater of reactor RB contained acetate, propionate and furthermore glucose, amino acids, starch and peptone. The granulation was worse in reactor RB than in RA, probably due to starch and peptone in the inflow. One objective was to determine the influence of those polymeric substances by removing them from the wastewater composition. It can be expected, that the granulation would improve with a shift to a monomeric medium.

The wastewater treatment including EBPR worked well in both reactors. However, it was not known which substrates sustain the biological dephosphatation activity. To answer this question, anaerobic batch tests with individual substrates were performed in both reactors.

As expected, acetate and propionate were taken up and led to phosphate release by the phosphate accumulating organisms (PAO) named *Accumulibacter*, which were abundant in both reactors.

Glucose was just consumed in reactor RB, which indicated an absence of fermenter in reactor RA. Glucose induced phosphate release, but it is unclear whether this was due to its uptake by the PAO *Tetrasphaera* or through fermentation products like acetate.

Starch and peptone did not lead to phosphate release in both reactors, not even as their concentration decreased in reactor RB. Probably these polymeric substances were just adsorbed to the surface of the granules and degraded later on in the aerobic phase.

The amino acids tested consisted of a mix of seven amino acids: alanine, arginine, aspartic acid, glutamic acid, glycine, leucine and proline. This mix led to phosphate release during the anaerobic phase not just in reactor RB but as well in RA. Tests with the individual amino acids in reactor RA showed: aspartic acid and glutamic acid were substantially consumed and led to phosphate release, alanine and arginine were slightly consumed and induced phosphate release and leucine and proline had no effect. Glycine was not consumed in reactor RA but led to a high release of phosphate. As the only PAO abundant in that reactor, the release can be assigned to *Accumulibacter*. The phosphate release rate could be described with Michaelis-Menten kinetics as a function of the glycine concentration.

The anaerobic batch tests lead to the conclusion that different substrates induce biological dephosphatation activity, depending on the bacteria available.

After the batch tests, the polymeric substances starch and peptone were removed from the influent of reactor RB which led against the expectations to a loss of granules. Even after twelve weeks of operation the reactor was dominated by flocs and the biomass behaved similar to conventional activated sludge.

So removing the polymeric substances from reactor RB showed that a significant adaption of the inflow, even a simplification, can lead to fundamental loss of the granules.

All in all more research is needed to further understand the processes in AGS to build full scale treatment plants with stable granules and reliable treatment performance.

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Nomenclature

NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
$P_0_4^{-3}$	Orthophosphate
SO_4^{2-}	Sulfate
AGS	Aerobic granular sludge
ATP	Adeonosine triphosphate
COD	Chemical oxygen demand
D member	Derivative member of PID
DNA	Deoxyribonucleic acid
DPAO	Denitrifying polyphosphate accumulating organisms
EBPR	Enhanced biological phosphorus removal
EBPR.ANA	Anaerobic EBPR batch activity tests
EPFL	Ecole polytechnique fédérale de Lausanne
EPS	Extracellular polymeric substances
FISH	Fluorescent in-situ hybridization
GAO	Glycogen accumulating organisms
HPLC	High performance liquid chromatography
I member	Integral member of PID
IC	Ion chromatography
Kp	Gain of PID
LBE	Laboratory of environmental biotechnology
NADH	Nicotinamide adenine dinucleotide
P member	Proportional member of PID
P/C ratio	Anaerobic phosphate release to carbon source uptake ratio [P-mol/C-mol]
PAO	Polyphosphate accumulating organisms
Pap	polyP:AMP phosphotransferase
pCOD	Particulate COD
PH2MB	Polyhydroxy-2-methylbutyrate
PH2MV	Polyhydroxy-2-methylvalerate
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
PID controller	Proportional-integral-derivative controller
Poly-P	Polyphosphate
Ppx	Exopoly-phosphate gene
QS	Quorum sensing
RA	Reactor with the name RA

RB	Reactor with the name RB
rbCOD	Readily biodegradable carbon source
SBR	Sequencing batch reactor
SNSF	Swiss national science foundation
SVI	Sludge volume index
TOD	Theoretical oxygen demand
TS	Total solids
TVS	Total volatile solids
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant
Xp	Proportional band

1 Introduction

The biological part of a wastewater treatment plant (WWTP) works usually with activated sludge, consisting of bacteria suspended in the water and eliminating pollutants like carbon, nitrogen and phosphorus (Gujer, 2006, p.322).

The aerobic granular sludge (AGS) technology should improve this process. It is based on self-granulated aggregates of bacteria, the granules (Morgenroth *et al.*, 1997). They build fast settling and dense biomass, which allows to build compact WWTPs with reduced sludge production. AGS enables "*simultaneous removal of carbon, nitrogen and phosphorus in one sequencing batch reactor*" (De Kreuk *et al.*, 2005). Selection for polyphosphate accumulating organisms (PAO) improves granule stability (De Kreuk *et al.*, 2005). PAO are able to perform the so-called enhanced biological phosphorus removal (EBPR). EBPR involves the incorporation of polyphosphate in the biomass and removal with the excess sludge (Tchobanoglous *et al.*, 2014).

1.1 Aerobic granular sludge

At the first Aerobic Granular Sludge Workshop 2004, granules were defined as "*aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which subsequently settle significantly faster than activated sludge flocs*" (Weissbrodt, 2013, p.18). The granules formation is a process of cell-to-cell aggregation involving different forces like shear stress but including as well extracellular polymeric substances (EPS), which work like a glue (Winkler *et al.*, 2017). This EPS production is correlated with N-acyl-homoserine-lactone, molecules involved in quorum sensing (QS) (Tan *et al.*, 2014). So the granule formation is initially a biological phenomenon, influenced by the composition of substrate (Morgenroth *et al.*, 1997; Dolfing, 1987).

AGS can be cultivated in sequencing batch reactors (SBRs). Cultivation is influenced by hydraulic parameters like short sedimentation and draw phases to wash out slow settling flocs (Morgenroth *et al.*, 1997). The selection for slow-growing organisms such as PAO improves granule stability and can combine biological nitrogen and phosphorus removal. This can be achieved by "*alternating anaerobic feeding and aeration periods*" (De Kreuk *et al.*, 2005).

Many studies on aerobic granulation were performed with synthetic wastewater. But the formation of AGS is as well possible during the treatment of real and low-strength municipal wastewater in a SBR (Derlon *et al.*, 2016).

The formation of AGS is reduced by particulate organic matter like starch. This disadvantage can be overcome with a longer anaerobic phase to favor the hydrolysis of the polymers (Wagner *et al.*, 2015).

1.1.1 NEREDA® wastewater treatment

Aerobic granular sludge is implemented in full scale industrial and municipal WWTPs under the name Nereda® by the company Royal HaskoningDHV from the Netherlands. They advertise the technology as 30-50 % less energy consuming and 50-75 % less space consuming, due to fast settling granules including oxygen-rich and oxygen-poor conditions at the same time (DHV, 02.08.2018). In Switzerland two of those treatment plants are under construction (Wabag, 02.08.2018).

The typical Nereda® cycle consists of a simultaneous influent feeding and effluent discharge phase, followed by an aeration phase and a short sedimentation phase (Pronk *et al.*, 2015). The AGS WWTP Garmerwolde was started up with conventional sludge. The treatment quality was reached after three months, but the intended biomass concentration of 8 g/L only after nine months (Pronk *et al.*, 2015).

1.2 Enhanced biological phosphorus removal

Phosphorus has to be removed from wastewater to control eutrophication because it is a limiting nutrient in most freshwater ecosystems. Removal can be done with chemical and/or biological treatment.

Enhanced biological phosphorus removal saves chemical cost and declines the sludge production (Tchobanoglous *et al.*, 2014, p.648). It also recovers the phosphate in a form which is more amendable to reuse than traditional chemical phosphorus precipitation (Nielsen *et al.*, 2012). Unfortunately this technology suffers from unpredictable and sometimes unexplained failures. Therefore a deeper understanding of the process for more rational design, effective operation and troubleshooting is needed (He & McMahon, 2011).

Different PAO were identified but isolation was not successful. One of them is the bacteria '*Candidatus Accumulibacter Phosphatis*' (Betaproteobacteria), further referred to as *Accumulibacter*, (Tchobanoglous *et al.*, 2014, p651). Their metabolism is well known and further described below. Another PAO abundant in domestic WWTPs is '*Tetrasphaera*' (Actinobacteria). It has a different metabolism, which is not fully understood yet (Mino *et al.*, 1998; McIlroy *et al.*, 2017; Tchobanoglous *et al.*, 2014, p.651).

1.2.1 Accumulibacter

A simplified model of the metabolism of the PAO *Accumulibacter* is presented in Figure 1.1 (Lochmatter, 2013, p.21). In the anaerobic phase (A), they degrade glycogen and hydrolyze polyphosphate (Poly-P), which they release as orthophosphate (PO_4^{3-}) to generate energy for taking up volatile fatty acids (VFA) and to store them as polyhydroxyalkanoates (PHA). These substrates are oxidized in a following aerobic phase (B) and used up for cell growth and to take up orthophosphate again (Morgenroth, 2016). Withdrawing excess sludge at the end of the aerobic phase with high polyphosphate content leads to high phosphate removal efficiency (Mino *et al.*, 1998). For a more detailed figure of the model see appendix: Figure A.1.

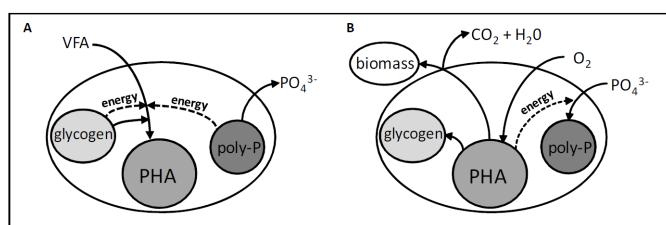


Fig. 1.1: Metabolism of PAO (Lochmatter, 2013, p.21)

Accumulibacter Type I is a denitrifying PAO (DPAO) (Nielsen *et al.*, 2010). Instead of oxygen, it can use nitrite or nitrate as electron acceptor.

According to different sources, *Accumulibacter* are able to take up the volatile fatty acids acetate and propionate and also pyruvate and glutamic acid (He & McMahon, 2011; Kong *et al.*, 2004; Martín *et al.*, 2006; Tchobanoglous *et al.*, 2014, p.653). Other substrates like formate, butyrate, oleic acid, ethanol, leucine, glycine, aspartic acid and thymidine can not be consumed (Kong *et al.*, 2004). The carbon sources taken up are stored in the form of different polyhydroxyalkanoates (PHA) (Mino *et al.*, 1998). Those are Polyhydroxybutyrate (PHB) from acetate, polyhydroxyvalerate (PHV) from acetate and propionate, polyhydroxy-2-methylbutyrate (PH2MB) from acetate and propionate and polyhydroxy-2-methylvalerate (PH2MV) from propionate only (Martín *et al.*, 2006).

Synthesizing PHA requires energy and reducing power. Energy in the form of adenosine triphosphate (ATP) is provided by hydrolyzing poly-P. In a lesser degree energy as ATP and reducing power as nicotinamide adenine dinucleotide (NADH) are generated, according to 'the Mino model', by the degradation of intracellular stored glycogen to acetyl-CoA (Mino *et al.*, 1998; Martín *et al.*, 2006).

There are different pathways for hydrolyzing polyphosphate. Either through the exopoly-phosphate gene (ppx)

or through polyP:AMP phosphotransferase (pap) to generate ATP (Martín *et al.*, 2006; He & McMahon, 2011). Hydrogen ions are released with the phosphate to build a gradient to take up the substrates (Martín *et al.*, 2006; Smolders *et al.*, 1994).

In the aerobic phase, phosphate is taken up. It has to be converted to ATP before being incorporated into poly-P (He & McMahon, 2011). Required energy is provided over the citrate cycle by metabolizing PHA (Martín *et al.*, 2006; Tchobanoglous *et al.*, 2014, p.650). Additionally energy is used for cell growth and maintenance (Morgenroth, 2016; Tchobanoglous *et al.*, 2014, p.650).

1.2.2 Tetrasphaera

The metabolism of the PAO *Tetrasphaera* is not fully known yet (Tchobanoglous *et al.*, 2014, p.651). They can take up acetate as well, but it seems that they utilize sugars and amino acids as main substrates in a fermentative metabolism and do not accumulate PHAs (Nguyen *et al.*, 2011; McIlroy *et al.*, 2017; He & McMahon, 2011; Kristiansen *et al.*, 2013). Those bacteria need more research attention, since their preferred substrates, amino acids, constitute a large fraction of the total organic carbon in the wastewater and they were prominent in many treatment plants with EBPR, where they account for up to 30% of the total bacterial volume (Nielsen *et al.*, 2010; He & McMahon, 2011; Mielczarek *et al.*, 2013; Nguyen *et al.*, 2015)(Stokholm-Bjerregaard *et al.*, 2017; Seviour *et al.*, 2008). Furthermore, most types of *Tetrasphaera* are able to denitrify and to couple nitrite and nitrate reduction with phosphorus uptake (Barnard *et al.*, 2017).

According to the metabolic model of Kristiansen *et al.* (2013), *Tetrasphaera* ferment glucose to succinate, lactate, acetate and alanine. They also synthesize glycogen as a storage polymer, using energy generated from the degradation of stored polyphosphate and substrate fermentation (Kristiansen *et al.*, 2013). But during more recent experiments at Per Nielsens laboratory in Aalborg, glycogen could not be found in *Tetrasphaera* (Adler, personal communication).

At the same laboratory, twenty different amino acids were tested on fresh biomass from a full scale EBPR plant. The biomass consisted of the two PAO *Tetrasphaera* (27%) and *Accumulibacter* (4%) (Nguyen *et al.*, 2015). Eleven of the amino acids induced a release of phosphate. Glycine showed the highest release. The molar ratio of phosphate released to glycine uptake was around 0.5, which is similar to the uptake of acetate by *Accumulibacter* (Nguyen *et al.*, 2015; Hesselmann *et al.*, 2000).

In a more recent paper, seven carbon sources (acetate, propionate, glucose, glutamate, aspartate and glycine) were tested on an enriched culture of *Tetrasphaera* (60%) and *Accumulibacter* (20%) (Marques *et al.*, 2017). Consumption of acetate and propionate led to phosphate release. *Accumulibacter* were mostly responsible for the uptake of the VFAs (Marques *et al.*, 2017). Regarding the other carbon sources tested, glucose, glutamate, aspartate and glycine consumed anaerobically can lead to either a negligible or very small level of anaerobic phosphate release or even to an uptake (Marques *et al.*, 2017). They suggest that *Tetrasphaera* are able to take up phosphate through energy generated by fermentation of carbon sources, avoiding the need to release phosphate for energy generation (Marques *et al.*, 2017).

1.2.3 Glycogen accumulating organisms

Achieving enhanced biological phosphorus removal (EBPR) relies on the design of sludge beds and wastewater feeding conditions to optimally load the biomass and to select for PAO (Weissbrodt *et al.*, 2017).

PAO have a competitive advantage: most of the other aerobic heterotrophic bacteria have none such mechanism to take up substrate in an anaerobic state and to use them later on in aerobic or anoxic state. But a group of competitors of PAO are the glycogen accumulating organisms (GAO). GAO have a similar metabolism like PAO but do not store phosphate, they just synthesize glycogen as an energy storage (Zeng *et al.*, 2003; Tchobanoglous *et al.*,

2014, p.649).

From a phosphorus removal perspective, GAO are undesired because they take up substrate but do not contribute to the EBPR process (Lopez-Vazquez *et al.*, 2009; Nielsen *et al.*, 2010). So one EBPR failure could be that PAO are outcompeted by GAO (Gonzalez-Gil & Holliger, 2011). Therefore, to achieve optimal EBPR performance, it is necessary to create conditions that are favorable for PAOs and disadvantageous for GAOs (Oehmen *et al.*, 2005). PAO can take up acetate and propionate at a similar rate, whereas the GAO *Candidatus Competibacter Phosphatis* (Gammaproteobacteria) are much slower at taking up propionate.

Other GAO known are *Defluviicoccus* (Alphaproteobacteria) (Tchobanoglous *et al.*, 2014). Those Alphaproteobacteria-GAO on the other hand can take up propionate at a similar rate as *Accumulibacter*, but acetate just at half their rate (Lopez-Vazquez *et al.*, 2009). Furthermore PAO prefer low temperatures (10 °C), where GAO are dominant around (30 °C). High pH (7.5) favors PAO as well (Lopez-Vazquez *et al.*, 2009).

Those GAO mentioned are both classical GAO competing with *Accumulibacter* on volatile fatty acids. More recent research focus as well on competitors of the PAO *Tetrasphaera*. One of them is *Micropruina*, which takes up sugars and amino acids under anaerobic conditions and ferments them further (McIlroy *et al.*, 2018).

All those PAO and GAO mentioned are summarized in Figure 1.2.

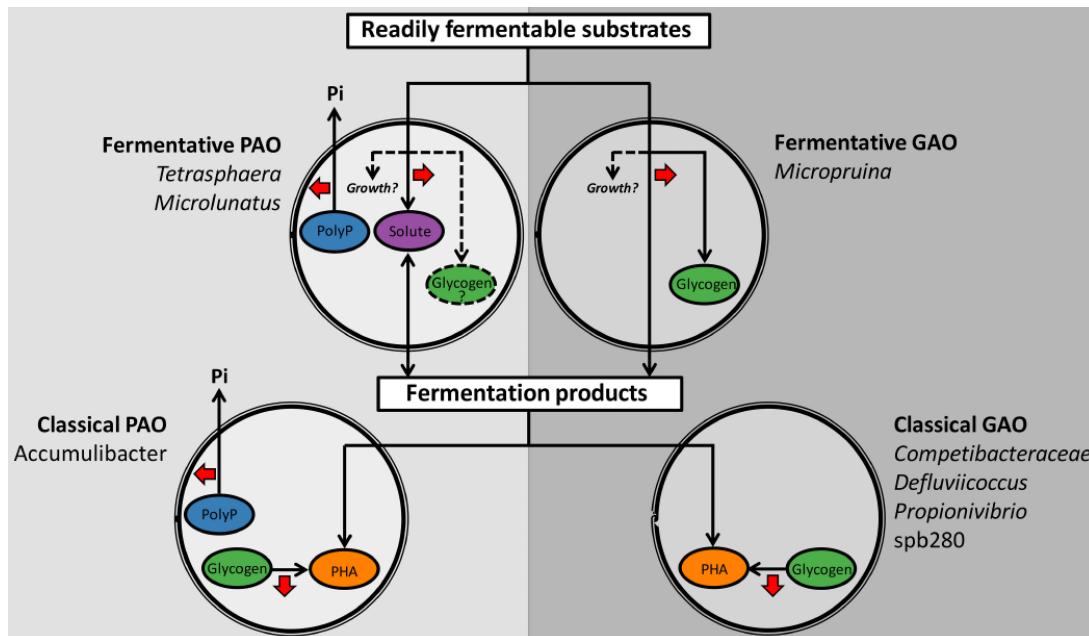


Fig. 1.2: PAO and GAO important for EBPR (McIlroy *et al.*, 2018)

1.3 Research at EPFL on AGS

The laboratory of Environmental Biotechnology (LBE) at EPFL has been doing research about aerobic granular sludge since over ten years: Around 2005 the first experiments about aerobic granular sludge were implemented. From 2008 on, Samuel Lochmatter and David Weissbrodt performed their PhD on aerobic granular sludge. Lochmatter worked on optimization of reactor start-up and intermittent aeration (Lochmatter, 2013). Weissbrodt investigated the bacterial resource management for nutrient removal (Weissbrodt, 2013). After Lochmatter and Weissbrodt, Aline Adler and Arnaud Geld started their PhD on aerobic granular sludge.

The ongoing project is in collaboration with the Department of Process Engineering of Prof. Eberhard Morgenroth at Eawag. It has the title 'aerobic granular sludge for wastewater treatment: from microbial ecology to application'. Thereby the effect of particulate substrates on granulation and nutrient removal efficiency is investigated. The research project is founded by the swiss national science foundation (SNSF).

1.3.1 Research on AGS-SBRs at LBE

An aerobic granular sludge sequencing batch reactor (AGS-SBR) was started up by Lochmatter and Weissbrodt with sludge from a WWTP with EBPR. It was initially fed with synthetic wastewater consisting of the carbon sources acetate and propionate (simple monomeric) (Lochmatter, 2013, p.38). Later on the composition of the wastewater medium was changed by Adler to a mixture of acetate, propionate, glucose and amino acids (complex monomeric). This shift is shown in Figure 1.3. It was done step-by-step with intermediate media to adapt the biomass slowly to new conditions (see Table A.1 for detailed composition of intermediate media).

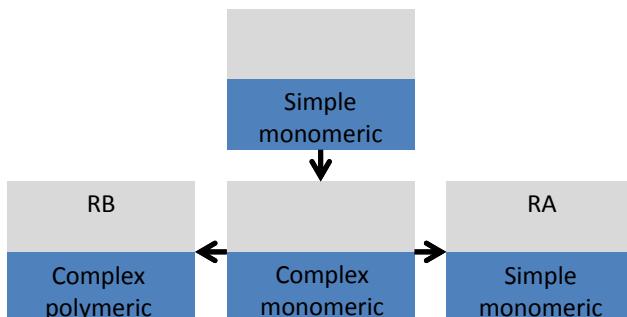


Fig. 1.3: Development of the reactors

Once stable operation was achieved with the complex monomeric medium, the biomass was divided in half and two reactors were operated. For the reactor with the name

RA, the medium composition was changed step-by-step back to medium simple monomeric. For reactor RB, the medium was changed to an even more complex medium also containing starch and peptone (complex polymeric). Although nutrient removal efficiencies could be maintained in both reactors, the biomass of reactor RB lost its large granules and was dominated by flocs. After a few weeks small granules came back, but a significant proportion of flocs remained. This non-granulation phenomenon occurred probably due to the polymeric substances. But it is unknown whether the granules would improve by shifting reactor RB back to a monomeric medium without starch and peptone.

Microbial community analysis has shown that the bacteria present in the reactor changed with the medium. Different populations are present in reactor RA than in reactor RB (see results of DNA analysis in Figures A.2 and A.3). It is expected that different bacteria populations carry out the EBPR. The phosphate concentration of the synthetic wastewater is around 5-6 times higher compared to municipal wastewater. Nonetheless the phosphate removal works properly. However, it is not known which substrates sustain the biological dephosphatation activity.

2 Objectives

In general this master thesis consists of two objectives: to investigate the reversibility of the non-granulation phenomenon observed in previous experiments and to determine which substrates are sustaining biological dephosphatation activity.

2.1 Biological dephosphatation activity

Which of the substrates present in the synthetic wastewater sustain the biological dephosphatation activity? Will there be a difference between reactor RA and RB due to their different bacterial populations?

The goal is to investigate the nutrient removal activities with a specific emphasis on biological phosphorus removal. Some substrates are well known to lead to phosphorus removal if taken up anaerobically, like acetate and propionate by *Accumulibacter*. For others it is unclear how they behave. Some have to be hydrolyzed and/or fermented first (see anaerobic food web in Figure A.5). Fermentation, especially by *Tetrasphaera* can lead directly to phosphate release or even uptake. Fermentation products like VFAs would induce phosphate release as well.

2.2 Granulation of the biomass

Is the non-granulation phenomenon observed in the previous experiment reversible?

In the previous experiment, like described in Chapter 1.3.1, the complex polymeric medium led to a loss of granules in reactor RB. This non-granulation phenomenon was expected due to the polymeric substances starch and peptone which normally diminish the granulation (Wagner *et al.*, 2015).

Reactor RB shall therefore be adapted back to the treatment of a more simple wastewater composition without starch and peptone. A better granulation with better settling properties can be expected.

3 AGS-SBRs

The experiments described further on were performed on the lab-reactors RA and RB mentioned before (see Figure 3.1). These two identical AGS-SBRs had a filling volume of 2.4 liter with a volume exchange ratio of 50% and were kept at 18 °C permanently. They were operated independently of each other.



Fig. 3.1: Reactor RA

Wastewater composition

The synthetic wastewater treated consisted of medium with the carbon source (11.25 %), a nitrogen and phosphorus medium (11.25 %) and filtered water from Lake Geneva (77.5 %). The carbon sources for reactor RA were acetate and propionate, each in the same amount of chemical oxygen demand (COD) equivalent. The carbon sources of RB consisted of acetate, propionate, glucose, amino acids, peptone and starch, all in the same amount of COD equivalent (see as well Table A.1). Amino acids were a mix of seven different amino acids, chosen according to the ones

included in the peptone. Those seven amino acids were alanine, arginine, aspartic acid, glutamic acid, glycine, leucine and proline. All of them were included in the same amount of COD equivalents. Their chemical figures and the figures of acetate and propionate are plotted in Chapter A.2.1. Soluble starch from potatoes was used. The peptone was an enzymatic product of animal protein digestion. In contrast to the other substrates, starch and peptone are not readily biodegradable carbon sources (rb-COD). Like particulate matter (pCOD), they have to be hydrolysed to rbCOD to be available to bacteria (Jabari *et al.*, 2016).

The amount of COD, total nitrogen and phosphorus was kept independently of the medium always the same and listed in Table 3.1 below. The high concentration compared to a typical swiss wastewater composition (see reference values in Table 3.1 according to (Gujer, 2006, p.98)) should increase the growth of bacteria, including phosphate accumulating organisms. The entire compositions are in more detail in document 'Simple Monomeric Medium' and 'Complex Polymeric Medium' in the digital appendix.

Tab. 3.1: Inflow concentration

	RA/RB [mg/L]	Typical [mg/L]
COD	600	300
Nitrogen	56	33
Phosphorus	22	5.3

SBR sequences

The reactors perform the sequences according to Figure 3.2 each cycle. Anaerobic mixing is done by injecting nitrogen. In the aerobic phase, intermittent for 10 minutes oxygen is pumped in, followed by an anoxic phase without any gas input.

The length of the cycles are listed in Table A.2. The aerobic phase lasts at least 4 h. If there is still a decrease of the oxygen concentration higher than 20 % during the anoxic part, it can last up to 5 h (Reactor RB) resp. 5.6 h (Reactor RA).

There was no systematic removal of excess sludge. The settling time was adapted between 4 and 6 minutes to remove slow settling biomass into the clarifier and keep the amount of biomass in the reactors stable.

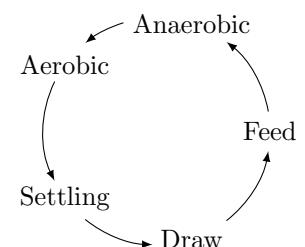


Fig. 3.2: Sequences

Reactor control and monitoring

The reactors were controlled and monitored with the program DAQFactory by Azeotech. The pH was controlled with two one-sided PID controllers managed by the digital multi-parameter transmitter Liquiline CM442 by Endress+Hauser further described in Chapter 4.2.1. Beside a pH sensor, an oxygen and a conductivity sensor were connected to DAQFactory. Those values were measured on-line and saved each cycle into a spreadsheet file. A typical cycle in reactor RA is plotted in Figure 3.3.

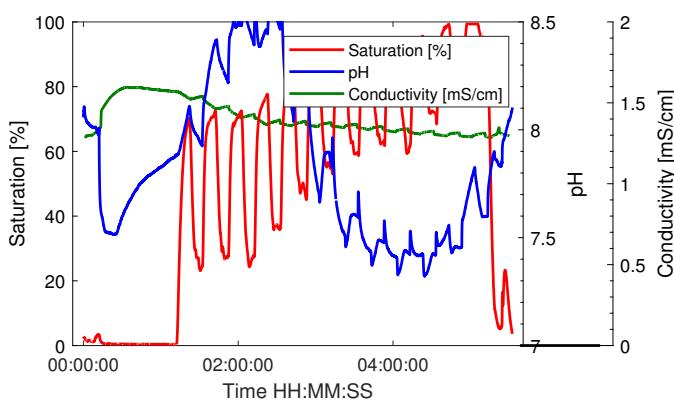


Fig. 3.3: Saturation, pH and conductivity in RA

The oxygen saturation in Figure 3.3 displays the different phases. During the anaerobic phase with nitrogen mixing, the oxygen saturation was around zero. Afterwards it increased during the ten minutes aerobic phases and decreased in the following anoxic phase. The lower the peak and the higher the decrease, the more oxygen was used up.

The conductivity was probably influenced by the uptake and release of phosphate but as well by the variation of the pH.

The pH itself was influenced by different processes. The VFAs in the inflow decreased the pH during the feeding phase. As they were taken up in the anaerobic phase the pH increased again. The strong pH increase in the aerobic phase correlated with the uptake of phosphate, which took place in the first 90 minutes of the aerobic phase (according to test by Adler, data not shown). It was probably due to the uptake of hydrogen ions with the phosphate and the aerobic respiration by the PAO. The respiration generated CO_2 , which decreases the pH. But this can be compensated if the aeration strips it out of the water.

Nitrification decreases the pH, which could explain the decrease after the first peak, whereas the second increase could be due to denitrification, which generates hydrogen carbonate (Morgenroth, 2016). This increase went

together with a much lower uptake of oxygen, indicating nitrification was already over at that point. The pH in reactor RB was in general lower, probably due to acidification by fermentation of glucose and amino acids, which produces CO_2 .

Water measurements

To monitor the performance of the reactors, every Monday and Thursday the inflow, outflow and the water at the end of the anaerobic phase were analyzed on COD, ammonium ($N - NH_4^+$) and with ion chromatography (IC) on nitrite ($N - NO_2^-$), nitrate ($N - NO_3^-$), orthophosphate ($P - PO_4^{3-}$) and sulfate (SO_4^{2-}). Additionally, in the inflow of reactor RB glucose was measured. For both inflows high performance liquid chromatography (HPLC) was been applied to measure the VFAs. See digital appendix 'Water_test' for protocol and results.

Biomass Properties

Every Tuesday the biomass was sampled according to the 'Protocol Biomass collection' (see digital appendix) to perform deoxyribonucleic acid (DNA) analysis to follow the development of the bacteria populations (see Figure A.2 and A.3).

The parameters 'total solids', 'volatile solids' and 'sludge volume index' were measured every few months and during the adaption of the medium in reactor RB weekly. They were measured according to 'Protocol SVL_TS_VS' in the digital appendix. Whereby the soluble solids emptied out after centrifugation were neglected. Their definitions are described as follow:

The **total solids (TS)** [g/mL] are defined as the residue remaining after a wastewater sample has been evaporated and dried at a specified temperature (103 °C-105 °C) (IWA, 31.07.2018; Tchobanoglous *et al.*, 2014, p.73).

The **volatile solids (VS)** [g/mL] are defined as those solids that can be volatilized and burned off when the TS are ignited (500 °C +- 50 °C) (IWA, 31.07.2018; Tchobanoglous *et al.*, 2014, p.73).

The **sludge volume index (SVI)** is a measurement of the compression of the activated sludge (Gujer, 2006, p.325). It is defined as the volume (in mL) occupied by 1 g of sludge after 30 min settling in a 1 L unstirred cylinder (see equation below) (van Loosdrecht *et al.*, 2016, p.237). SV_{30} is defined as the volume of biomass after 30 min of settling (van Loosdrecht *et al.*, 2016, p.237).

$$SVI[mL/gTS] = \frac{SV_{30}[mL]}{TS[g/mL] * V[mL]} \quad (3.1)$$

4 Biological dephosphatation activity

To answer the question, which substrates sustain the biological dephosphatation activity, anaerobic batch tests were performed in the reactors itself. They show the uptake of substrate and the release of phosphate induced by those substrates. The release of phosphate was measured with IC, the substrate concentration with COD measurements as an indicator.

The first experiments were performed with the current medium on both reactors (see Chapter 3), afterwards it was repeated on both reactors with every substrate (acetate, propionate, glucose, amino acids, starch and peptone) individually.

The pH was adapted during two experiments, to evaluate its influence, further described in Chapter 4.2.1. To explain the unexpected and substantial uptake of amino acids and release of phosphate in reactor RA, tests with glycine and other individual amino acids were performed as well, described further in Chapter 4.1.2.

4.1 Material and Methods

The tests generally followed the protocol of anaerobic EBPR batch activity tests EBPR.ANA.1, EBPR.ANA.2 and EBPR.ANA.3 according to the book 'Experimental Methods in Wastewater Treatment' by van Loosdrecht *et al.* (2016). The duration of the tests was given by the current length of the anaerobic phase of each reactor, which was one hour in reactor RA and one and a half hours in reactor RB.

Measurements

The defined amount of carbon source equals the mass of COD-equivalent added every cycle so far (720 mgCOD), which results in an initial concentration of 300 mgCOD/L. The substrate should be added as fast as possible and therefore fit in one reactor-syringe (50 mL). So a total substrate volume of 40 mL was chosen with a concentration of 18'000 mgCOD/L.

The parameters measured were, as mentioned before, the COD and the concentration of phosphate in the water. The concentration of phosphate ions ($P - PO_4^{3-}$) was analyzed with ion chromatography (ICS-90 Ion Chromatography System by Dionex), which measured the nitrate ($N - NO_3^-$) concentration as well. The measurement ranges were 0.7-16.7 mg $P - PO_4^{3-}$ /L and 0.5-11.5 mg $N - NO_3^-$ /L. The COD was measured as an indicator for the substrate concentration. It shows often more than 95 % of the theoretical value, the theoretical oxygen demand (TOD) (Gujer, 2006, 2008, p.50). It can be calculated as the amount of oxygen used to oxidize the substrates based on the in-

dividual chemical elements (Gujer, 2008, p.98). The TOD can be used as an approximative conversion factor between gCOD and mol concentration of a substrate. The values of the substrates used are listed in Table A.4. The COD can be measured easily, but below a concentration of 20 mg/L it is quite inaccurate (Gujer, 2006, p.50). COD measurements were done with the Kit LCK 314 by Hach Lange which measures in the range 15-150 mgCOD/L.

Dilutions were chosen according to the measurement ranges of phosphate and COD (see 'Batch_Tests.xls' in the digital appendix for corresponding dilutions).

Samples were taken during the whole period of anaerobic mixing. First every five, later on every 10 and 15 minutes. The exact procedure is described in the protocol (see appendix Chapter A.3.5).

Stoichiometric parameters

For every experiment, the phosphate release in mg $P - PO_4^{3-}$ /L was calculated as the difference between the last and the first measurement during the anaerobic phase. To define the uptake of substrates, the COD concentration at the begin and the end were compared. For both values the initial concentration before adding the substrate was subtracted

The simpler substrates like the VFAs were expected to be fully consumed, which confirms test EBPR.ANA.2 (van Loosdrecht *et al.*, 2016, p.30). Those tests allow to determine the anaerobic phosphate release to carbon source uptake ratio (P-mol/C-mol), short P/C ratio.

$$P/C \text{ ratio} = \frac{P - PO_4 \text{ release [mmol/L]}}{COD \text{ uptake [mmol/L]}} \quad (4.1)$$

The phosphate release, the substrate uptake and the ratio are described in detail in Chapter A.3.2. Most of the other substrates were not expected to be fully consumed or consumed at all in the given anaerobic time. This corresponds to test EBPR.ANA.3, where the carbon source is added in excess (van Loosdrecht *et al.*, 2016, p.30). Thus the P/C ratio can be calculated as well, but it has to be evaluated with caution because it is strongly influenced by the length of the experiment or by measurement uncertainties, if the substrate was not consumed at all.

For reactor RA, additionally test EBPR.ANA.1 was conducted (van Loosdrecht *et al.*, 2016, p.30). In this test no carbon source was added, which allows to determine the anaerobic endogenous maintenance phosphate release rate and behave as a reference measurement (van Loosdrecht *et al.*, 2016, p.30).

4.1.1 Influence of pH

In this chapter, the influence of the pH on biological dephosphatation activity during the anaerobic batch experiments is analyzed further.

As mentioned earlier, the pH was controlled with two one-sided PID controllers. These standard automatic controllers combine the instantaneous condition (P member),

with the past history (I member) and the possible future development (D member) into one single control rule (Gujer, 2008). In this case, just the P member, the proportional controller was activated.

The calibrated parameters are listed in Table 4.1 according to the operation instructions of Liquiline CM442 (Endress+Hauser, n.d.).

Tab. 4.1: Parameter of the PID controller

Name	Description	Regular Values	First Adaption	Second Adaption
w	Set Point w	[pH]	7.5	7.8
Xn (1)	Neutral Zone up	[pH]	1	0.1
Xn (2)	Neutral Zone down	[pH]	0.3	0.1
Rate	Rate	[/s]	0.1	0.3
Xp	Proportional band	[/pH]	6	6

With the regular calibrated parameters, the pH is controlled between 7.2 and 8.5. They lead to a pH variation according to Figure 4.1 (Regular Values) during the anaerobic batch test with acetate.

PID parameter were set back to the regular values after the anaerobic phase.

4.1.2 Glycine and other amino acids

The amino acid mix tested on reactor RA was partly consumed and led to a high release of phosphate (discussed further on in Chapter 4.2). Every amino acid included in the amino acid mix was tested individually on reactor RA as well. The simple amino acid glycine lead to high release of phosphate tested on biomass consisting of a big proportion of *Tetrasphaera* (Nguyen *et al.*, 2015). Therefore the first tests were performed with glycine. As the results were unexpected, the test was repeated at different concentrations: first with the initial inflow concentration of 600 mgCOD/L, later on with half as much (300 mgCOD/L) and even lower (150 mgCOD/L and 75 mgCOD/L). Three times the anaerobic phase was extended to two hours and samples were taken during the aerobic phase as well.

The other amino acids included in the mix (alanine, arginine, aspartic acid, glutamic acid, leucine, proline) were tested once on reactor RA. There fewer samples were taken as a clear trend could be seen easily.

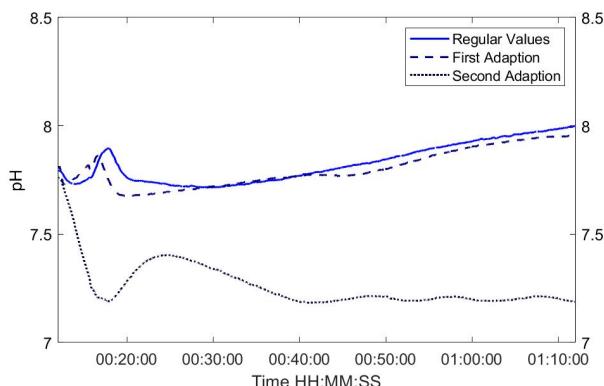


Fig. 4.1: pH variation during anaerobic phase

To analyze the influence of pH, the set point and the neutral zone were adapted and the rate slightly increased to keep the pH between 7.7 and 7.9. But the pH still fluctuated quite strongly up to 8 (see Figure 4.1, First Adaption). Therefore the proportional band Xp, which is equal to the reciprocal value of the gain Kp ($Xp = 1/Kp$) was decreased as well. A larger gain leads to a larger and more rapid change of the control variable (Gujer, 2008, p.337). The pH variation according to this second parameter adaption can be seen in Figure 4.1 as well. The pH variation during the whole cycle, as well the oxygen saturation and conductivity are plotted in Figure A.22. The

4.2 Results and Discussion

Figure 4.2 presents the results of the anaerobic batch tests EBPR.ANA with the different carbon sources on reactor RA and RB. All numbers can be found in the appendix Table A.6. The detailed plots of each test are in the appendix Figures A.10 for RA and Figures A.11 for reactor RB. Plots with smaller phosphate axis for the ones with no release are in Figure A.12.

The results of the COD control measurements of the substrates are listed in Table A.5 in the appendix.

The P/C ratios of the substrates fully used up (COD end below 20 mgCOD/L) are listed in Table 4.2.

Tab. 4.2: P/C ratios [mol-P/mol-C]

	RA	RB
Current medium	0.35	0.23
Acetate	0.35	0.58
Propionate	0.27	0.38
Glucose		0.29

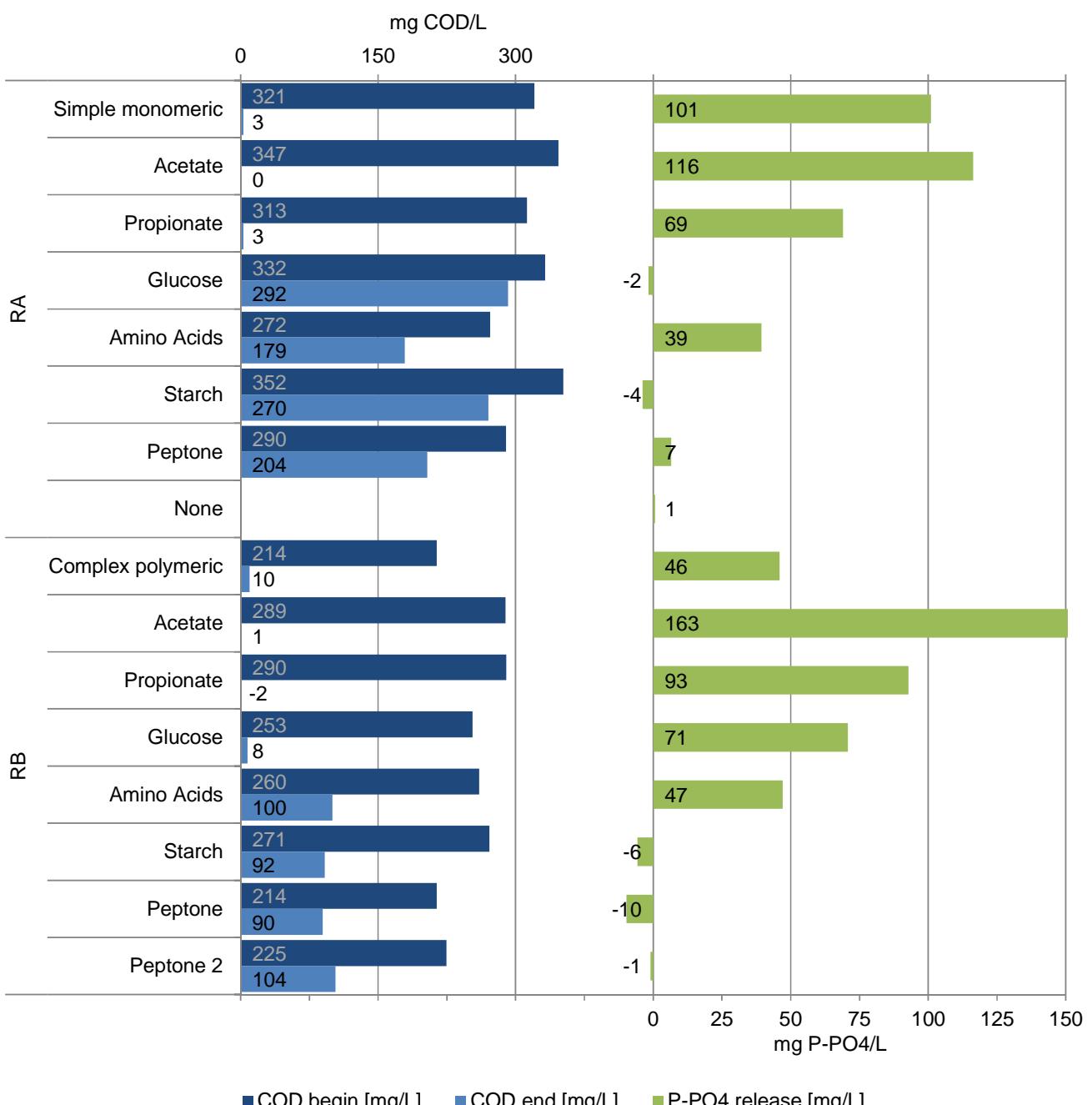


Fig. 4.2: Phosphate release induced by different substrates on reactor RA and RB

Discussion of uncertainties

The value of 'COD begin' (in Figure 4.2 displayed in dark blue) was calculated as the difference between the first measurement right after adding the substrate (AN0) and the initial measurement before adding the substrate (AN-). In general, it should be around 300 mgCOD/L for every substrate. Its variation depends on:

- The COD concentration before adding the substrate, consisting of COD from previous cycle and from the pipes. It could not be measured precisely because the values were mostly below the measurement limit of 15 mgCOD/L.
- The uncertainty in adding the correct mass of substrate
- The uncertainty in measuring the COD of certain substrates. To control this uncertainty, during each test the substrate itself was analyzed on COD as well (see Table A.5). Only the COD of peptone was significantly below 300 mgCOD/L. The peptone used was a mixture of different peptides and amino acids, which were just roughly known. The rough conversion factor 1.4 gCOD/g peptone was used. This can explain the 'COD begin' value for peptone below 300 mgCOD/L.
- Insufficient mixing before taking sample 'COD begin'. This fourth point could be an indicator, if just the first sample was much higher than the others and clearly above 300 mgCOD/L. This is visible, for example, in RA starch: according to the whole pathway just the first value was quite high and then the concentration stayed stable which leads to the hypothesis that it was not used up (see Figure A.10).

Discussion of the tests with acetate and propionate

The uptake of the whole simple monomeric medium in RA was expected, as it is equal to the regular inflow. The same was expected with acetate and propionate in RA and RB as individual substrates, as they were in the regular inflow of both reactors. All led to phosphate release, which can be assigned to the bacteria *Accumulibacter*, which take up acetate and propionate during the anaerobic phase (see introduction Chapter 1.2.1). According to the DNA analysis by Adler (see Figure A.2 and Figure A.3) and the fluorescent in-situ hybridization (FISH) analysis by Gelb (see Figure A.4), there were a lot of *Accumulibacter* in RA and some in RB. In reactor RA there was also a significant number of the GAO *Competibacter*, which could not be found in RB. Instead, the fermentative GAO *Microprruina* was abundant in RB but not in RA. The GAO *Defluviicoccus* could not be found in both reactors.

The substrate uptake velocity in reactor RA was $0.2 \text{ C} - \text{mol} * \text{C}^{-1} * \text{mol}^{-1} * \text{h}^{-1}$ for acetate and $0.17 \text{ C} - \text{mol} * \text{C}^{-1} * \text{mol}^{-1} * \text{h}^{-1}$ for propionate, in RB 0.13 resp. $0.12 \text{ C} - \text{mol} * \text{C}^{-1} * \text{mol}^{-1} * \text{h}^{-1}$ (3500 mgVS/L, see Chapter 5, 26 mg/C-mmol (van Loosdrecht *et al.*, 2016, p.48)). The faster uptake in RA can be explained by the higher concentration of *Accumulibacter* and the *Competibacter*. The presence of *Competibacter* explains as well the lower release in RA compared to RB, as it probably takes up part of the VFAs in RA.

A higher phosphate release for propionate can be expected, because *Competibacter* are faster in taking up acetate (Lopez-Vazquez *et al.*, 2009; Oehmen *et al.*, 2005). This contradicts the results here, where the release was lower for propionate (see Table A.6).

The Alphaproteobacteria-GAO *Defluviicoccus* could explain this effect as they take up propionate at a similar rate as *Accumulibacter*, but acetate just at half their rate (Lopez-Vazquez *et al.*, 2009). But like mentioned above, they were not abundant in both reactors.

Naturally, the influence of the GAO concentration is reflected in the P/C ratio. This will be discussed further with the pH in Chapter 4.2.1.

Discussion of the results in Reactor RA

Glucose, starch and peptone were not used up, which was expected, as those substrates were not included in the regular inflow medium. Therefore bacteria which can hydrolyze and ferment them were not expected to grow. Furthermore there was no phosphate release meaning the phosphate concentration behaved like the reference plot 'RA None' with no carbon source (see Figures A.12). The phosphate concentration decreased, as long as nitrate was available, then increased slowly (3.2 mgP/L/50min), which can be considered as the endogenous anaerobic maintenance release rate. The phosphate release rate was around $1.2 * 10^{-4} \text{ P-mol/h}$ or $8 * 10^{-4} \text{ P-mol} * \text{C} - \text{mol}^{-1} * \text{h}^{-1}$, which is in the expected magnitude (van Loosdrecht *et al.*, 2016, p.49).

As glucose was not consumed, this confirms the results from DNA and FISH analysis (see Figure A.2 and Figure A.4) that no *Tetrasphaera* were available in reactor RA. The substantial consumption of the amino acids mix was unexpected. One third (34 %, see Table A.6) was consumed, despite there usually were no amino acids in the regular inflow. The amino acids led to a phosphate release of almost $42 \text{ mgP} - \text{PO}_4^{3-}/\text{L}$ (see Table A.6) and still increasing (see Figure A.10). This phenomenon was analyzed further with tests with glycine only in Chapter 4.2.2 and with the other amino acids individually in Chapter 4.2.3.

Discussion of the results in Reactor RB

In reactor RB, the concentration of all substrates decreased. The consumption of amino acids, starch and peptone was limited by the duration of the anaerobic phase. This was expected, as in the regular inflow medium just one sixth of each substrate was included.

Based on the given results, it can not be concluded if the release of phosphate by amino acids and glucose was due to their direct uptake by *Tetrasphaera* or/and because they were fermented to acetate which led to the phosphate release (Kristiansen *et al.*, 2013). HPLC analysis to detect an intermediate concentration of acetate or propionate can not be done, because they would be faster consumed than produced (was tested, data not shown).

Starch and peptone did not lead to phosphate release. As in reactor RA, there was always an uptake as long as nitrate was available. The first test with peptone even led, due to a permanent anoxic state, to the full uptake of phosphate (see Figures A.12).

In contrast to reactor RA, the concentration of the polymeric substances decreased substantially. If they were hydrolyzed, intermediate products like glucose or amino acids would have led to phosphate release. It can be assumed that the molecules were adsorbed to the biomass,

an effect known from particulate matter (Morgenroth *et al.*, 2002). This would explain their decrease in the water not connected to phosphate release. As a proof, the experiment can be repeated with inactivated biomass, though it would be difficult to perform. Probably the polymeric substances did not get adsorbed to the biomass in reactor RA due to a lower concentration of flocs and smother granules (Adler and Derlon, personal communication). In conclusion, in these reactors starch and peptone behave like insoluble particulate matter and do not affect the rate of anaerobic phosphate release (Jabari *et al.*, 2016). Their influence depends on the rate of hydrolysis which is lower than the uptake of VFAs particularly under anaerobic conditions (Morgenroth *et al.*, 2002).

4.2.1 Influence of pH

The anaerobic batch test with acetate was repeated with adapted parameters of the pH controller according to Table 4.1. In all tests, the acetate was mostly used up. But a significantly lower phosphate release can be seen at lower pH in Figure 4.3, which led to a lower P/C ratio of just 0.25 P-mol/C-mol compared to 0.35 and 0.37 in the first two experiments with acetate. The COD and phosphate concentration during the anaerobic phase are plotted in Figure A.13 in the appendix.

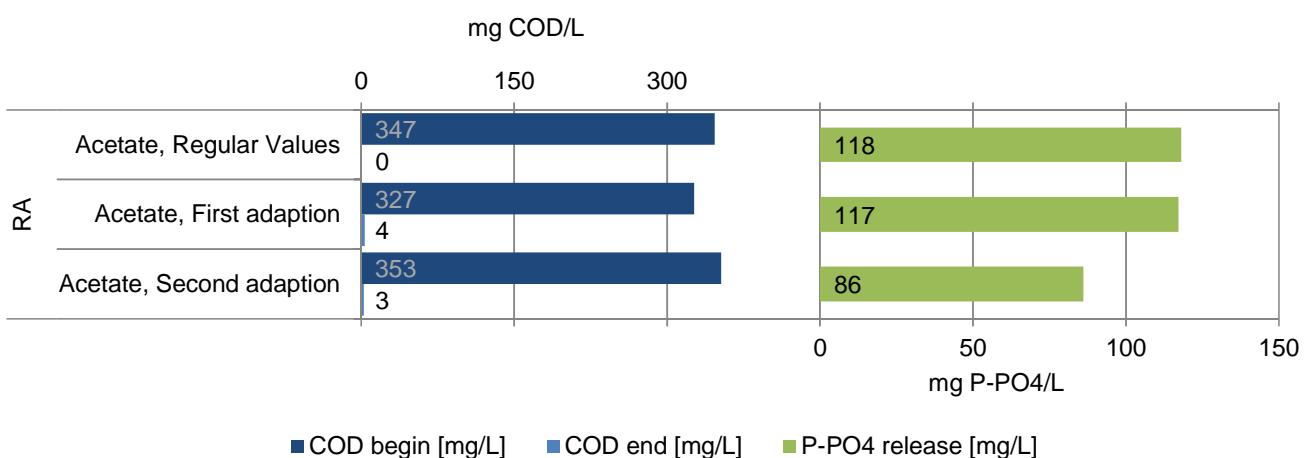


Fig. 4.3: Phosphate release induced by acetate with adapted pH parameter

Discussion of influence on pH

The pH influences the biological dephosphatation in different ways:

On one hand, a quite significant amount of phosphate can be removed by precipitation (see Figure A.7). In the anaerobic phase the phosphate concentration as well as the pH increases, both leading to higher precipitation rates (Ferguson *et al.*, 1973; Morgenroth, 2016).

On the other hand, the pH influences the anaerobic metabolism of the biological phosphorus removal. At low pH the uptake of acetate requires no energy. All phosphate released originates from the ATP required to convert acetate to acetyl-CoA (Smolders *et al.*, 1994). But at higher pH, up to 0.5 ATP is used to take up 1 mol of acetate, leading to a higher phosphate release (Smolders *et al.*, 1994).

This is due to the pH gradient between the inside and outside of the cell. At a higher pH outside, much more work has to be done to take up VFAs, because they are transported into the cells with a hydrogen ion and their number is limited at a high pH (Smolders *et al.*, 1994).

The pH decreases by releasing phosphate, which releases hydrogen ions as well (see Figure A.1).

Precipitation could be an explanation for the decrease of the phosphate concentration during some anaerobic batch tests after the substrate was used up. This is most clearly visible with acetate in reactor RA (see Figure 4.2(b)). Already at the second test with almost the same pH, this trend was weaker (see Figure 4.3(b)).

The third test with acetate on reactor RA at clearly lower pH does not show a decrease during the last one or two measurement points. This indicates the effect of precipitation, which is stronger at higher pH and as well at higher concentration. But no clear trend can be stated due to too few measurements.

On the other hand, the lower pH led to a clearly lower release of phosphate (see Figure 4.3). So the effect of pH on the anaerobic metabolism of the PAO is much higher than on chemical precipitation. So the P/C ratio was lower at lower pH. Literature values show the same phenomena, but in general a higher release (see Figure A.8 (Smolders *et al.*, 1994)).

On the other side, the P/C ratio can be considered as one of the most suitable indicators to assess the PAO and GAO activity of EBPR cultures. At standard conditions of 20 °C and pH 7.0 it indicates whether the metabolism is more GAO or PAO dominated, see Figure 4.4.

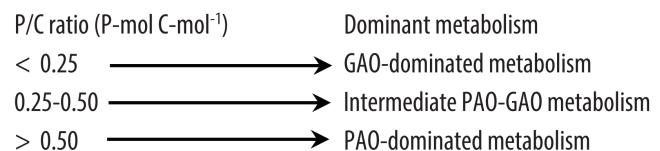


Fig. 4.4: P/C ratio (van Loosdrecht *et al.*, 2016, p.42)

The result of the second adaption (pH around 7.2) showed a ratio of 0.25 P-mol/C-mol. It can be expected, that at pH 7.0, the ratio would be even lower, which means below 0.25. This indicates that in reactor RA the bacteria population was slightly GAO-dominated.

In reactor RB, the P/C ratio for acetate was higher at 0.58 P-mol/C-mol (see Table A.6), at a lower pH between 7.2 and 7.6 as well (see Figure A.21(c)). This higher P/C ratio of RB compared to RA indicates a increased PAO metabolism. This can be confirmed by the DNA analysis, which showed some GAO, type *Competibacter* in RA but not in RB (see Figure A.2 and A.3).

4.2.2 Glycine

The consumption of amino acids coupled with phosphate release in reactor RA was surprising, as mentioned in the previous chapter. No amino acids were in the regular influent of reactor RA, bacteria which can consume them were not expected to be abundant.

Glycine, the smallest amino acid, was tested at different initial concentrations in reactor RA. It led to a significant release of phosphate while it was not used up (see Figure 4.5).

The phosphate release is limited by the length of the anaerobic phase, which is shown with three tests when the anaerobic phase was extended to two hours (see dark green values in Figure 4.5). Thereby the aerobic phase was analyzed as well (see Figure A.17).

The detailed figures are in appendix in Figures A.15 and Figures A.16. They show a stable phosphate release rate during the first hour of anaerobic phase.

The values itself are listed in Table A.7. The COD uptake and the P/C ratio in Table A.7 depend on measurement uncertainties and can not be compared.

Glycine was also tested on reactor RB. The substrate concentration decreased and phosphate was released, but less compared to reactor RA (see Figure A.14). The oxygen saturation, pH and conductivity during those tests are plotted in Figures A.24 and Figures A.25.

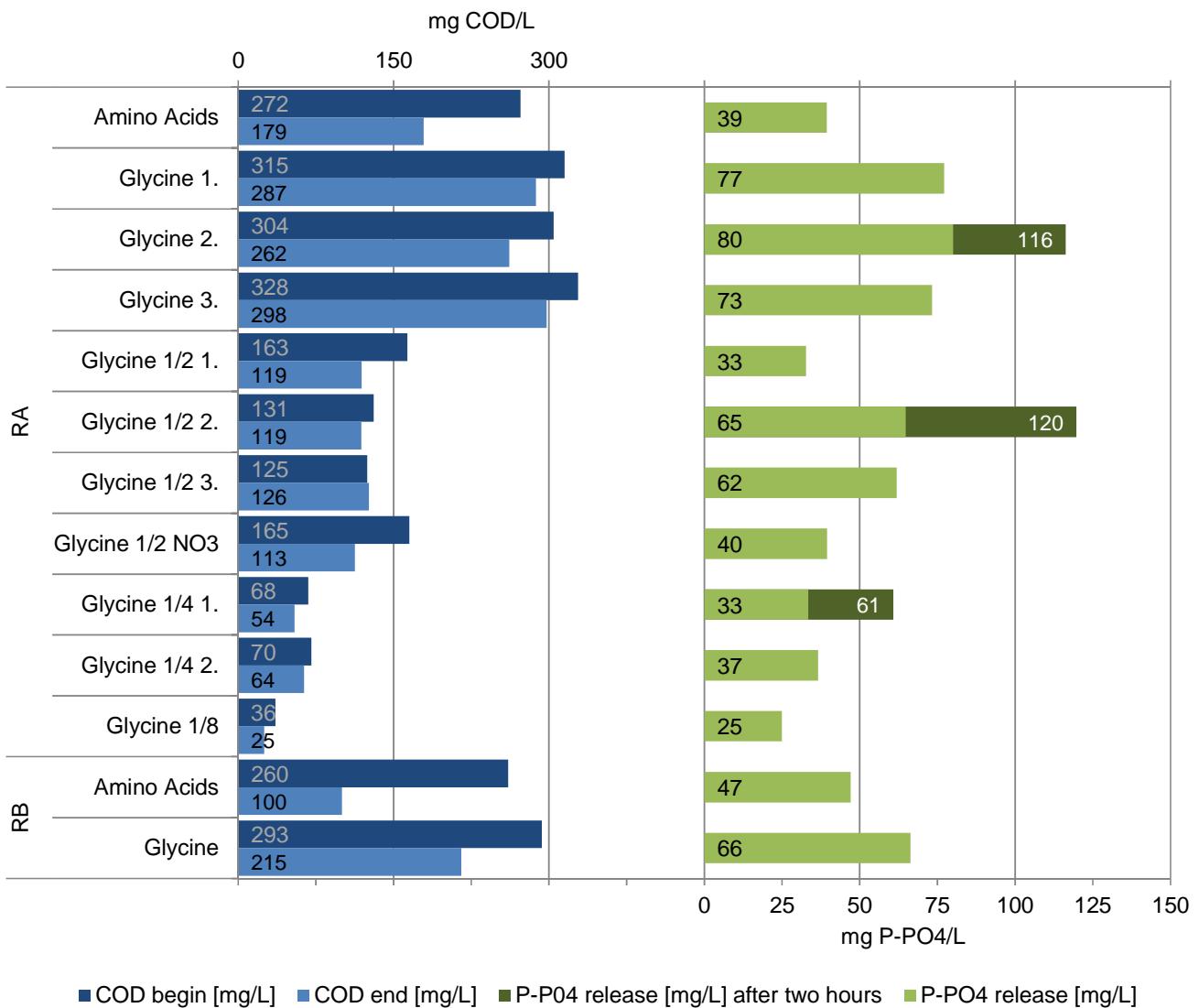


Fig. 4.5: Phosphate release induced by glycine at different concentrations on reactor RA

Discussion

Glycine with a concentration of 300 mgCOD/L (dark blue in Figure 4.5) led three times to a comparable release of phosphate. The first test at half concentration (Glycine 1/2 1.) led to a very low release. The whole plot in Figure A.16(c) shows a high concentration of nitrate due to a malfunction in the previous cycle. Another test with glycine and manually added nitrate could confirm the lower release due to nitrate (see 'Glycine 1/2 NO3' and Figure A.15(e)). Based on those results, it can be derived that at least some of the *Accumulibacter* in RA were Type I, able for denitrification linked to phosphate uptake (Nielsen *et al.*, 2010).

A second and third repetition of 'Glycine 1/2' led to a much higher release of phosphate, which was just slightly less compared to the release of 'Glycine 1. 2. and 3.'

The phosphate release of 'Glycine 1/4' and 'Glycine 1/8' was lower compared to the initial concentration of glycine. *Accumulibacter* was the only PAO abundant in RA, so the release has to be by *Accumulibacter*. The phosphate release was not connected to an uptake of substrate and was ongoing, even after two hours. Therefore in an unlimited anaerobic phase, it can be expected that the release would keep going on up to emptied phosphate storage in the bacteria.

For the test in reactor RB, it is not possible to distinguish between a release due to *Accumulibacter* like in RA or by *Tetrasphaera*, which can consume it connected to phosphate release (Nguyen *et al.*, 2015). Both are abundant in RB according to the DNA analysis (see Figure A.3).

Michaelis-Menten kinetics

Based on the results above (Figure 4.5) can be concluded that the phosphate release rate increases with the concentration of glycine. Therefore the release rate in $mgP - P04 * L^{-1} * h^{-1}$ was plotted against the average concentration of the COD measurements during each test, see Figure 4.6.

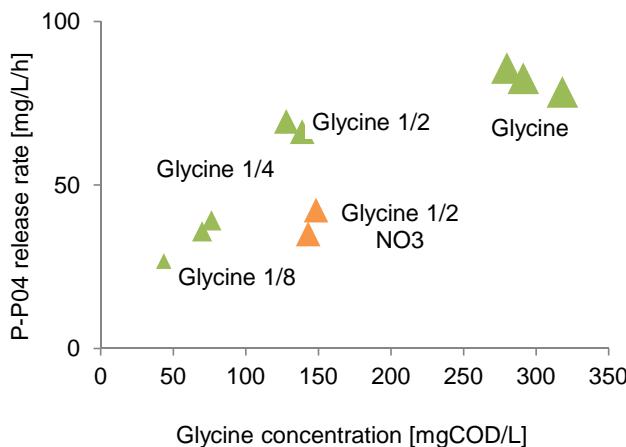


Fig. 4.6: Correlation glycine and phosphate release rate

The values in orange were lower due to the high nitrate concentration. For the others, the dependence of the release rate to the concentration seems to be higher at low concentration. Therefore, the relation can be described with Michaelis-Menten kinetics. The appropriate equation was proposed to model enzymatic reactions (Stryer, 1988, p.198).

The reaction rate V is described as a function of the maximum reaction rate V_{max} , the substrate concentration $[S]$ and the Michaelis constant K_M according to the equation below:

$$V = V_{max} \frac{[S]}{[S] + K_M} \quad (4.2)$$

The constants V_{max} and K_M can be derived easily from the Lineweaver-Burk plot, where the reciprocal of the release rate (V) is plotted as a function of the reciprocal of the glycine concentration ($[S]$) (see Figure 4.7).

The plot is described with the following equation (Stryer, 1988, p.198):

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} * \frac{1}{[S]} \quad (4.3)$$

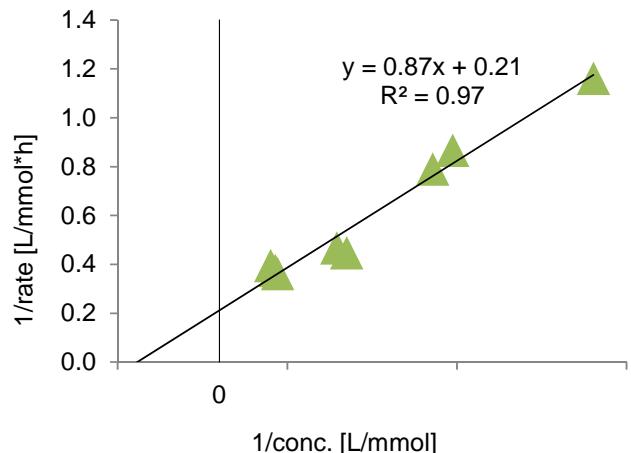


Fig. 4.7: Lineweaver-Burk plot

Accordingly, based on the trendline in Figure 4.7, the constants can be calculated: $V_{max} = 4.7 \text{ mmol/L/h}$ and $K_M = 4.1 * 10^{-3} \text{ mol/L}$. The concentration K_M leads to half of the maximum release rate (Stryer, 1988, p.198). The sampling points, the constants and the Michaelis-Menten equation are plotted in Figure 4.8.

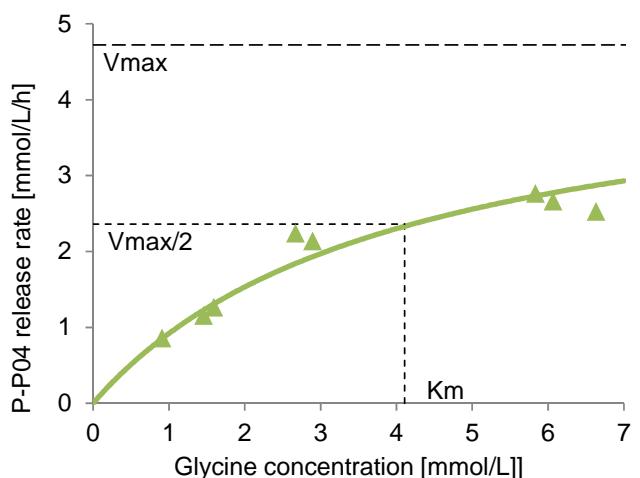


Fig. 4.8: Michaelis-Menten kinetics

A K_M value of $4 * 10^{-3} \text{ mol/L}$ is quite high, which indicates a low affinity of glycine to the PAO. For comparison, nitrate taken up by denitrifier has a value around around 10^{-5} mol/L , indicating a high affinity (Lin, 2008; Shaw, 2015).

Influence on phosphate uptake in the aerobic cycle

During the experiments 'Glycine 2.', 'Glycine 1/2 2.' and 'Glycine 1/4' samples were taken during the aerobic phase as well, for 'Glycine 2.' and 'Glycine 1/4' up to the end (see Figures A.17). The COD concentration decreased slightly. The phosphate was taken up, but slower than in a regular cycle (regular cycle measured by Adler, data not shown). For 'Glycine 2.' there was a residual concentration of around $7 \text{ mgP} - P_0^{-3}/\text{L}$. For 'Glycine 1/4' the residual concentration was just $0.3 \text{ mgP} - P_0^{-3}/\text{L}$. Both of which were lower than the phosphate concentration in the inflow. It is assumed that the phosphate was taken up by energy generated by oxidizing PHA produced in previous cycles. There was probably even enough energy for further growth, which could lead to more phosphate uptake than previously released.

Figure 4.9 (the others in Figure A.24 and A.25) show a high oxygen uptake. The aerobic phase was always extended to their maximum of 5.6 h, even at further cycles. The NH_4^+ concentration of the effluent was high according to the regular measurement kit. But the kit just interfered with the glycine in the water. Control measurements with IC showed NH_4^+ concentrations below the measurement limit (see 'water results' in digital appendix). So nitrification was working properly even with glycine and with NH_4^+ produced from glycine degradation.

The higher oxygen demand shown in the longer aerobic phase was probably due to the respiration of bacteria consuming glycine and due to an increased nitrification based on NH_4^+ produced out of glycine.

Influence on pH

Normally, the phosphate gets released with hydrogen ions. Those induced a pH gradient and were then taken up again with the volatile fatty acids (Smolders *et al.*, 1994; Martín *et al.*, 2006). Therefore during tests with glycine, the hydrogen ions stayed in the water, which led to a decrease of the pH during the anaerobic phase (visible in Figure 4.9 and the others A.24. The pH was regulated at 7.2, see Table 4.1). The height of the peaks was as well influenced by the current state of the reactor.

Additionally, the regular peak of the pH during the first hour of the aerobic phase did not appear (compare with a regular cycle Figure ??). As the PAO didn't get nourishment, they were just oxidizing PHA from previous cycles and taking up phosphate slowly. Therefore the peak due to the uptake of hydrogen ions with phosphate could be distributed over the whole aerobic phase, which makes it invisible.

An influence of the pH itself on the phosphate release by

glycine is not expected, as it is not connected to an uptake. Further tests at different pH concentrations would be recommended to investigate this influence.

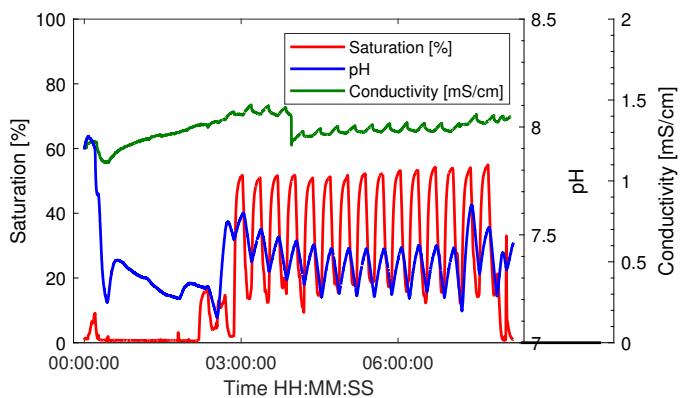


Fig. 4.9: RA Glycine 2.

Possible Explanations for the phosphate release

As previously discussed, according to DNA and FISH analysis, reactor RA consists mostly of the PAO *Accumulibacter*. Therefore it can be assumed that glycine induced a phosphate release on them.

The COD measurements show a constant substrate concentration. But detailed measurements would be necessary to guarantee that the COD measured was still glycine. Fermentation processes like the Stickland reaction are not expected, as they would have lowered the COD and lead to acetate which would be used up fast (Marques *et al.*, 2017; Nisman, 1954). It is possible that glycine was taken up but released again, an effect known as overflow metabolism which is due to ineffectively regulated substrate uptake (Burkovski & Krämer, 2002). But this would probably include a decrease in the beginning, which could not be detected.

Glycine is the simplest possible amino acid and looks similar to acetate or propionate (see Figures 4.10). Maybe the receptors mistook it with volatile fatty acids and therefore released phosphate. As it still is an amino acid, the symporter could not take them up.

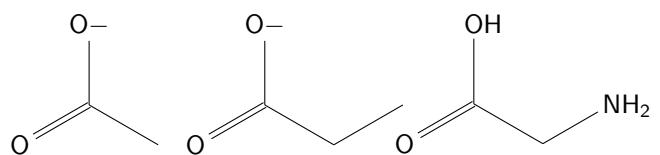
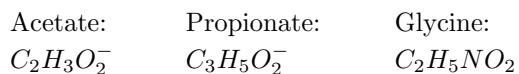


Fig. 4.10: Chemical structural formula

4.2.3 Other Amino Acids

The results for the other different amino acids are shown in Figure 4.11. The values themselves are listed in Table A.7. The detailed Figures are in appendix in Figure A.18. The oxygen saturation, pH and conductivity during those tests are plotted in Figure A.26.

Alanine and Arginine were slightly used up and led to a slight release of phosphate.

Aspartic acid and glutamic acid were used up for 85% resp. 73% (see Table A.7) and phosphate release was measured. Leucine and proline were not used up and did not induce a release of phosphate (see as well Figure A.19, with lower phosphate scale).

Discussion

The slight uptake of alanine and arginine coupled with phosphate release was unexpected. Further experiments are necessary to prove those results.

Aspartic and glutamic acid were both partly consumed. Their consumption seems not to be limited by time (see Figure A.18). Maybe the amino acids were fermented

or *Accumulibacter* could use a certain amount of those amino acids and the uptake stopped as the bacteria were saturated, an effect known as the overflow metabolism (Burkowsky & Krämer, 2002). Kong *et al.* (2004) tested glutamic acid and aspartic acid as well on biomass with *Accumulibacter*. In their experiment, glutamic acid was consumed, but not aspartic acid. Here both were partly consumed. Further research seems to be necessary to understand the consumption of these amino acids.

The high 'COD begin' concentration of leucine is, according to Figure A.18(e), due to insufficient mixing before taking the sample. Therefore it can be assumed that leucine and also proline can not be consumed by *Accumulibacter* bacteria and do not lead to release of phosphate. This goes together with tests by (Kong *et al.*, 2004), who tested leucine on *Accumulibacter* as well and no consumption was measured. According to the same paper, leucine can be consumed as co-substrate with acetate, which was not tested in this case, but could be further investigated to better understand the consumption of amino acids by *Accumulibacter*.

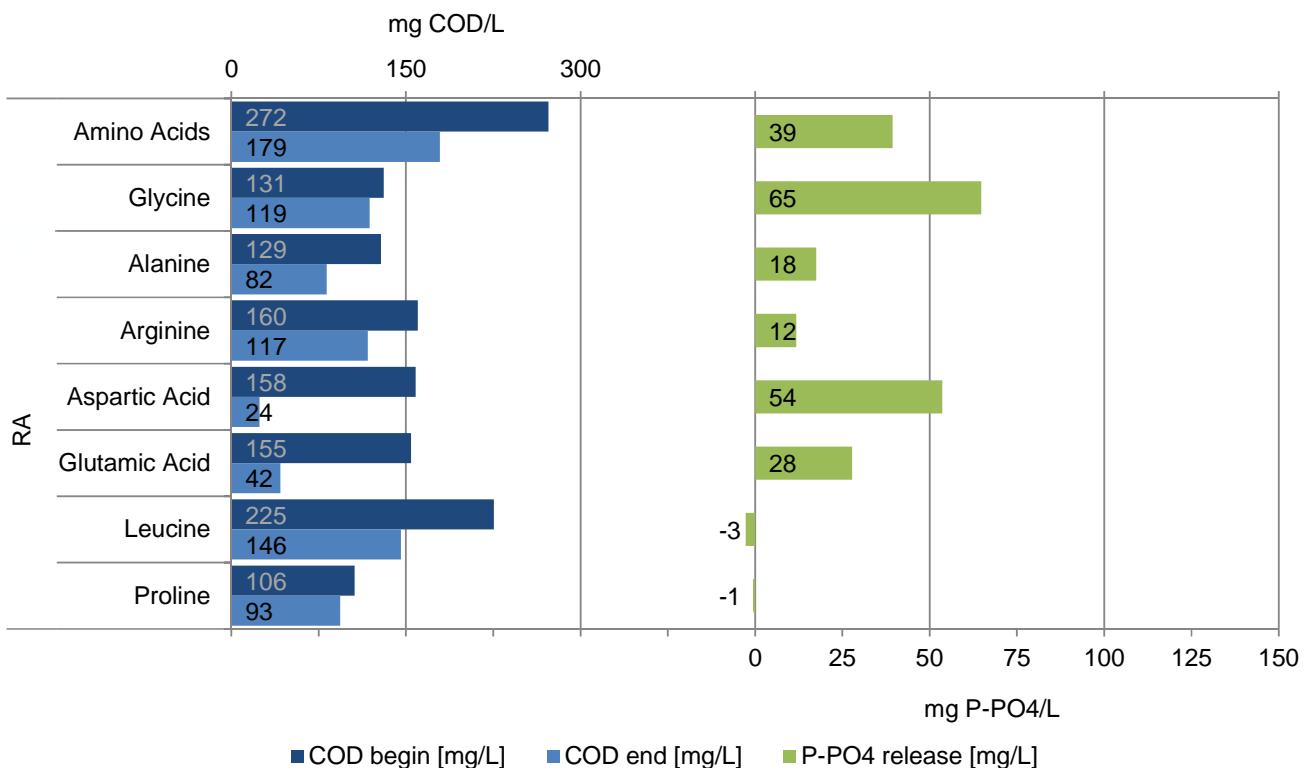


Fig. 4.11: Phosphate release induced by different amino acids

4.3 Conclusion

The anaerobic batch tests confirmed that acetate and propionate sustain the biological dephosphatation activity. If fermenter are available, glucose does sustain the dephosphatation activity as well. The concentration of starch and peptone decreased just in reactor RB and did not lead to phosphate release. It is assumed that those molecules got adsorbed to the biomass, a phenomenon known for particulate matter. So in this case polymers did not sustain the biological dephosphatation activity. In real wastewater, polymeric substances account for a big portion of the total COD. But normally enough monomeric substances are available to have a stable EBPR (Derlon, personal communication).

The amino acid mix was partly consumed in both reactors and led to phosphate release. No consumer of amino acids were expected in RA, therefore they were tested individually. Proline and leucine were not consumed, alanine and arginine slightly and aspartic acid and glutamic acid in a high amount. Further tests are necessary to confirm those results.

The most interesting part was the release of phosphate by *Accumulibacter* induced by glycine, as glycine was not consumed. The release rate can be described with monod kinetics as a function of the glycine concentration. The Michaelis constant K_m has a value of 4mmol/L which shows a low affinity. Impacts of those results on a full scale treatment plant are not expected. The glycine concentration is normally much lower and due to the low affinity the effect would be very small. As well, in a real WWTP, VFAs would be available for *Accumulibacter* to be taken up and glycine would be fermented by *Tetrasphaera* or other fermenter. But by tests with amino acids on sludge of full-scale plants, like tested by Nguyen *et al.* (2015), see Figure A.9, this effect should be considered as there were as well *Accumulibacter* in the water.

A further investigation of the phosphate release by *Accumulibacter* induced by glycine can help to better understand the metabolism of those organisms in the EBPR process.

All in all, different substrates sustain the biological dephosphatation activity depending on the bacteria available. A more diverse inflow composition leads to a higher diversity of bacteria whereby more substrates induce the biological dephosphatation activity.

5 Adaption of the reactor

5.1 Material and Methods

To evaluate the reversibility of the non-granulation phenomenon, reactor RB with the complex polymeric medium (acetate, propionate, glucose, amino acids, starch and peptone) was adapted back to complex monomeric without starch and peptone (see Figure 5.1). Reactor RA was not adapted to perform further tests with amino acids especially glycine described in the previous chapter.

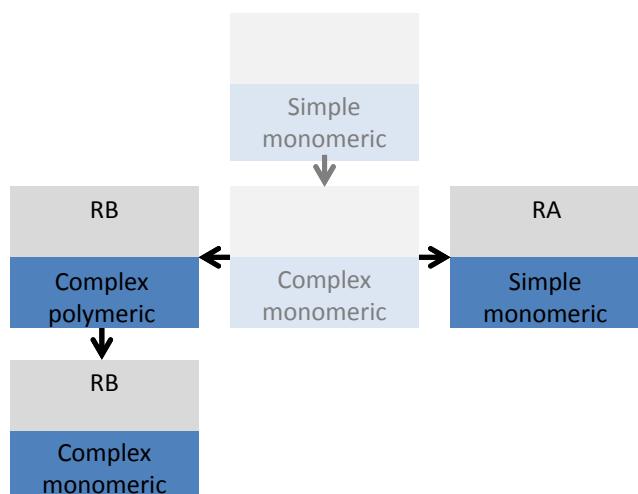


Fig. 5.1: Adaption of the reactors

5.1.1 Change in reactor control (DAQFactory)

The cycle times of reactor RB were changed from a flexible length to a fixed length including an idle phase at the end of the cycle without mixing. This led to a fix number of cycles per week and helped to perform measurements easier as cycles were predictable. The total cycle time of reactor RB was fixed to a total time of seven hours including a flexible idle phase at the end between 6min and 1h6min. The resulting cycle times are listed in Table A.8. Nine weeks after the substrate adaption, the idle phase was undone, as the long sedimentation of the biomass could be a factor for bad granulation up to that point.

As the COD concentration was already below 20mg-COD/L after 60 min of anaerobic time, the anaerobic phase was shortened to 1h15min after ten weeks and to 1h after eleven weeks (COD measurements in 'water results' in the digital appendix).

The settling time was adapted between the goal of 3 minutes and up to 8 minutes to keep the height of biomass quite stable and to sustain the granulation.

5.1.2 Additional biomass properties

Additionally to the water measurements and the biomass properties 'total solids', 'volatile solids' and 'sludge volume index' described in Chapter 3, the following values were measured as well:

Height of the biomass

Almost daily the height of the biomass in reactor RB was noted after 8min of settling during an anoxic phase. Those values helped to monitor the development of the biomass and to adapt the settling time. A short settling time helps to select for fast settling biomass, but increases the risk of reducing the biomass below a certain amount needed for a proper wastewater treatment. A height of 23 cm, which was around 1/3 of the total reactor height was defined by Adler as a target value.

Granulation

It is visible by eye whether the granulation improves or not, but a quantitative method to describe the amount of granules and how they change is still needed.

One option would be image analyzing: pictures of the biomass are analyzed to define the size distribution of granules (see 'Protocol_ImageJ' in the digital appendix). First attempts on reactor RA did not show reliable trustworthy results, different photos on the same day of the same reactor showed totally different results (see appendix Figure A.27). Therefore this method was not applied during the adaption of the reactor.

The method applied was sieving of the biomass according to 'Protocol Sieving' (see digital appendix) developed with explanations given by Nicolas Derlon. 100 mL of mixed reactor volume from the aerobic phase were washed through a sieve with a pore size of 500 µm and one with 250 µm. The caught and remaining biomass fractions were analyzed on the 'total solids' resp. 'volatile solids' concentration. They sum up to the 'total' resp. 'volatile solids' concentration in the reactor. The biomass was separated from water by sedimentation (bigger granules) and centrifugation (flocs), whereby part of the biomass could get lost. Hence and because of the high uncertainty, total solids tests of the total biomass were performed as control measurements.

Additionally the SVI can be used to evaluate sludge granulation and to characterize the sludge settling properties (Zhao *et al.*, 2014).

Biomass production and solids retention time

During the adaption of the reactor, the biomass production was analyzed. Therefore the biomass removed from the reactor and the biomass caught in the clarifier were analyzed on 'total solids' and 'volatile solids' weekly (according to protocol 'SVL_TS_VS' in the digital appendix). Some removed biomass could not be analyzed on total solids, for example those used for DNA analysis. Its total solids were calculated based on the mixed volume and on TS measurements that week. Biomass, which could not be analyzed at all, for example because it was lost in the filter during water experiments, was neglected. So probably the effective biomass production was slightly higher. Based on the measured and calculated biomass production, the 'TS/COD removed ratio' could be calculated.

The same goes for the solids retention time, which is the average time the activated sludge stays in the system. It is determined by dividing the total solids in the reactor by the solids removed per day (Tchobanoglous *et al.*, 2014, p.598). In this case, the biomass was either removed for measurements or mostly caught in the clarifier.

$$SRT[d] = \frac{TS_{Reactor}}{TS_{Clarifier} + TS_{removed}} \quad (5.1)$$

5.2 Results

The water treatment was still working well after the adaption of the medium (see 'Water test' in the digital appendix). All COD was used up (below 20 mgCOD/L) in the anaerobic phase.

A big difference was the phosphate release during the anaerobic phase. The average phosphate concentration at the end of the anaerobic phase decreased from 64 to 16 mgP – P04^-3/L (see Figure A.28).

Figure 5.2 shows the total solids concentration in reactor RB after the adaption of the medium. In green the biomass concentration of the granules bigger than 500 µm diameter, in orange the small granules between 250 µm and 500 µm diameter and in red the biomass below which was considered as flocs. The first two bars show the result of reactor RA as reference value. TS 1, TS 2 and TS 3 are total solids measurements of the whole biomass. The sludge volume index SVI describes the settling properties of the biomass. The values itself are listed in Table A.9, the values of the volatile solids in Table A.10. The ratio of VS/TS was on average around 72% (see Table A.10).

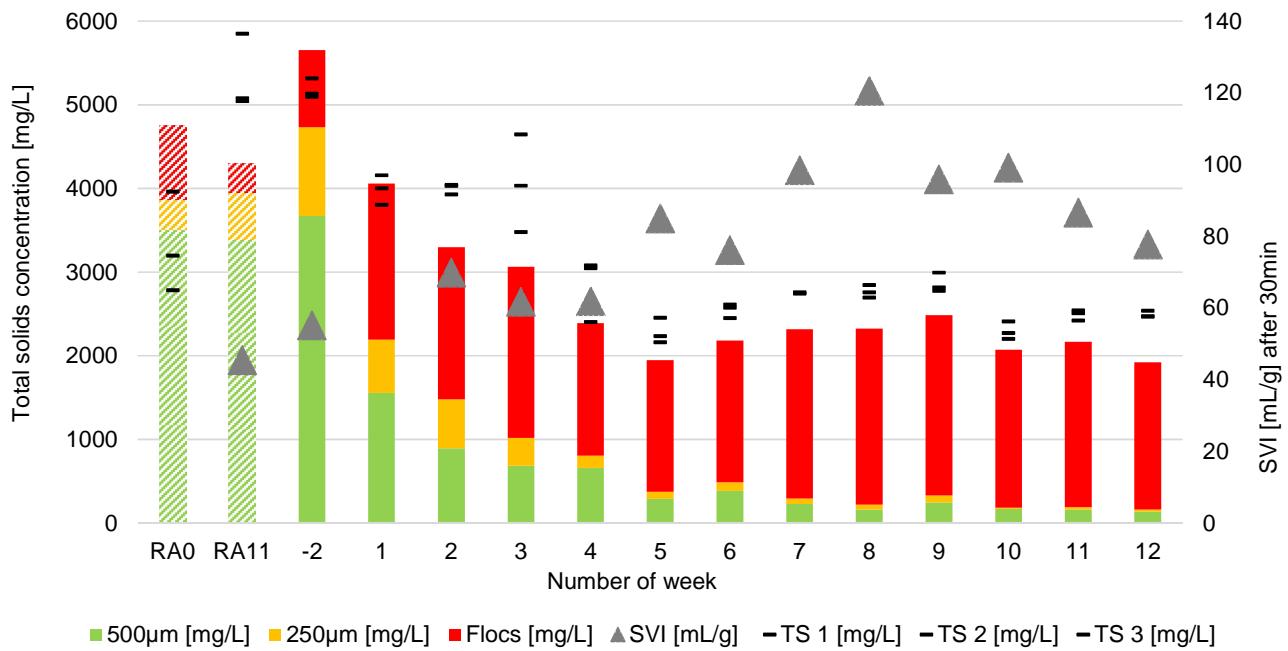


Fig. 5.2: Solids concentration in reactor RB during medium adaption

Figure 5.3 shows the mass of solids in the reactor (TS Reactor), the mass caught in the clarifier (TS Clarifier) and removed manually for analysis each week (TS removed). The missing values in week 11 and 12 were due to a malfunction of the clarifier. Based on those values the solids retention time SRT was calculated. The 'TS/-COD removed ratio' increased from around 0.13 up to 0.30 gTS/gCOD. All the values are listed in Table A.11.

In general, more biomass was caught in the clarifier than

removed manually (compare 'TS Clarifier' to 'TS removed' in Figure 5.3). 'TS removed' was quite high in week 1 only, when a lot was removed as disposal option to decrease the bed height. Afterwards, the bed height was controlled by varying the settling time. Both the bed height and the settling time are plotted in Figure A.30. The settling time was adapted between 4 and 6 minutes to reach a bed height around 23 cm.

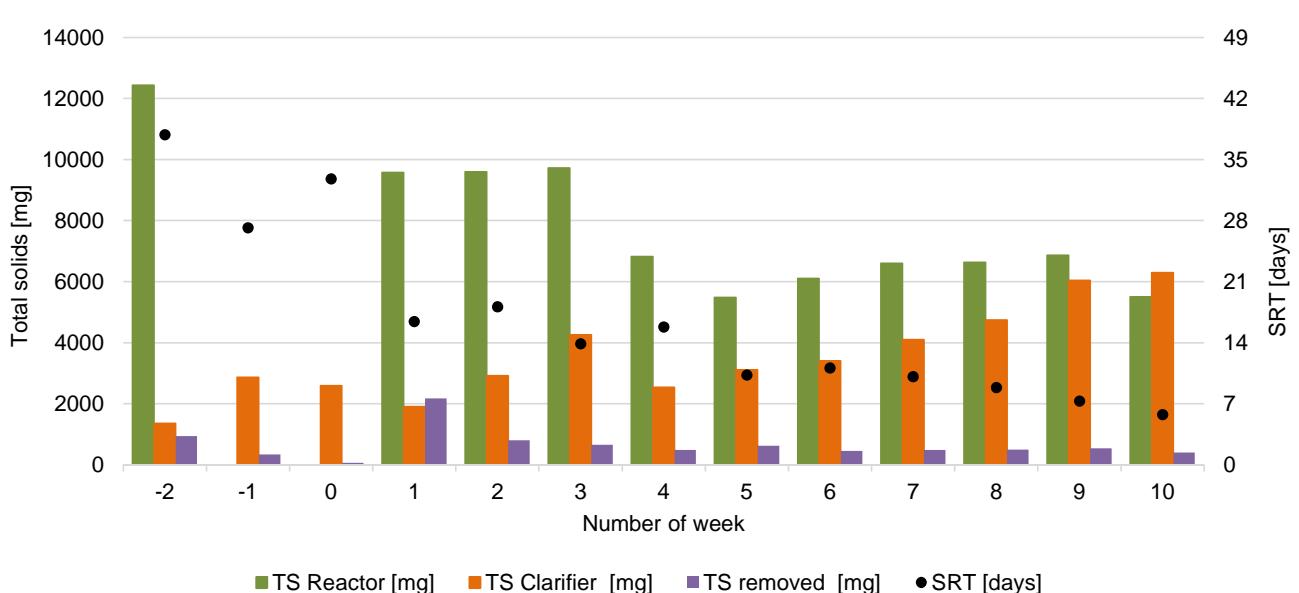


Fig. 5.3: Biomass in the reactor, clarifier and removed

5.3 Discussion

A proper treatment of the wastewater was expected as the medium just got simpler. The decrease of the phosphate release during the anaerobic phase was surprising. Starch and peptone were the only substrates, which did not induce a phosphate release in the anaerobic phase (see Chapter 4.2). Therefore an increase in phosphate release was expected by removing them from the medium. Maybe the microbial community changed rapidly, for example *Accumulibacter* or other PAO were washed out. Probably the results of the ongoing DNA analysis can answer this question.

The ratio VS/TS around 72 % was quite stable and comparable to Gujer, who assumes a loss of ignition of around 70 % (Gujer, 2006, p.67). Therefore just the total solids concentration was used for detailed analysis.

The aerobic granular sludge in reactor RB was in a good state before changing the medium. Even if the granules in RA were bigger and the SVI was lower, reactor RB was comparable (see Figure 5.2, RA and -2). Afterwards, the amount of granules in RB decreased and the flocs increased. As the density of flocs is much lower than the density of granules, the bed height increased and biomass was washed out. The increasing bed height can be seen in Figure A.30. After two weeks, the settling time was decreased stepwise to get back to the target bed height, which led a high washout of biomass between week 3 and 4 (see Figure 5.2).

After 5 weeks, most of the granules were lost. Just a few big granules stayed in the reactor but there was no regranulation. In the following weeks the composition and the concentration of the biomass was stable, but behaved like conventional activated sludge, which can be seen according to different parameters:

Initially the **sludge volume index** was around 60 mL/g which is still quite high as density of aerobic granular sludge. The SVI of RA was below 50 mL/g and in a full scale AGS treatment plant, SVI values of 35 mL/g were reached (Pronk *et al.*, 2015). But it increased due to the high amount of flocs up to more than 100 mL/g. Values which are more comparable to conventional activated sludge (100-150 mL/g, (Gujer, 2006, p.327)).

The **sludge production** increased from around 0.13 up to 0.30 gTS/gCOD removed. The values can be compared to literature, which assume 0.19-0.43 gTSS/gCOD removed IWA (31.07.2018). The increase can be explained due to the lower sludge production of AGS with its higher SRT (Weissbrodt, 2013, p.18).

The **solids retention time (SRT)** was initially always

above 20 days, comparable to a full scale AGS treatment plant (SRT between 20-38 days, Pronk *et al.* (2015)). After the adaption of the medium the SRT decreased to around ten days or even lower, comparable to the SRT of conventional activated sludge (3-15 days according to (Gujer, 2006, p.330)).

The transformation of the granular sludge into flocculent sludge is hard to explain. Maybe the bacteria were used to the polymeric substances and the shock due to their absence let the granules fall apart. But in any case, regranulation would be expected. Normally granulation can be observed within 30 to 50 days of operation (Gonzalez-Gil & Holliger, 2011; Lochmatter, 2013, p.66).

Another reason for the non-granulation could be the idle phase. During the idle phase the biomass was settling up to an hour, which maybe due to the missing shear stress disturbed the granulation. The idle phase was undone nine weeks after the adaption, which maybe can explain the decrease in the SVI in the following weeks.

5.4 Conclusion

It can be concluded, that the non-granulation phenomenon was not reversible. The granulation got even much worse with the change to the monomeric medium. The experiment could show that a big adaption of the inflow, even a simplification can lead to a fundamental loss of the granules. There was no regranulation with the monomeric medium which leads to the hypothesis, that other factors than the substrate composition disturbed the granulation.

6 Outlook

Different aspects of aerobic granular sludge are still unknown. Further experiments are needed to better understand the influence of the individual substrates on granulation. In particular the behavior based on polymers is difficult to predict. How are those substrates consumed and how do they interfere with biological dephosphatation? Concerning biological dephosphatation, still more research has to be done to better understand the metabolism of bacteria involved and how they interact. The metabolism of *Accumulibacter* is well known, but even then unexpected phenomena can occur, as seen with the amino acids, especially glycine. The influence of glycine on biological dephosphatation and on *Accumulibacter* in particular should be investigated in more detail to better understand the metabolism of *Accumulibacter*.

All in all more research is needed to further understand the processes in AGS to build full scale treatment plants with stable granules and reliable treatment performance.

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A Appendix

A.1 Introduction

Figure A.1 shows the EBPR metabolism of the *Accumulibacter* phosphatis composite genome according to Martín *et al.* (2006).

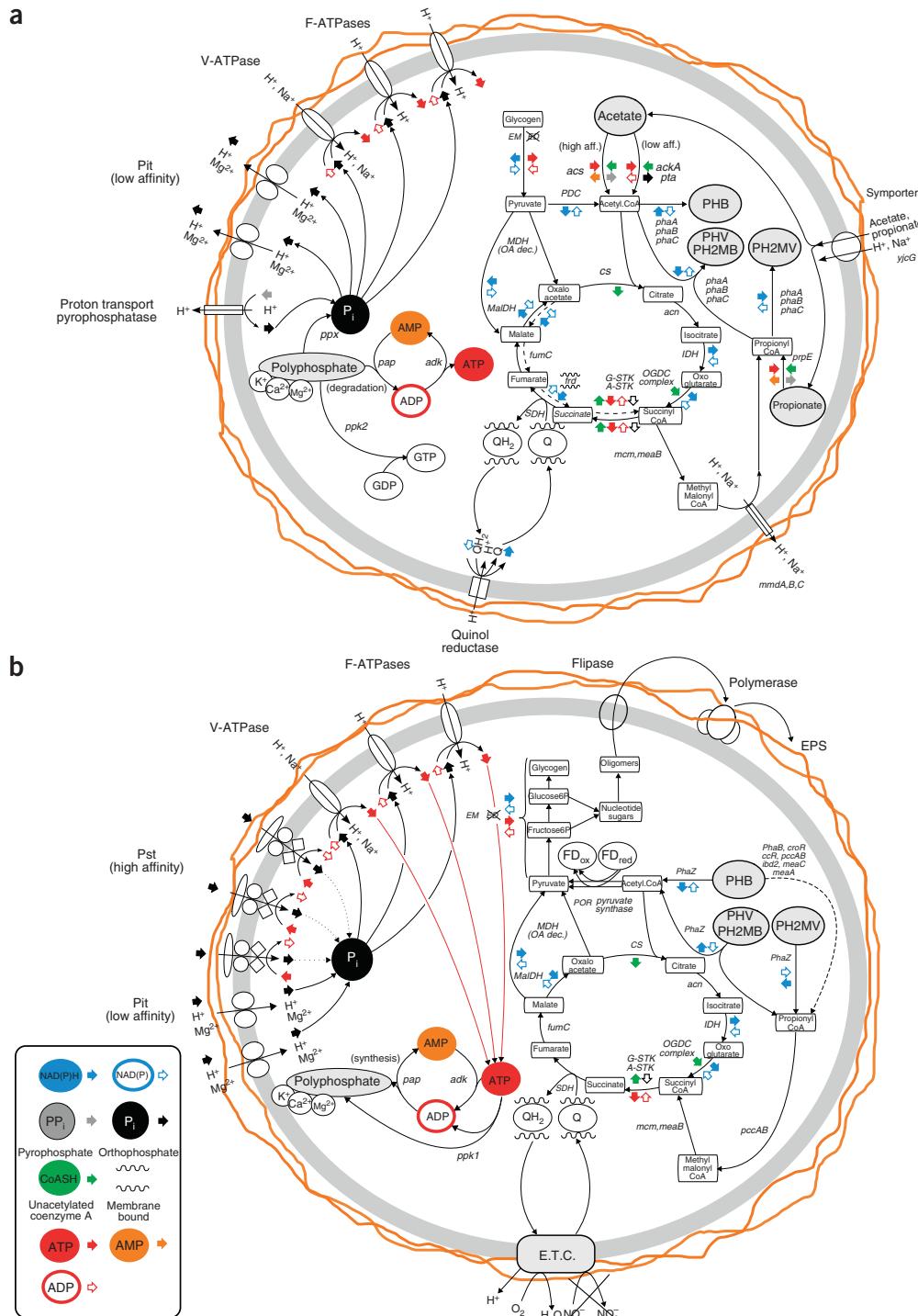


Fig. A.1: EBPR metabolism of *Accumulibacter* (Martín *et al.*, 2006)

Figure A.2 and A.3 show the relative abundance of different bacteria in reactor RA and RB. Up to the change to the simple monomeric resp. complex polymeric medium, there was just one reactor (see Figure 1.3). The measurements began in October 2015 and the figure ends in April 2018. More results were not yet available during the period of the master thesis. The measurements continued up to now.

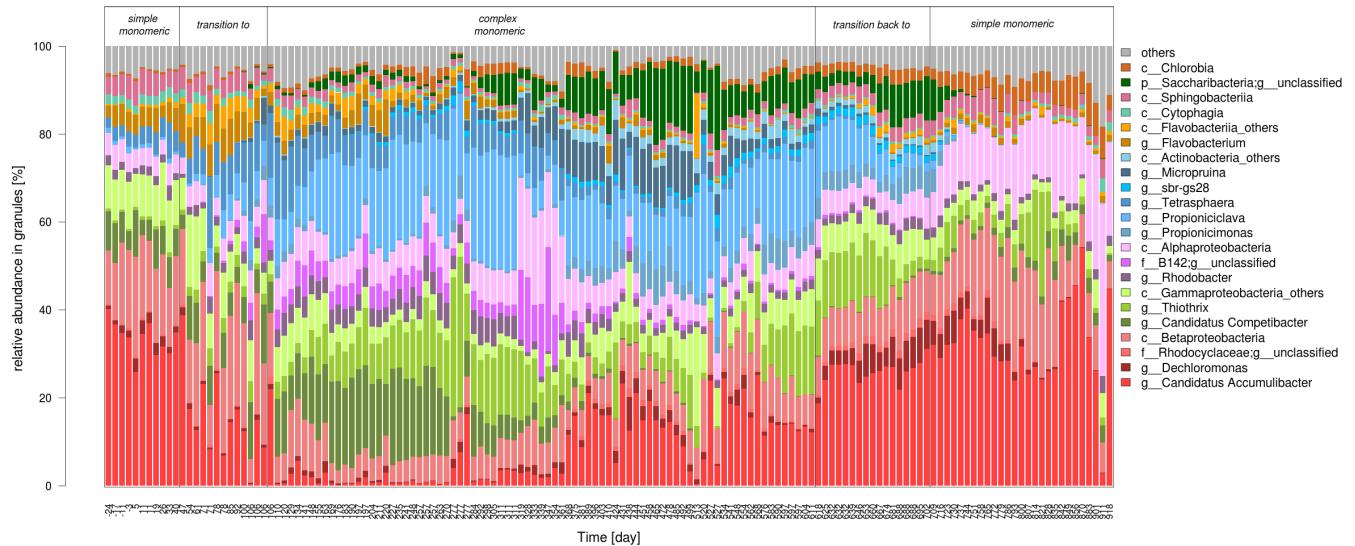


Fig. A.2: Development of bacteria abundance in reactor RA

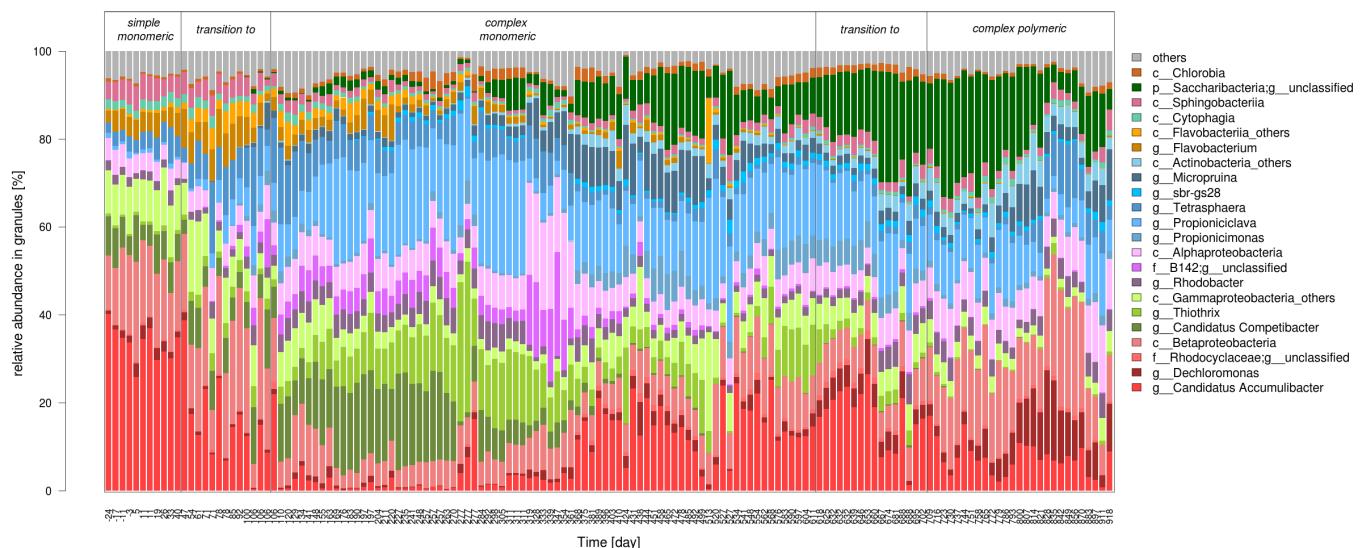


Fig. A.3: Development of bacteria abundance in reactor RB

Figure A.4 shows the result of a FISH analysis performed on reactor RA by Arnaud Gelb in July 2018. *Accumulibacter* were marked red, the others white, accordingly *Accumulibacter* were very abundant. None *Tetrasphaeraea* could be found in a similar test (data not shown). This goes together with the DNA analysis above, which couldn't find *Tetrasphaeraea* but a lot of *Accumulibacter* in RA.

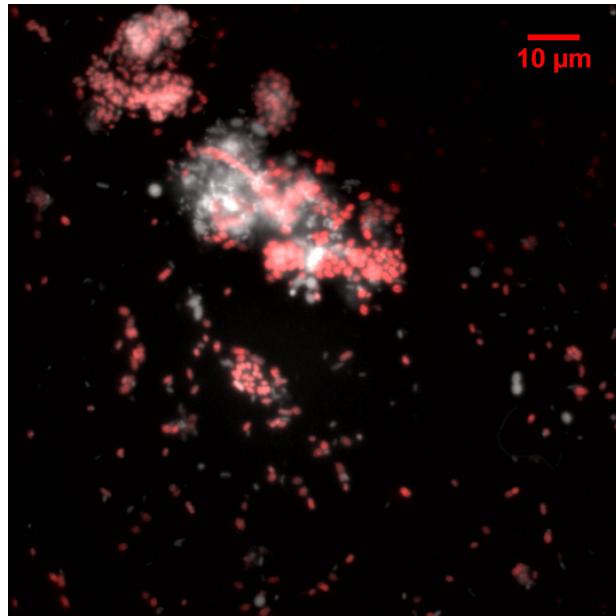


Fig. A.4: FISH analysis of reactor RA by Arnaud Gelb

The following Figure A.5 shows the anaerobic food web. It describes the processes in an anaerobic digester but also indicates the processes required to consume the substrates in the anaerobic phase of the reactor. Complex polymers have to be hydrolyzed before fermentation. As at latest acetate and propionate can be taken up directly by PAO or GAO, the last step (methanogenesis) is not expected in these reactors. The figure was copied from the lecture slides of the course process engineering Ia at ETH fall 2016, Anaerobic processes slide 48/81.

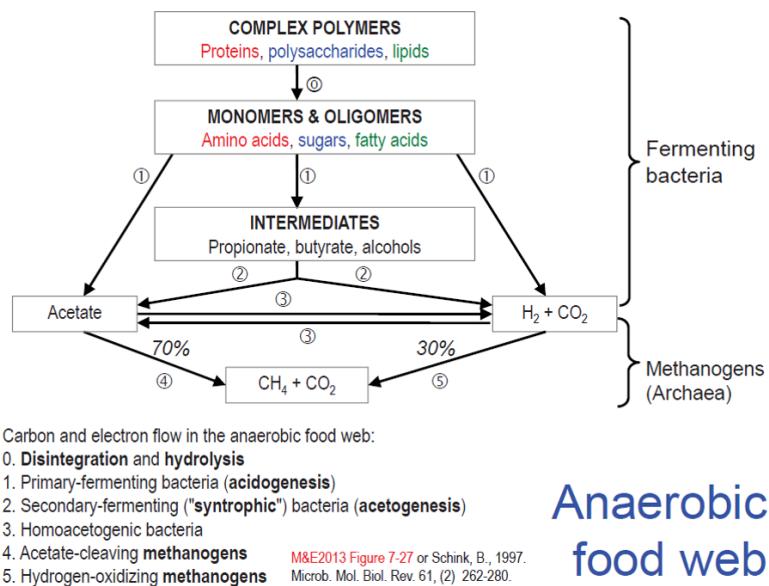


Fig. A.5: Anaerobic food web (Morgenroth, 2016)

A.2 AGS-SBRs

Table A.1 below shows the different synthetic wastewater used in the lab AGS-SBRs. During the previous adaptions shown in Figure 1.3, the following intermediate medium were used:

Tab. A.1: Different synthetic media used for the lab reactors

	Simple monomeric		Complex monomeric			Complex polymeric	
Acetate	50.0%	40.0%	30.0%	16.7%	16.7%	16.7%	16.7%
Propionate	50.0%	40.0%	30.0%	16.7%	16.7%	16.7%	16.7%
VFAs	100.0%	80.0%	60.0%	33.3%	33.3%	33.3%	33.3%
Glucose		10.0%	20.0%	33.3%	28.3%	23.3%	16.7%
Amino Acids		10.0%	20.0%	33.3%	28.3%	23.3%	16.7%
Peptones					5.0%	10.0%	16.7%
Starch					5.0%	10.0%	16.7%

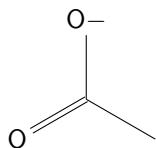
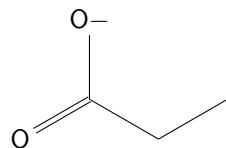
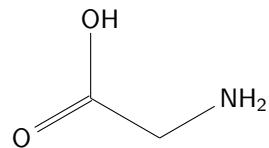
Table A.2 shows the cycle times of the reactor. As the aerobic phase changes flexibly with the consumption of oxygen in the anoxic phase, it can last up to 5h in RB resp. 5h40min in RA.

Tab. A.2: Cycle times of reactor RA and RB

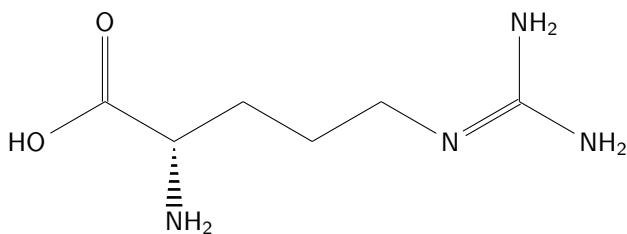
	RB [h:min:sec]	RA [h:min:sec]
Anaerobic feed	00:12:00	00:12:00
Anaerobic	01:30:00	01:00:00
Aerobic min.	04:00:00	04:00:00
Aerobic max.	05:00:00	05:40:00
Settling	00:06:00	00:04:00
Withdraw	00:07:00	00:08:00
Total min.	05:55:00	05:24:00
Total max.	06:55:00	07:04:00

A.2.1 Chemical structural formula

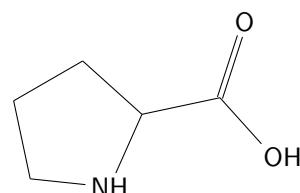
The following chemical figures represent the two volatile fatty acids acetate and propionate and the seven amino acids included in the amino acid mix. They were plotted with the web page Mol2chemfig (26.07.2018) according to information from the website Pubchem (25.07.2018).

Acetate: $C_2H_3O_2^-$ Propionate: $C_3H_5O_2^-$ Glycine: $C_2H_5NO_2$ 

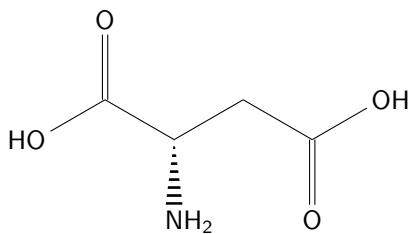
Arginine



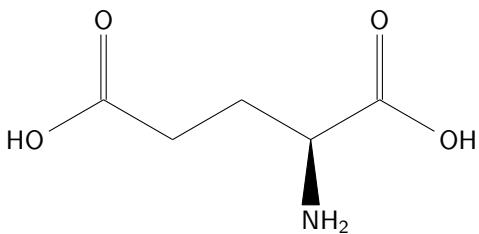
Proline



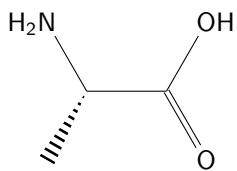
Aspartic Acid



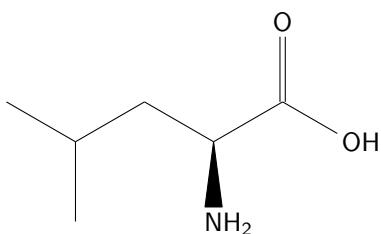
Glutamic acid



Alanine



Leucine

**Fig. A.6:** Chemical structural formula of acetate, propionate and different amino acids

A.3 Biological dephosphatation activity

A.3.1 Material and Methods

Table A.3 shows the inflow concentrations during the cycles of the anaerobic batch tests. Both media contribute each 11.25 % to the total inflow volume, the third part is industrial water (filtered water from Lake Geneva). The medium NP includes all nitrogen and phosphorus compounds and is equal to the inflow NP medium of reactor RA. Medium C is equal to medium C of both reactors without the carbon sources.

Tab. A.3: Inflow concentration during anaerobic batch tests

Medium NP	Formula	Molar Mass [g/mol]	Concentration [g/L]
Ammonium chloride	NH4Cl	53.492	1.893
Potassium hydrogen phosphate	K2HPO4	174.174	0.7305
Potassium phosphate monobasic	KH2PO4	136.084	0.2855
<hr/>			
Medium C			
Magnesium sulfate heptahydrate	MgSO4*7H2O	246.472	0.177
Calcium chloride dihydrate	CaCl2*2H2O	147.02	0.165
Potassium chloride	KCl	74.551	0.3575

The carbon sources listed in Table A.4 were tested individually on both reactors. Each amino acid is included in the same amount in the amino acid mix. These amino acids were chosen because they are all included in the peptones mixture available at the laboratory. The molar mass (MM) and the COD/Sub. values were provided over the laboratory server by Stéphane Marquis. For peptone, the conversion factor 1.4 mgCOD/mg peptones according to Aline Adler was used.

Tab. A.4: Substrates tested during anaerobic batch tests

		Formula	Mass COD mg	COD/ Sub. gCOD/ mol	Amount mmol	MM g/mol	Mass Sub. mg	Amount C- mmol	Conc. C /L
RA/RB	Sodium acetate trihydrate	C2H3Na * 3H2O	720	64	11.3	136.08	1531	22.5	9.4
RA/RB	Sodium propionate	C3H5O2Na	720	112	6.4	96.06	618	19.3	8.0
RA/RB	Glucose monohydrate	C6H12O6 * H2O	720	192	3.8	198.17	743	22.5	9.4
RA/RB	Starch	(C6H12O6)n	720	192	3.8	162.14	608	22.5	9.4
RA/RB	Peptones		720				514	24.4	10.2
RA	Alanine	C3H7NO2	103	96	1.0714	89.09	95	3.21	1.3
RA	Arginine	C6H14N4O2	103	176	0.5844	174.20	102	3.51	1.5
RA	Aspartic acid	C4H7NO4	103	96	1.0714	133.10	143	4.29	1.8
RA	Glutamic acid	C5H9NO4	103	144	0.7143	147.13	105	3.57	1.5
RA/RB	Glycine	C2H5NO2	103	48	2.1429	75.07	161	4.29	1.8
RA	Leucine	C6H13NO2	103	240	0.4286	131.18	56	2.57	1.1
RA	Proline	C5H9NO2	103	176	0.5844	115.13	67	2.92	1.2
RA/RB	Total Amino Acids		720	139.43	0.9425			24.4	10.1
RA	Simple Monomeric		720	88	8.8			1074.2	20.9
RB	Complex Polymeric		720	139.89				9.4	8.7

During each batch test, the substrate itself was measured as well. It has a COD concentration of 18'000 mgCOD/L or 9'000 mgCOD/L. Either COD kit 314 or kit 514 has been used with a dilution of 200x resp. 20x or 10x.

The results were multiplied with the dilution factor and the volume of the syringe (40 mL) and divided by the volume of the reactor (2.4L) to get the initial concentration in the reactor itself, which should equal 300 mg/L or 150 mg/L.

All values are listed in Table A.5. The cursive values seem to be unrealistically low. Just peptones were systematically below 300 mg/L, probably due to the unclear composition and vague conversion factor.

Tab. A.5: Control COD measurements of carbon sources tested

	Sample 1 COD [mg/L]	Sample 2 COD [mg/L]	Sample 3 COD [mg/L]
Medium 1	288		
Acetate	297	246	271
Medium 7	232	311	
Glucose	284	314	
Amino Acids	221	281	
Starch	164	254	
Peptones	238	238	252
Glycine	309	321	293
Glycine 2.	134	159	155
Aspartic Acid	153		
Glutamic Acid	156		
Alanine	154		
Leucine	155		

A.3.2 Stoichiometric parameters of anaerobic batch tests

The amount of phosphate release is calculated as the difference between the concentration in the end of the anaerobic phase (AN60 or AN90) and the concentration at the beginning (AN-).

$$P\text{-PO}_4 \text{ release[mg/L]} = P\text{-PO}_4 [\text{mg/L}] (\text{AN60; AN90}) - P\text{-PO}_4 [\text{mg/L}] (\text{AN-}) \quad (\text{A.1})$$

COD begin is the difference between the COD concentration after adding the substrate (AN0) and the initial concentration (AN-) before. It should represent the amount of substrate added into the reactor in COD equivalent.

$$\text{COD begin[mg/L]} = \text{COD} (\text{AN0}) - \text{COD} (\text{AN-}) \quad (\text{A.2})$$

The COD at the end of the experiment is the difference between the last COD measurement (AN60 or AN90) and the measurement before adding the substrate (AN-). It should represent the residual amount of substrate at the end of the anaerobic phase.

$$\text{COD end[mg/L]} = \text{COD} (\text{AN60; AN90}) - \text{COD} (\text{AN-}) \quad (\text{A.3})$$

The amount of substrate uptake is calculated as the difference between COD begin and COD end.

$$\text{COD uptake[mg/L]} = \text{COD begin[mg/L]} - \text{COD end[mg/L]} \quad (\text{A.4})$$

The amount of substrate uptake in percentage is the substrate uptake from above divided through the amount added.

$$\text{COD uptake[%]} = \frac{\text{COD uptake[mg/L]}}{\text{COD begin[mg/L]}} \quad (\text{A.5})$$

A stoichiometric parameter often mentioned is the anaerobic orthophosphate release to carbon source uptake ratio, short P/C ratio. The transition from mg/L to mmol/L is done with the molar mass for phosphate (31 g/mol) and the molar mass of the different substrates, which are listed in Table A.4.

$$P/\text{Cratio} = \frac{P\text{-PO}_4 \text{ release [mmol/L]}}{\text{COD uptake [mmol/L]}} \quad (\text{A.6})$$

A.3.3 Reference values for discussion

Figure A.7 shows the distribution of the products of the biological phosphate elimination according to a real example. It was copied from the lecture slides of the course 'Process Engineering Ia' at ETH fall 2016, EBPR slide 22/71.

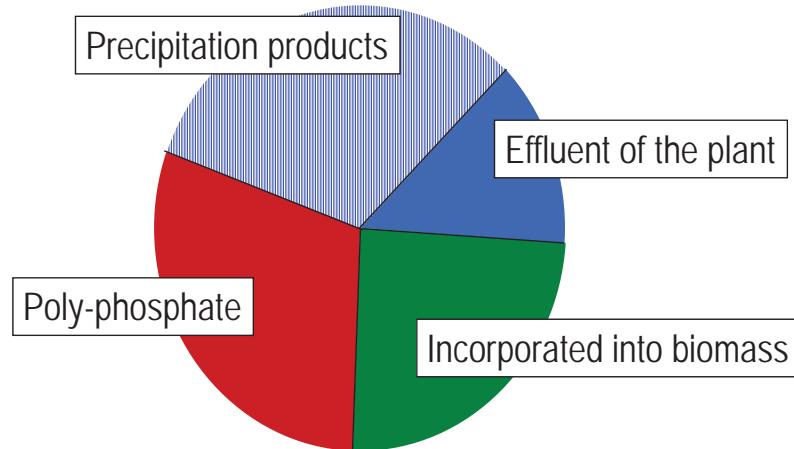


Fig. A.7: Distribution of the products of biological P elimination, a real example (Morgenroth, 2016)

Figure A.8 copied from the Paper (Smolders *et al.*, 1994), shows the relation between the ratio of phosphate to carbon source uptake as a function of pH. The influence of glycogen accumulating organisms was not mentioned there.

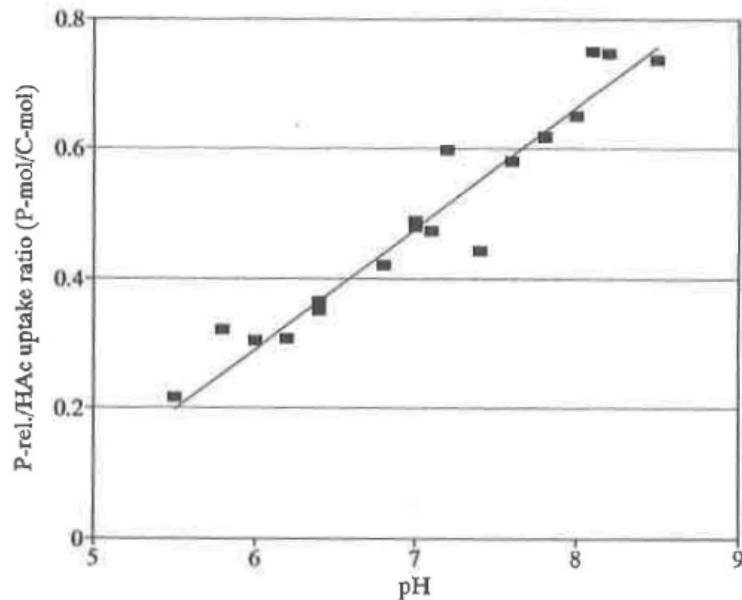


Fig. A.8: Phosphate release as a function of pH (Smolders *et al.*, 1994)

Figure A.9 shows the phosphate release due to different amino acids tested on biomass consisting mostly of the PAO Tetrasphaera and just a few Accumulibacter (Nguyen *et al.*, 2015). The yellow-marked amino acids are included in the amino acid mix tested on RA and RB as well.

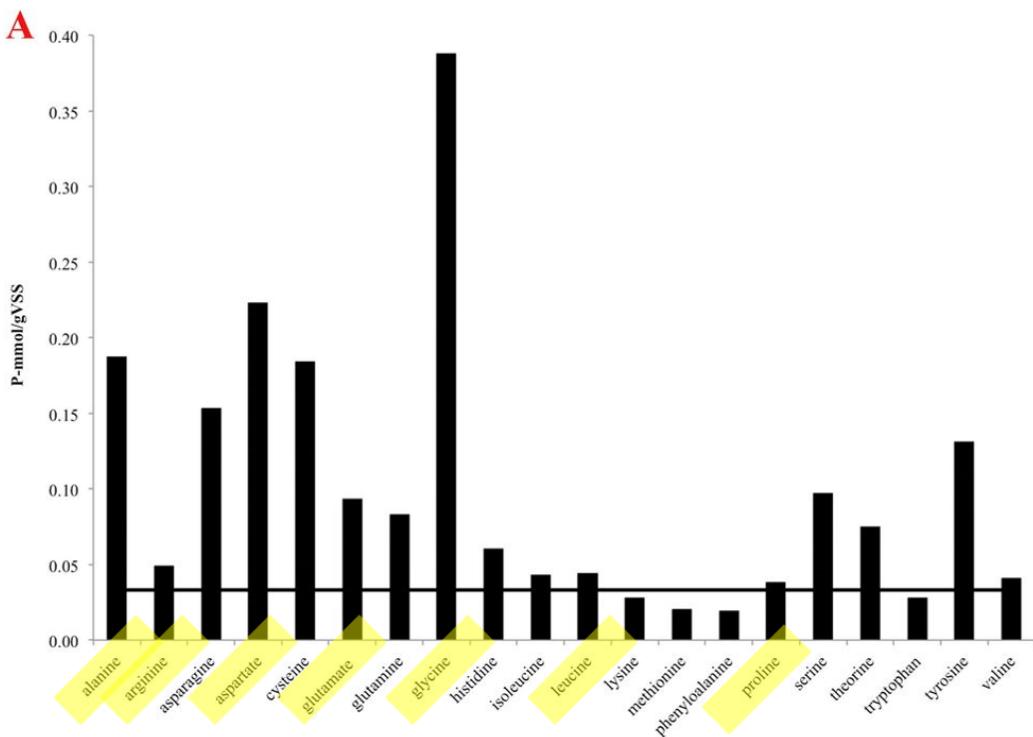


Fig. A.9: Phosphate release of different Amino Acids (Nguyen *et al.*, 2015)

A.3.4 Results

The following values are listed in the Tables below:

Table A.6 describes the results of the anaerobic batch tests with the six substrates of the complex polymeric medium tested on both reactors. Additionally the results of the tests with adapted pH controller are displayed.

If the phosphate release was insignificantly low (below 10 mg/L), the P/C ratio in the last column was not calculated. The p/C ratio values are cursive, if the substrate was not fully (below 20 mgCOD/L) consumed. Those values are influenced by the duration of the anaerobic phase and measurement uncertainties.

The following Table A.7 shows the results of the tests on RA with glycine and other amino acids.

The following figures in Figure A.10 - A.19 show the change in phosphate and COD concentration during the anaerobic batch tests.

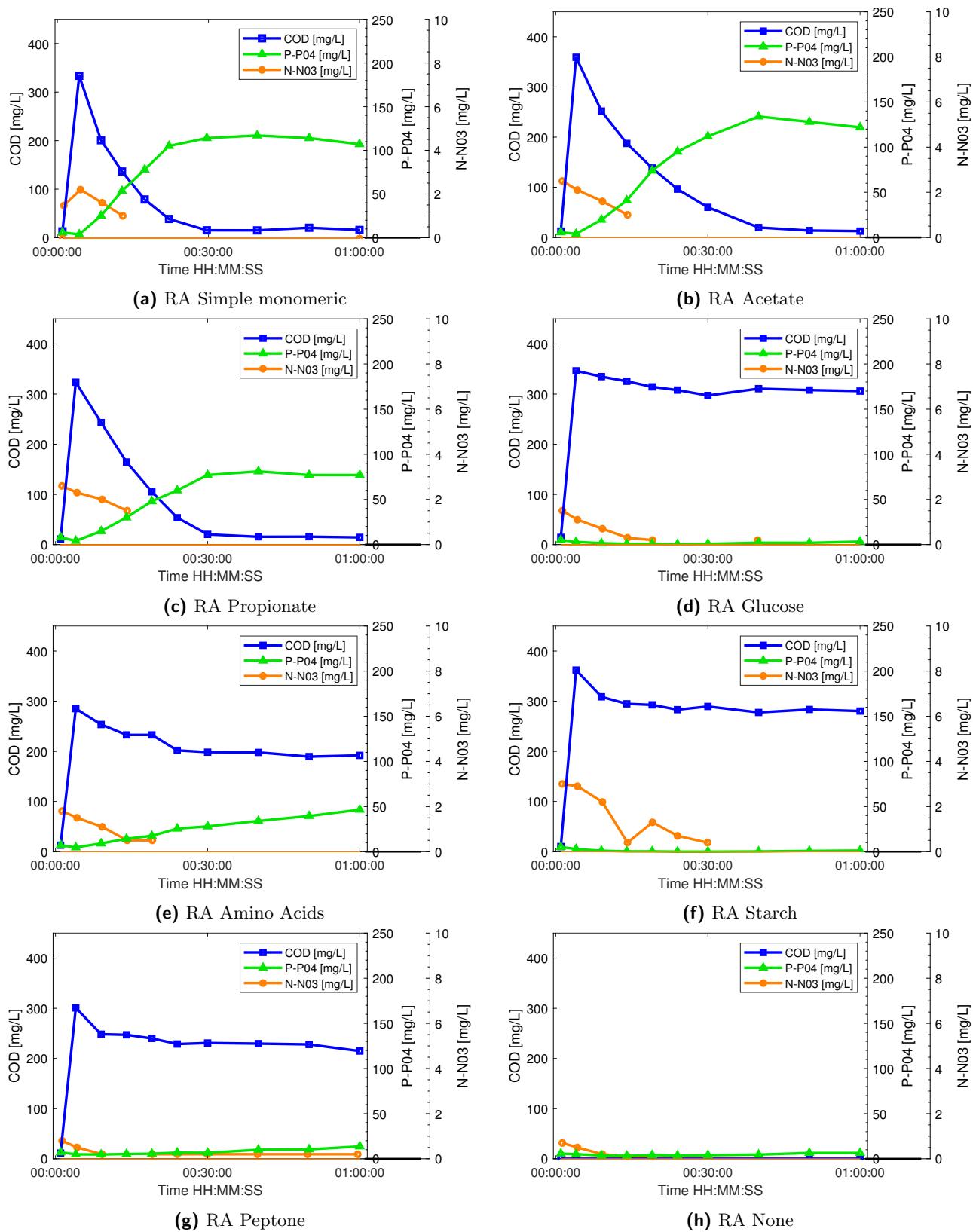
The following figures in Figure A.20-A.22 display the variation of oxygen saturation, conductivity and pH of the whole cycle during anaerobic batch tests.

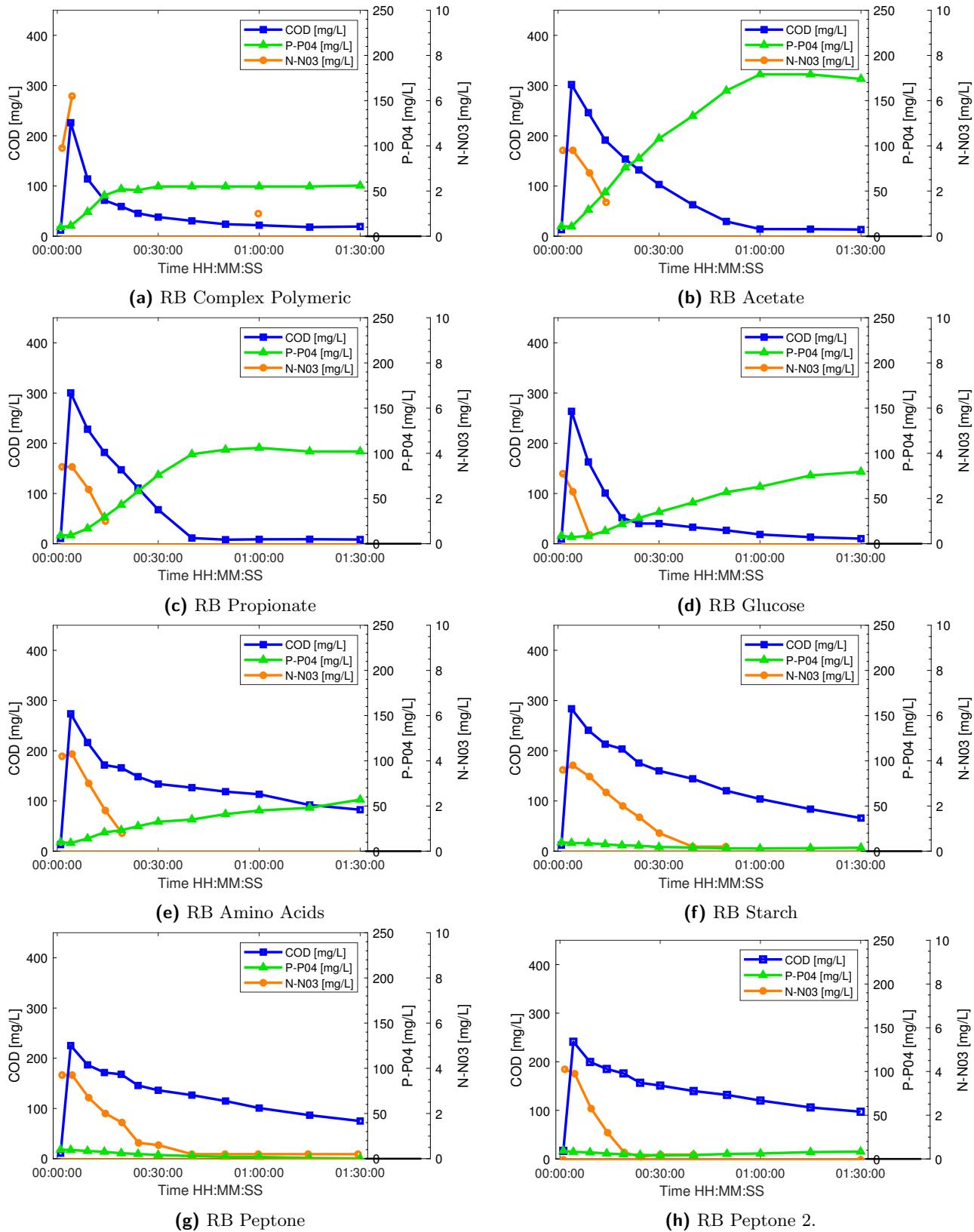
Tab. A.6: Parameters of the anaerobic batch tests on RA and RB

	P-P04 release	COD initial (AN0-AN-)	COD end (AN60;AN90-AN-)	COD uptake (AN0-AN60;AN90)	COD uptake %	P/C ratio
	mg P-P04 / L	mg COD / L	mg COD / L	mg COD / L	mg COD / L	P-mol / C-mol
RA	Simple monomeric	101.0	320.6	3.0	317.6	99%
	Acetate	116.3	346.6	0.0	346.6	100%
	Propionate	69.0	312.5	3.2	309.3	99%
	Glucose	-1.7	332.1	291.7	40.4	12%
	Amino Acids	39.4	272.3	179.1	93.2	34%
	Starch	-3.9	351.9	270.3	81.6	23%
	Peptone	6.6	289.5	203.5	20.4	7%
	None	0.7	0.0	0.0	0.0	0%
	Complex polymeric	46.0	213.8	9.7	206.7	97%
	Acetate	163.1	288.9	1.1	288.7	100%
RB	Propionate	92.8	289.8	-1.9	292.0	101%
	Glucose	70.8	253.0	7.8	253.5	100%
	Amino Acids	47.1	260.4	100.2	191.0	73%
	Starch	-5.8	271.4	91.8	217.6	80%
	Peptone	-9.7	214.2	89.8	159.2	74%
	Peptone 2	-1.0	224.6	103.6	144.0	64%
	Acetate, Regular Values	118.0	346.6	0.0	346.6	100%
	Acetate, First adaption	117.1	326.6	3.6	323.0	99%
RA	Acetate, Second adaption	86.1	353.1	2.5	350.6	99%
						0.25

Tab. A.7: Parameters of the anaerobic batch tests with glycine and other amino acids on RA and RB

	P-P04 release	P-P04 release after 2h	COD be- gin (AN0- AN-)	COD end (AN60;AN90- AN-)	COD uptake (AN0 AN60;AN90)	COD	P/C
	mg P-P04 / L	mg P-P04 / L	mg COD / L	mg COD / L	mg COD / L	mg COD / L	P-mol / C-mol
RA	Amino Acids	39.4	272.3	179.1	93.2	34%	0.40
	Glycine 1.	77.1	315.2	287.2	28.0	9%	2.11
	Glycine 2.	80.0	116.3	304.4	261.6	14%	1.44
	Glycine 3.	73.2	328.0	297.6	30.4	9%	1.85
	Glycine 1/2 1.	32.7	163.3	119.1	44.2	27%	0.57
	Glycine 1/2 2.	64.8	119.7	130.8	118.8	12.0	9%
	Glycine 1/2 3.	61.9	61.9	124.6	126.0	-1.4	-1%
	Glycine 1/2 NO3	39.5	60.8	165.0	112.6	52.4	32%
	Glycine 1/4 1.	33.4	67.7	54.4	13.3	20%	1.93
	Glycine 1/4 2.	36.6	70.4	63.5	6.9	10%	4.07
RB	Glycine 1/8	24.9	35.9	24.9	11.0	31%	1.74
	Alanine	17.5	128.7	81.7	47.0	37%	0.29
	Arginine	11.9	160.2	117.4	42.8	27%	0.21
	Aspartic Acid	53.6	158.4	24.2	134.2	85%	0.31
	Glutamic Acid	27.8	154.6	42.1	112.5	73%	0.19
	Leucine	-2.7	225.4	145.8	79.6	35%	
RB	Proline	-0.5	106.0	93.4	12.6	12%	
	Amino Acids	47.1	260.4	100.2	191.0	73%	0.23
	Glycine	66.3	292.9	215.3	92.4	32%	0.55

**Fig. A.10:** P-PO₄ and COD; EBPR.ANA. on RA

**Fig. A.11:** P-PO₄ and COD; EBPR.ANA. on RB

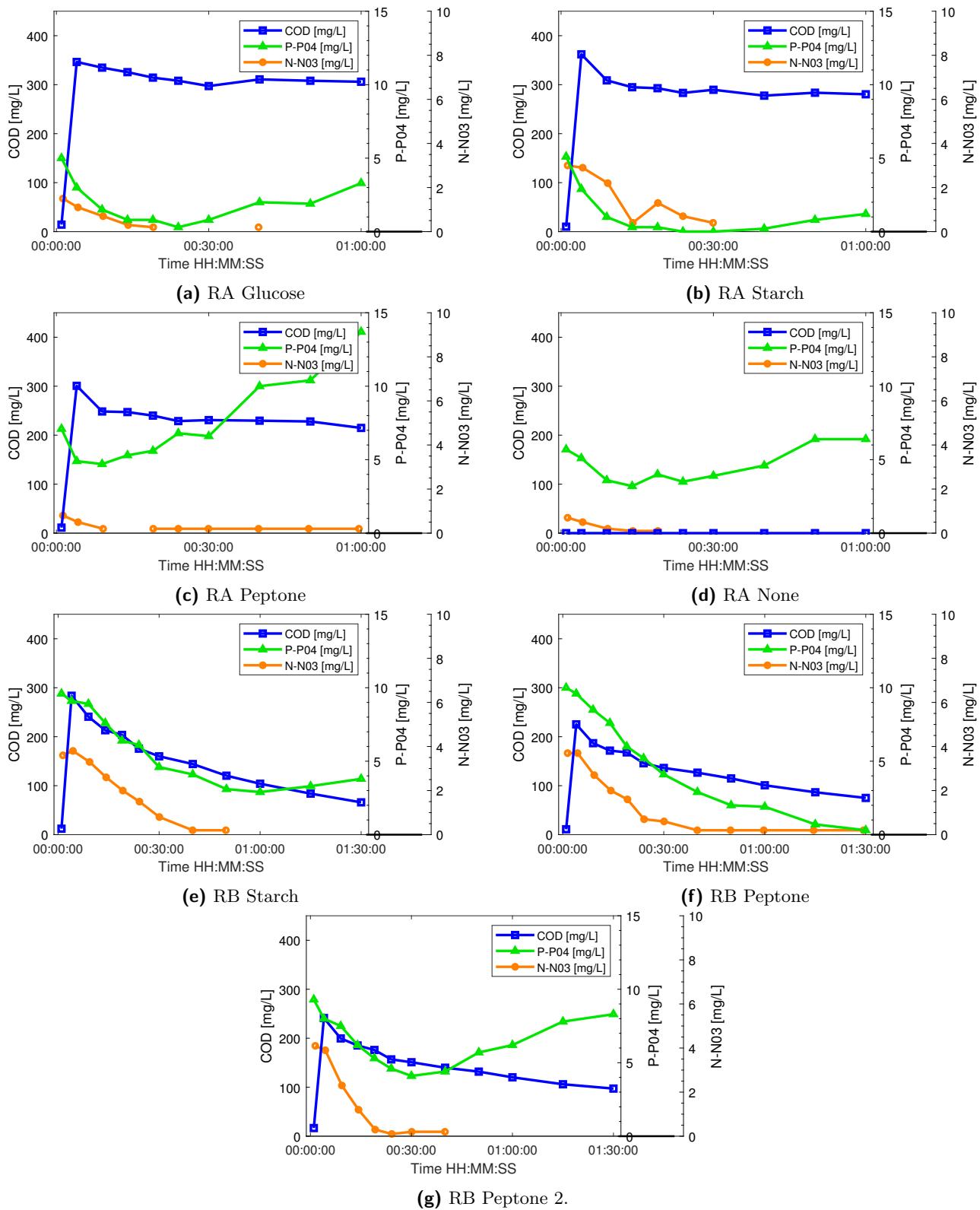


Fig. A.12: Small P-PO4 range; EBPR.ANA. on RA/RB

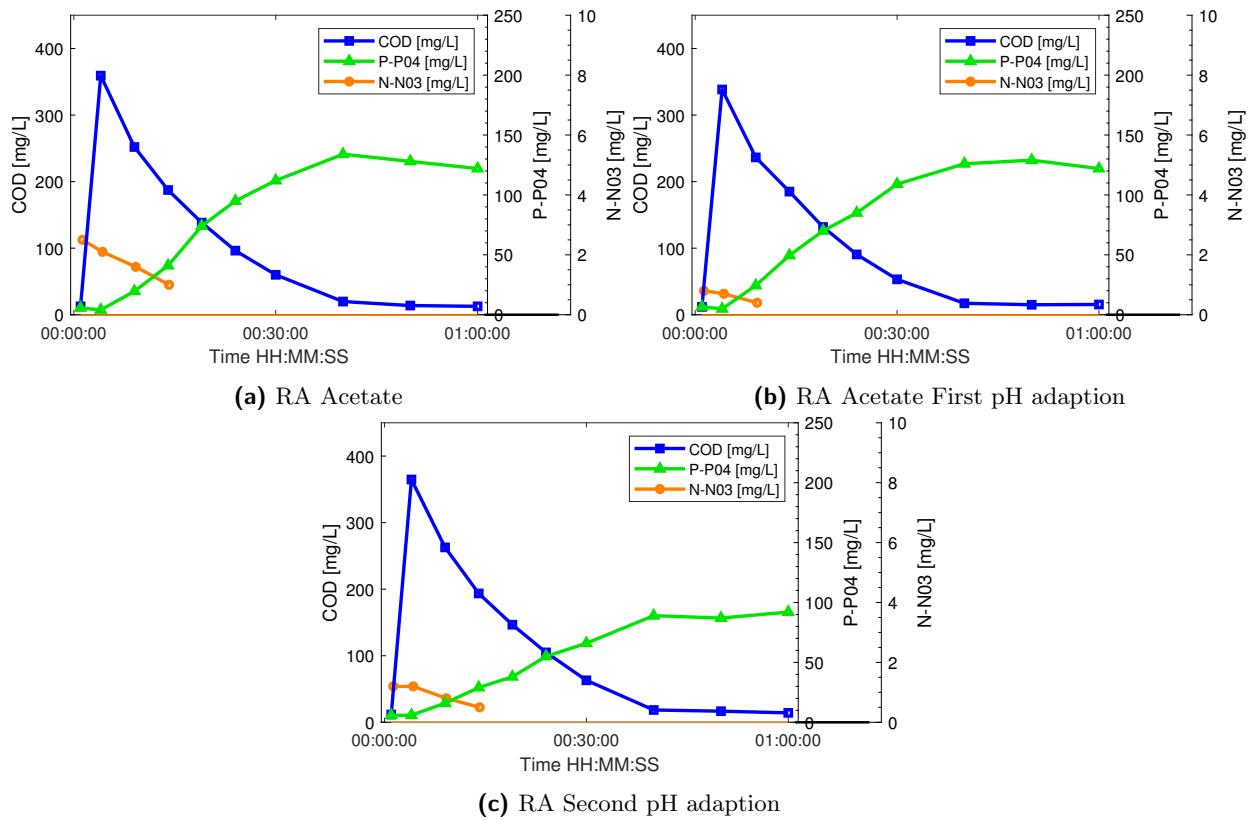


Fig. A.13: P-PO₄ and COD; EBPR.ANA. on RA with acetate and **adapted pH**

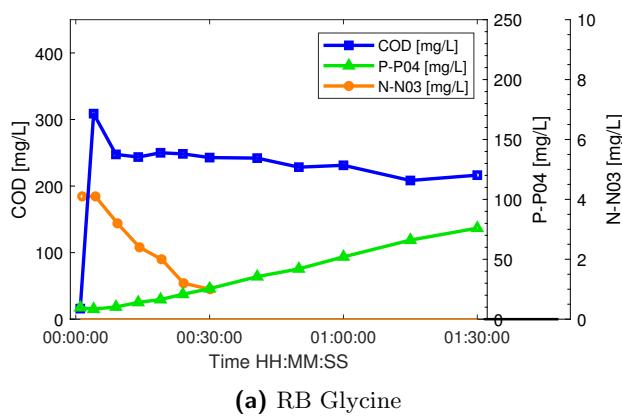


Fig. A.14: P-PO₄ and COD; EBPR.ANA. on RB with Glycine

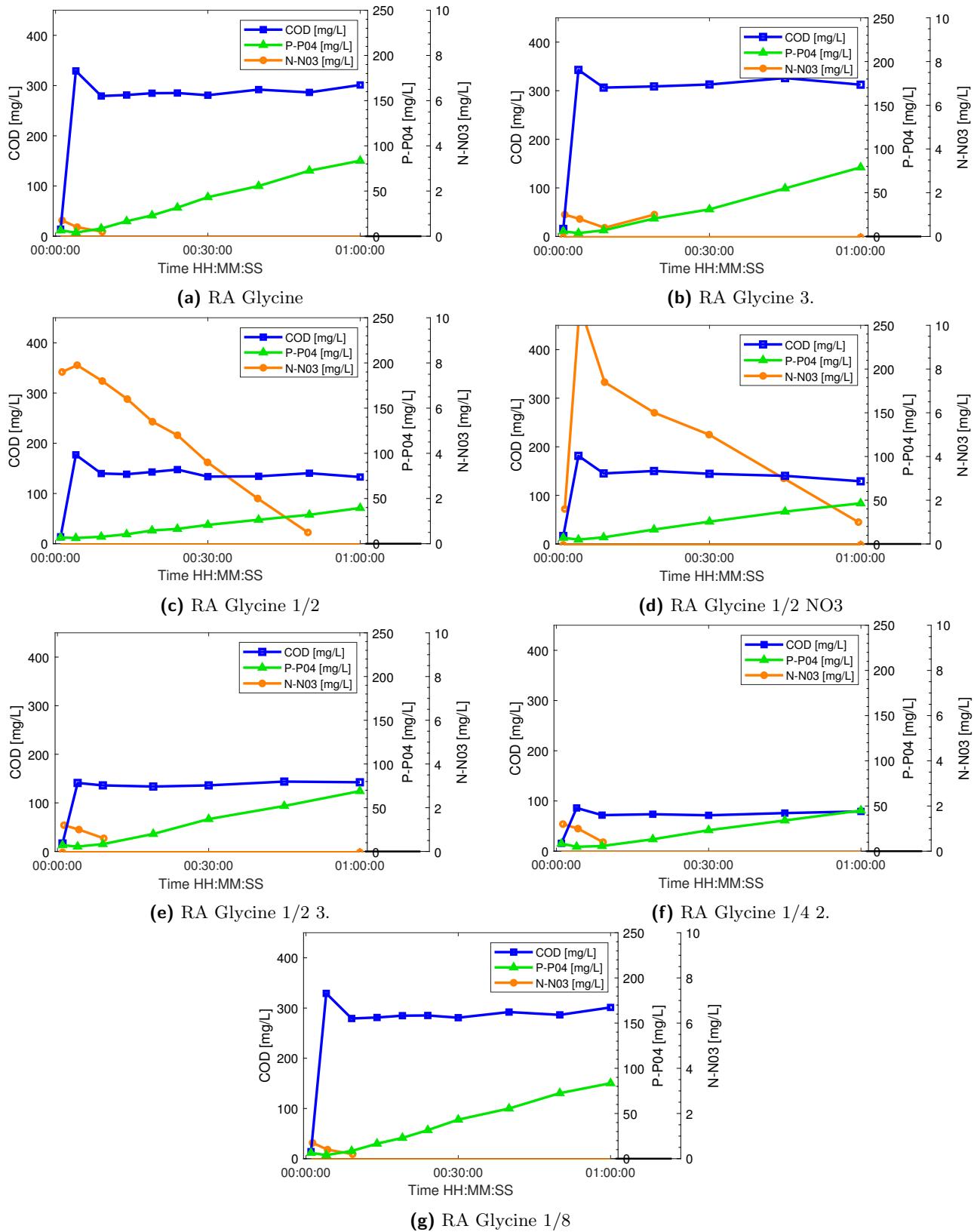


Fig. A.15: P-PO4 and COD; EBPR.ANA. on RA with Glycine Part I

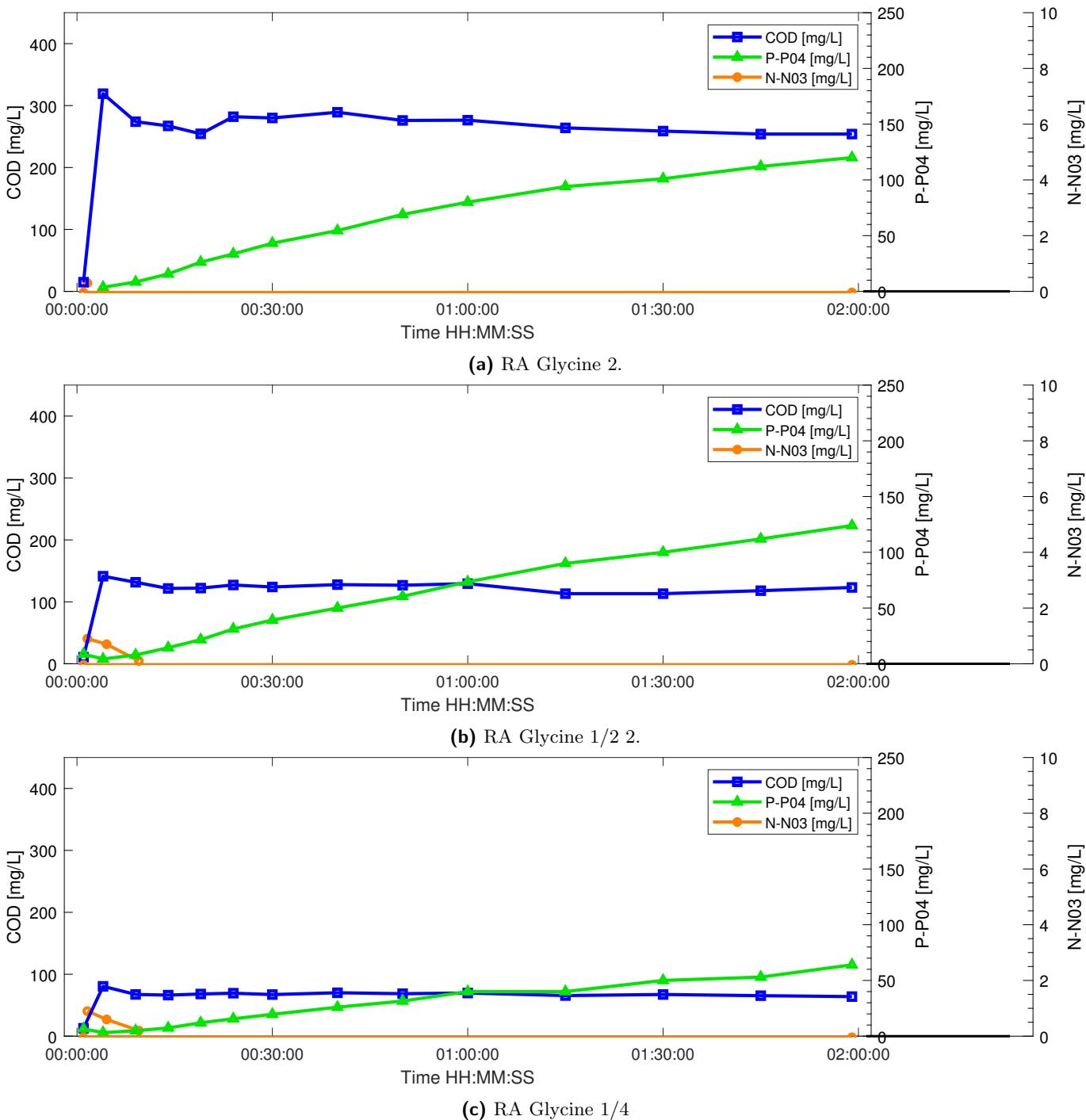


Fig. A.16: P-PO₄ and COD; EBPR.ANA. on RA with Glycine Part II

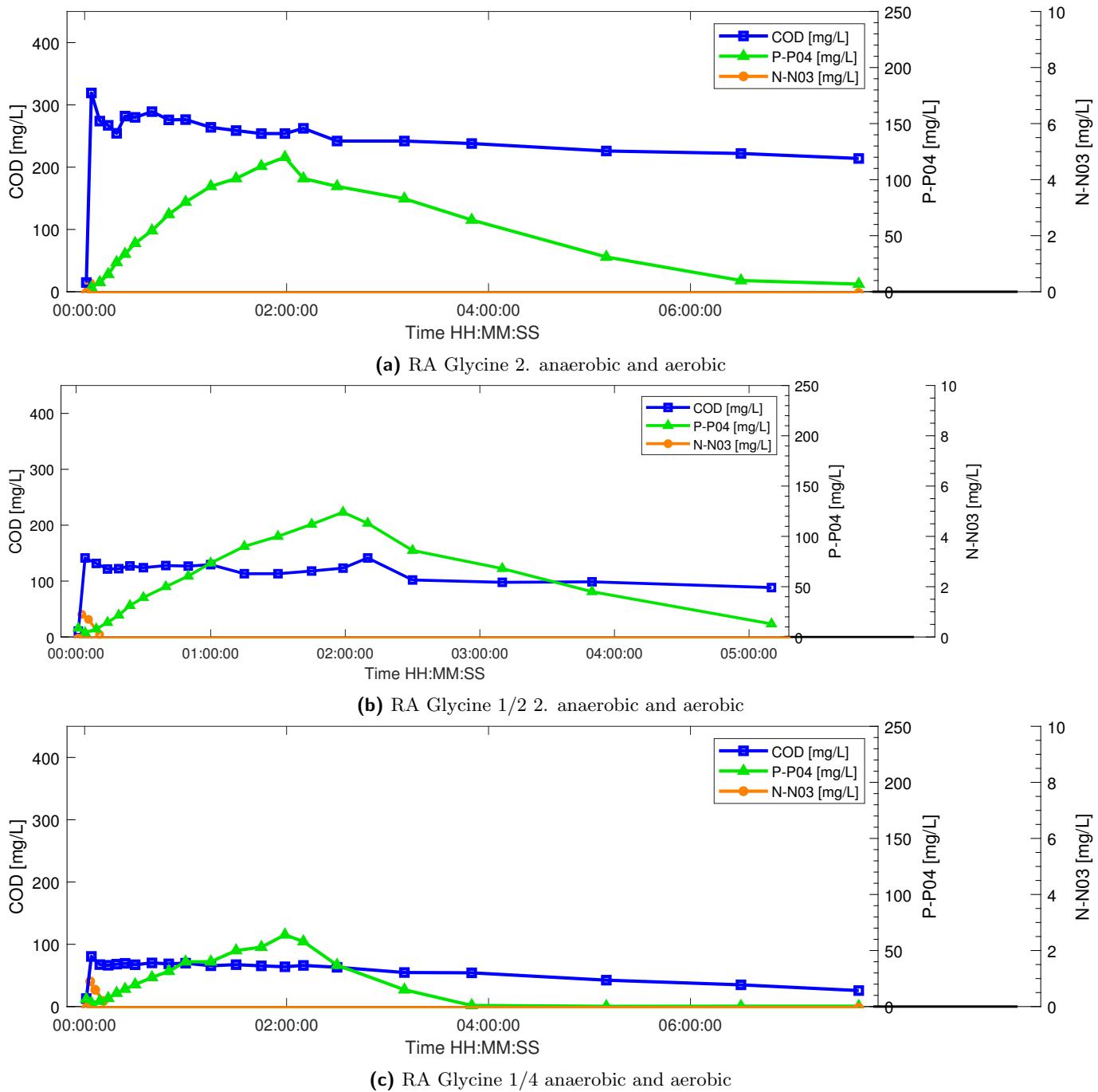
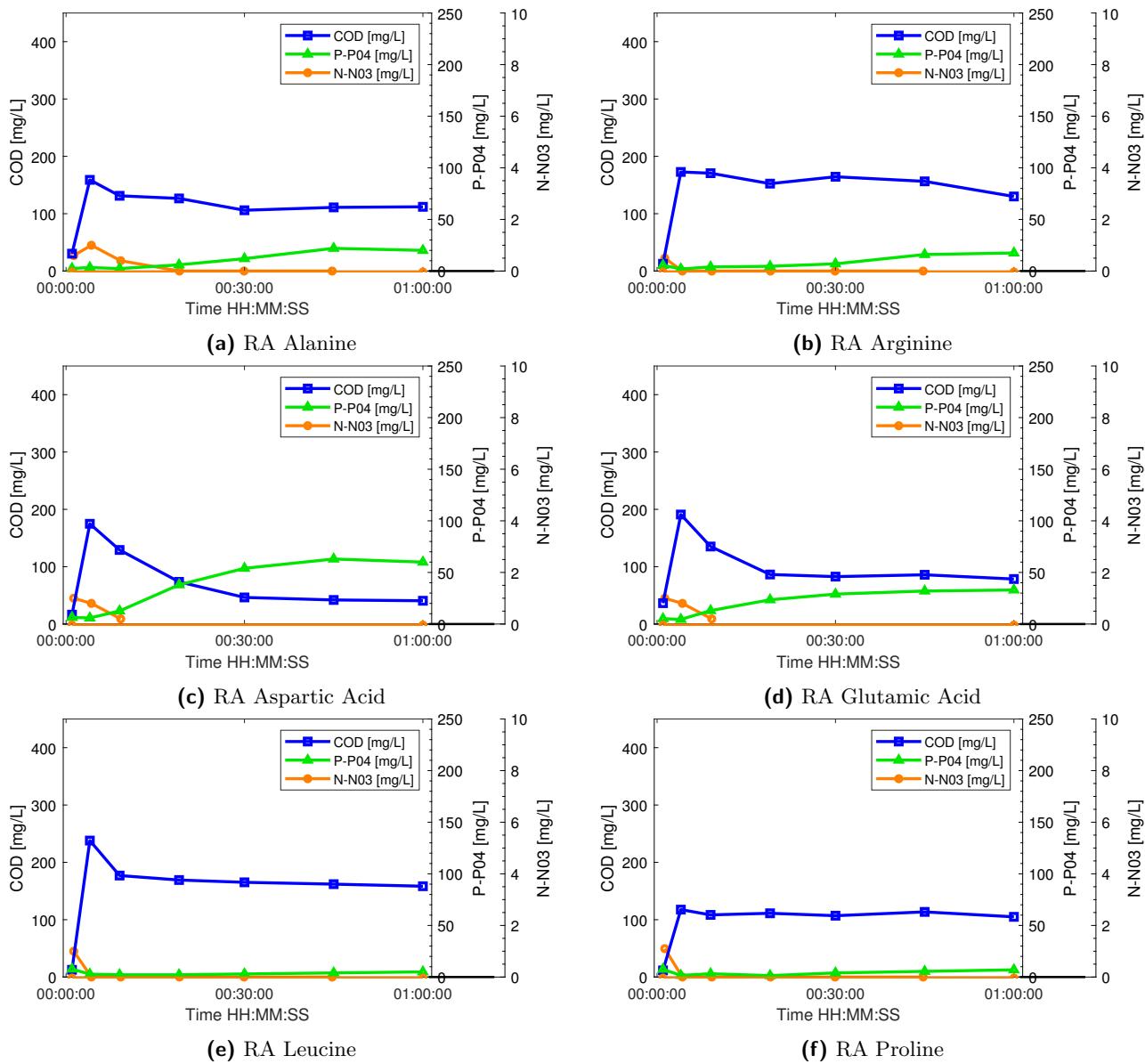
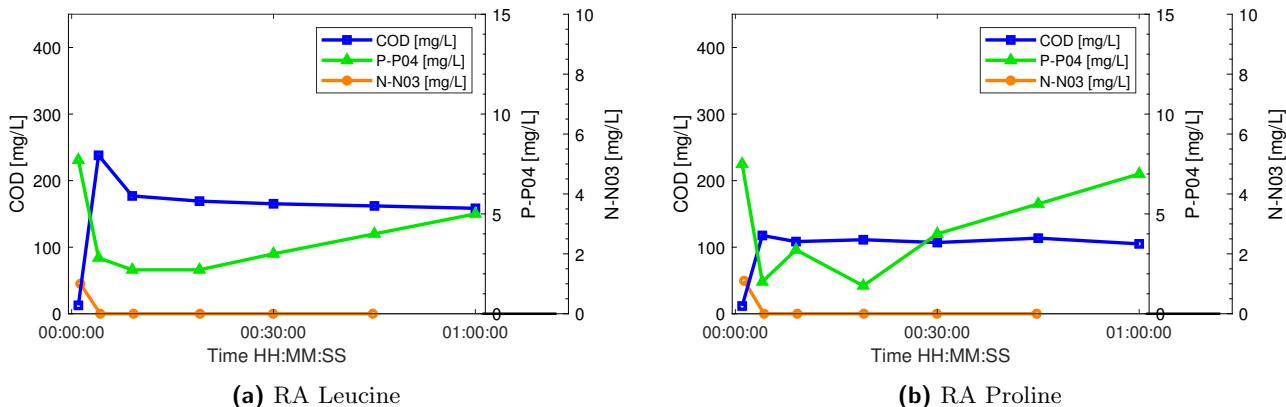


Fig. A.17: P-PO₄ and COD; EBPR.ANA. on RA tested with Glycine **into the aerobic phase**

**Fig. A.18:** P-PO4 and COD; EBPR.ANA. on RA with different amino acids**Fig. A.19:** Small P-PO4 range; EBPR.ANA. on RA with Leu./Pro.

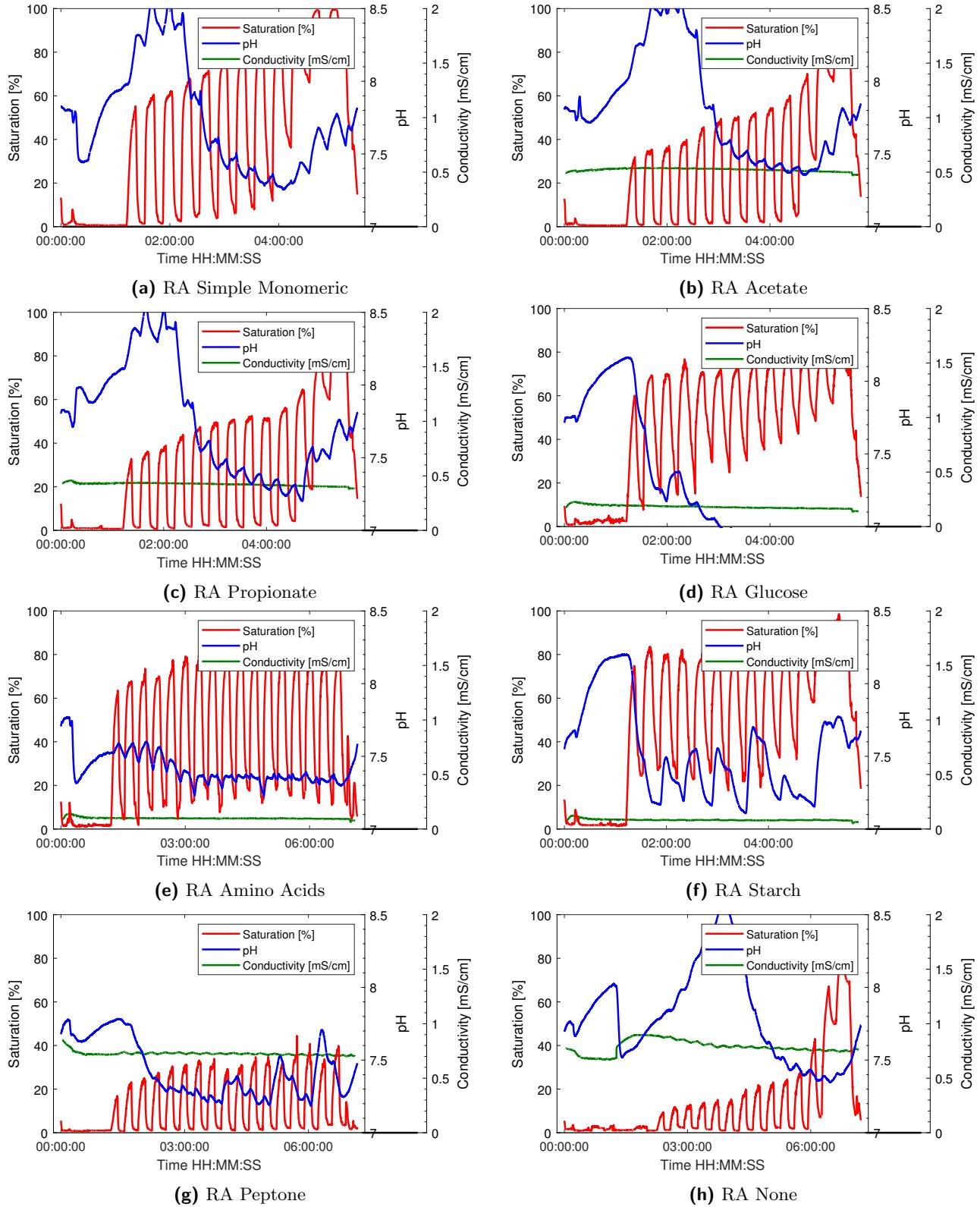


Fig. A.20: Oxygen saturation, pH and conductivity; EBPR.ANA. on RA

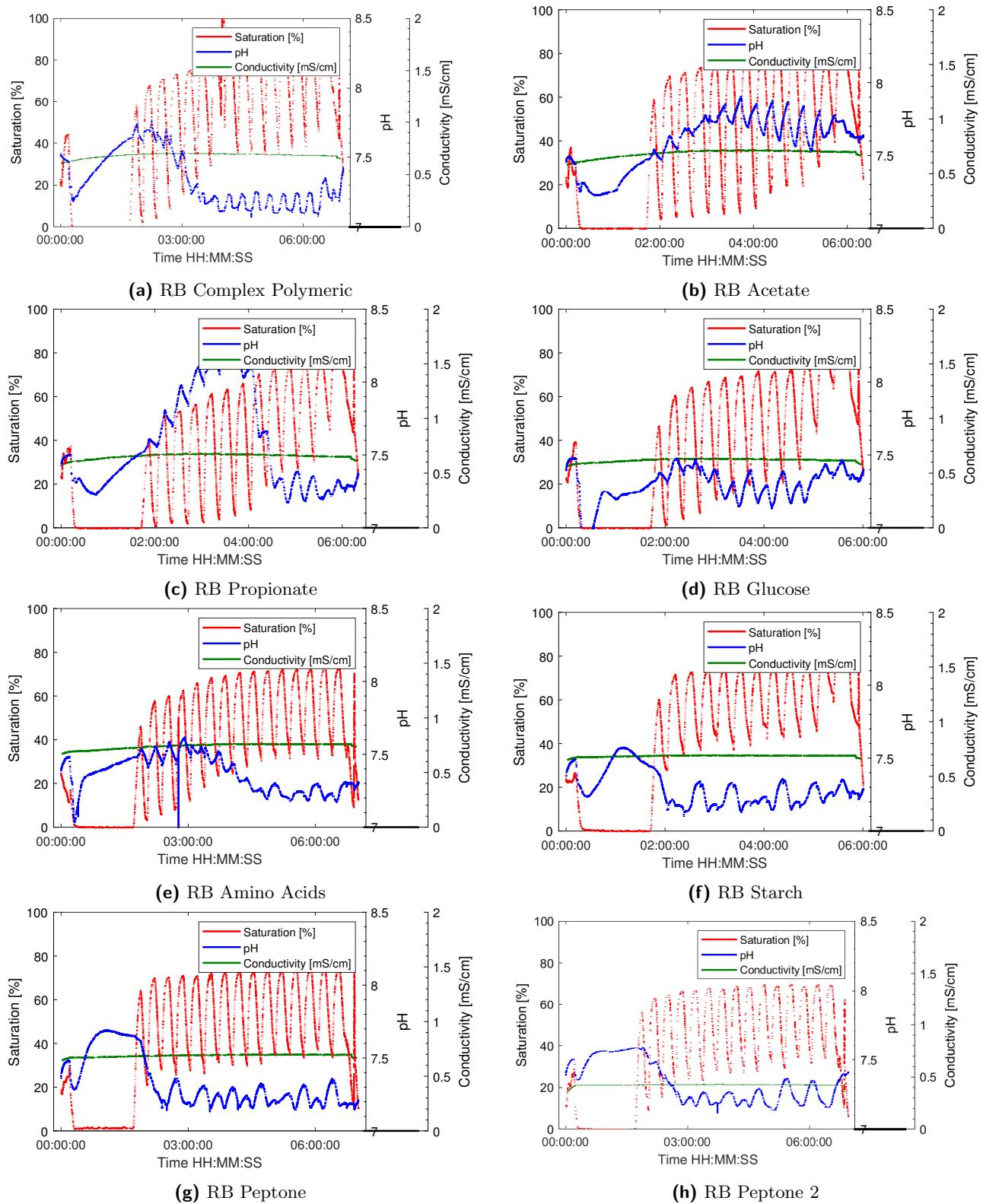


Fig. A.21: Oxygen saturation, pH and conductivity; EBPR.ANA. on RB

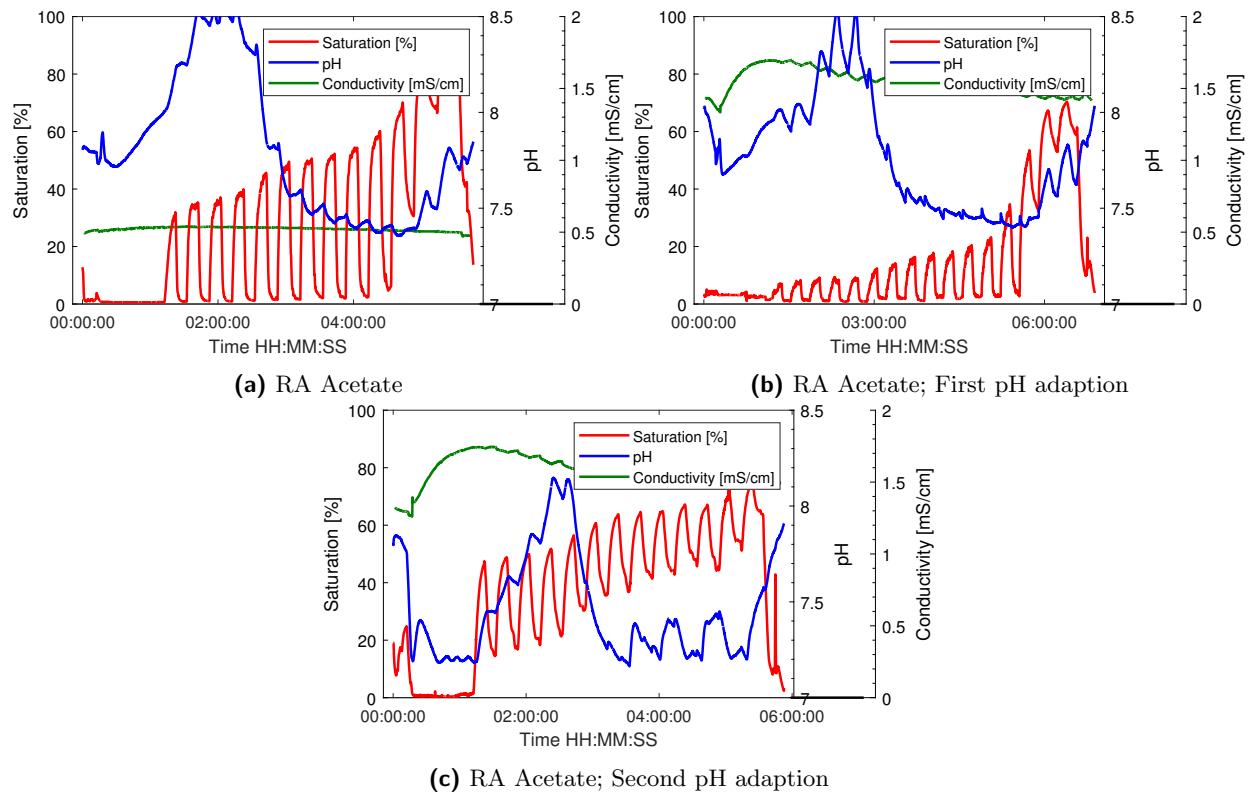


Fig. A.22: Oxygen saturation, pH and conductivity; EBPR.ANA. on RA with adapted pH

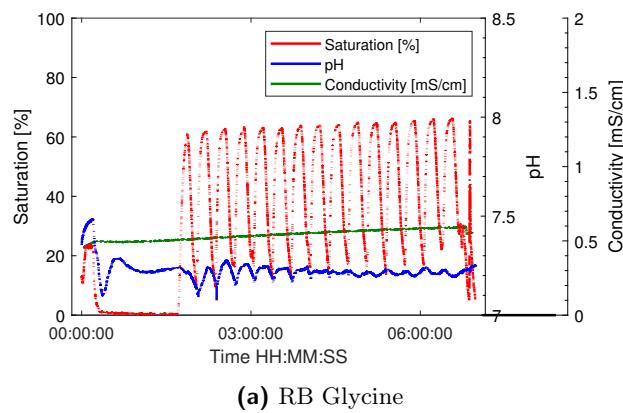


Fig. A.23: Oxygen saturation, pH and conductivity; EBPR.ANA. on RB with Glycine

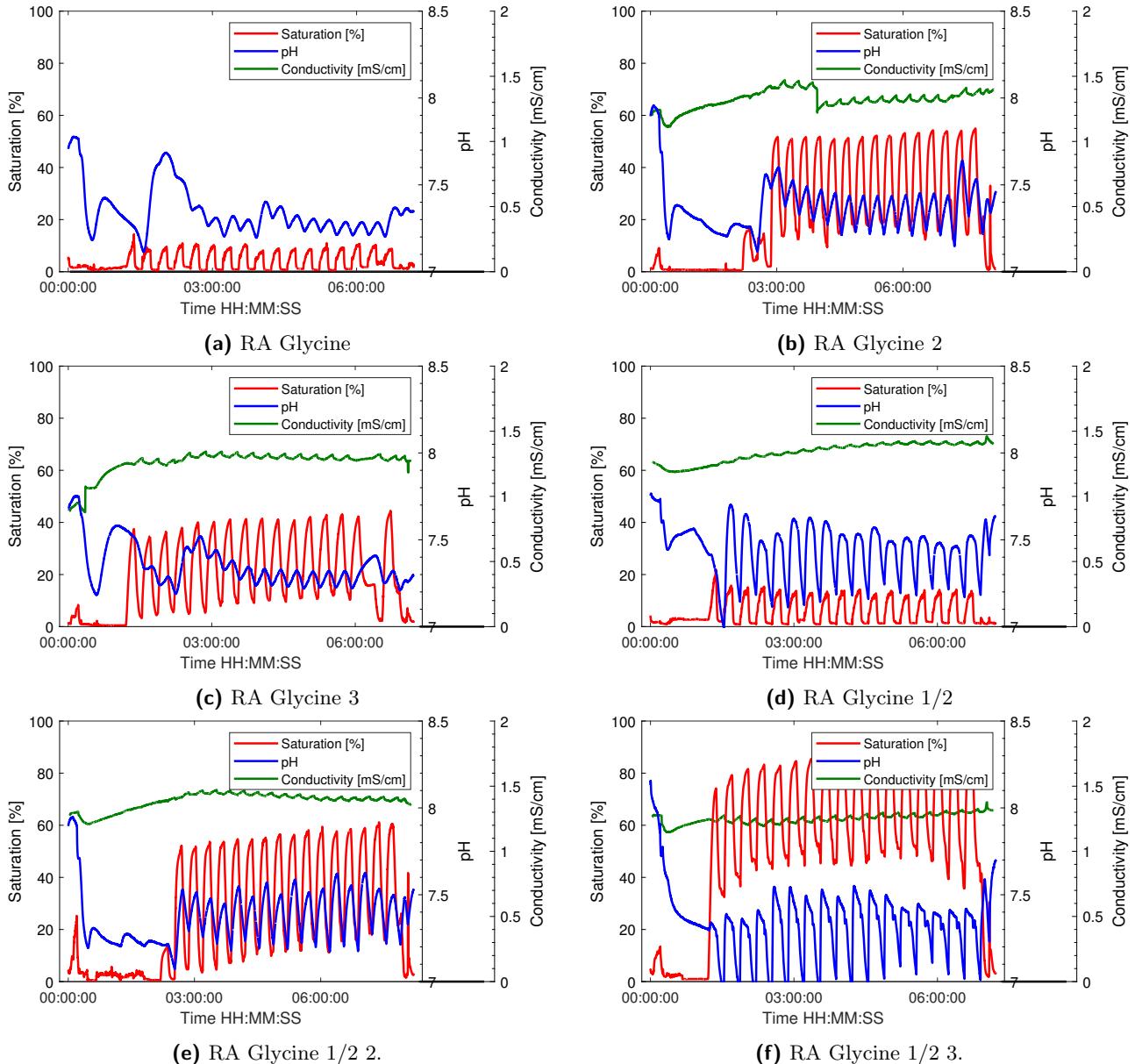


Fig. A.24: Oxygen saturation, pH and conductivity; EBPR.ANA. on RA with Glycine Part I

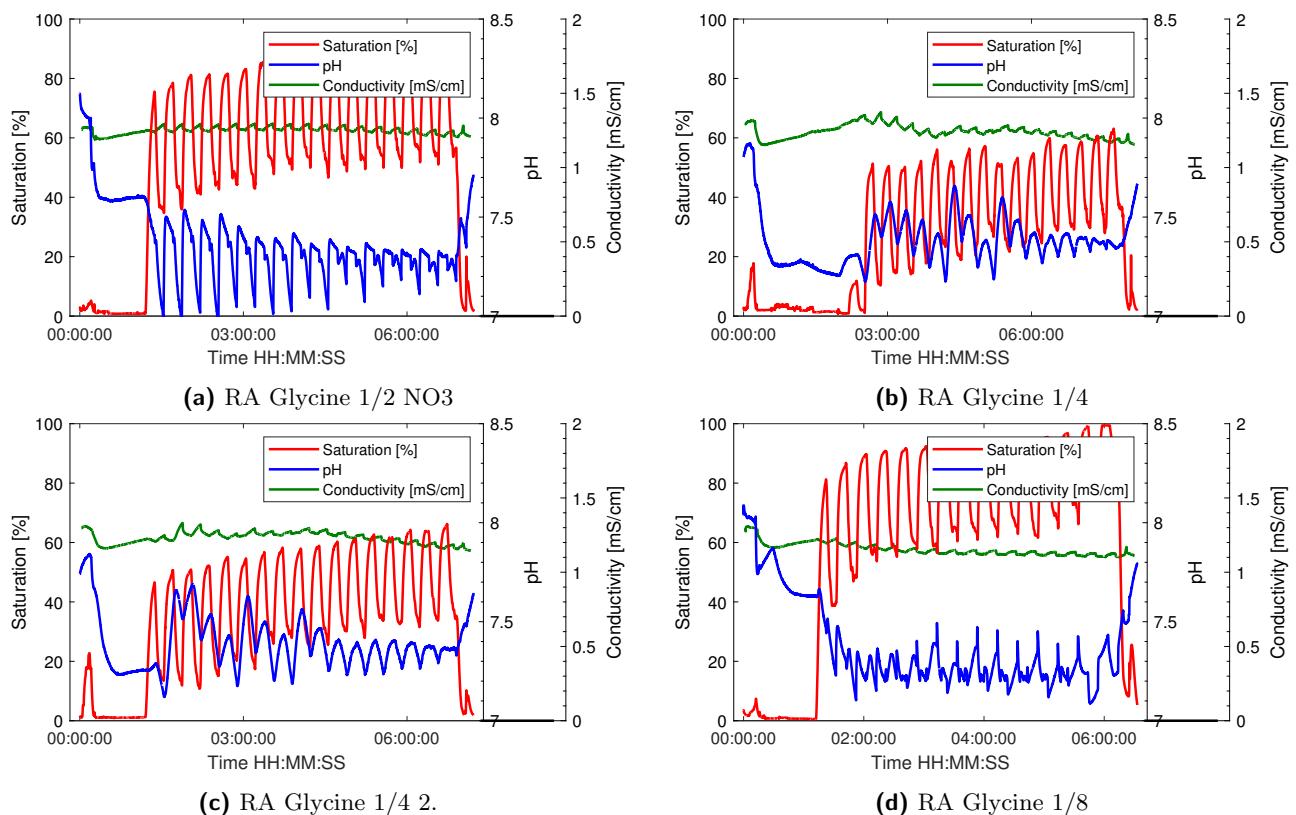


Fig. A.25: Oxygen saturation, pH and conductivity; EBPR.ANA. on RA with Glycine Part II

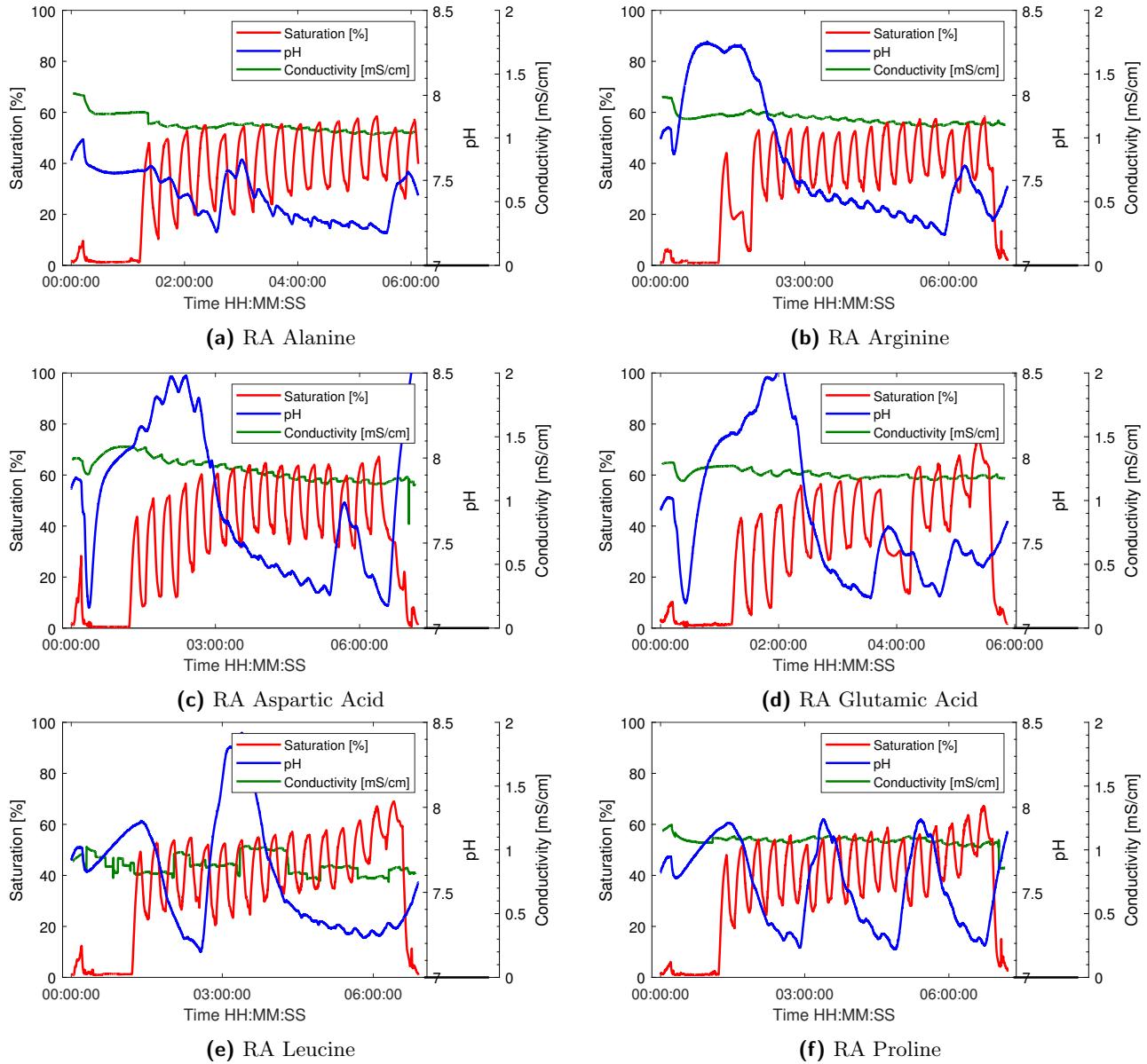


Fig. A.26: Oxygen saturation, pH and conductivity; EBPR.ANA. on RA with different amino acids

A.3.5 Protocol Anaerobic batch tests

Material

- Concentrated substrate solution
- Bottles with water, magnesium, calcium, potassium to replace the regular substrate inflow
- Around 30 Falcon tubes 15 mL
- 10-12 Kit LCK 314 COD (15.0-150.0 mg/L COD)
- 1 Kit LCK 514 COD (100-2000 mgCOD/L))
- Filter (45 µm) and small syringes (10 mL).

Procedure

1. At one minute: Take a first full-syringe sample at the reactor (AN-) to measure the state before adding the substrate. Let the syringe settle and put a bit in a small syringe (10 mL) and filter 10 mL in a tube. Empty the syringe back into the reactor.
2. Before 4 min: Add the substrate with the syringe. Measure 40 ml with a cylinder (uncertainty +/- 1 mL).
3. 15 sec around 4 min: Take the sample AN0 like before, but just 8 mL filter volume. Note the time from DAQFactory.
... Take all the other samples and filter the amount of volume according the dilution tables. The time has to be noted down.
4. Dilution for IC and COD into new tubes. For IC, 7 mL and for COD 2 mL of diluted liquid are required for the analysis. The dilution was chosen so that the values fit in the test range of the IC for P-P04 resp. the COD-Kit (0.7 mg/L - 16.7 mg/L resp. 15-150 mgCOD/L).

The dilutions for each test are listed in Batch Tests.xlsx/Dilution.

5. IC-samples preparation.
6. The COD-tests are done according to the Kit and like it is done usually:
 - Vortex the kit 15 sec
 - Add to 2 ml of diluted sample
 - Vortex 30 sec
 - Oven for 1h at 160 °C, shake after 30 min
 - Cool down for 15 min
 - Measure the kit with the spectrophotometer

At each anaerobic batch test, the substrate itself was tested as well. Either with the kit LCK 314 or LCK 514 with a dilution of 200x resp. 20x.

A.4 Adaption of the reactor

Table A.8 shows the initial cycle times of RB (equal to Table A.2, before the adaption of the medium and after 9 weeks) and with an idle phase and a fix length (in the first 9 weeks after medium adaption).

Tab. A.8: Cycle times of reactor RB

	RB initial [h:min:sec]	RB idle [h:min:sec]
Anaerobic feed	00:12:00	00:12:00
Anaerobic	01:30:00	01:30:00
Aerobic min.	04:00:00	04:00:00
Aerobic max.	05:00:00	05:00:00
Settling	00:06:00	00:06:00
Withdraw	00:07:00	00:07:00
Idle min.		01:05:00
Idle max.		00:05:00
Total min.	05:55:00	07:00:00
Total max.	06:55:00	07:00:00

A.4.1 ImageJ analysis

The following figures show the size distribution of the granules in reactor RA according to ImageJ analysis. It was performed with pictures from the sludge according to protocol 'Protocol ImageJ' in the digital appendix. As these three figures from different pictures, but the same reactor at the same date (29.05.2018) showed completely different results, this approach was not further investigated.

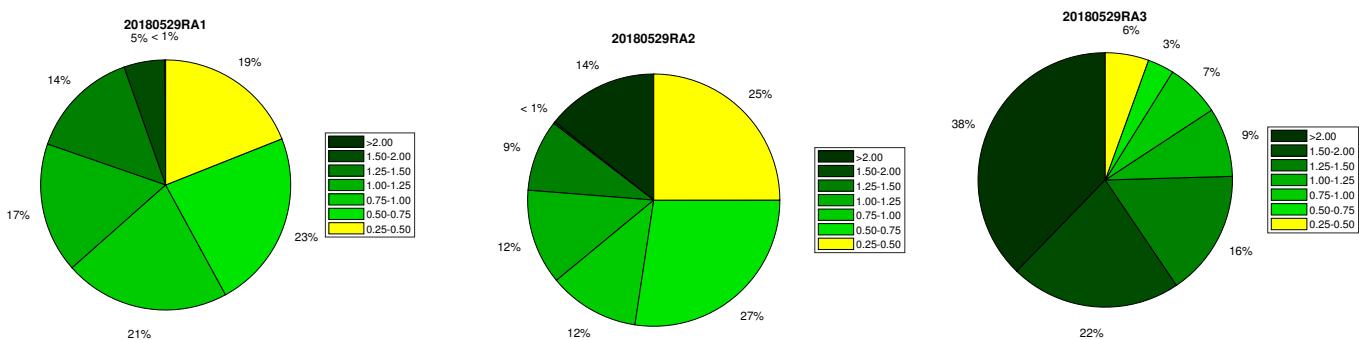


Fig. A.27: Image analysis on Reactor RA

A.4.2 Results

Every Monday and Thursday, the inflow, outflow and the water at the end of the anaerobic phase were analyzed on phosphate among others.

The phosphate concentration at the end of the anaerobic phase (AN90) decreased after adapting the wastewater composition (21.05.2018, week 0) from around $64 \text{ mgP} - P\text{O}_4^{-3}/\text{L}$ to $16 \text{ mgP} - P\text{O}_4^{-3}/\text{L}$.

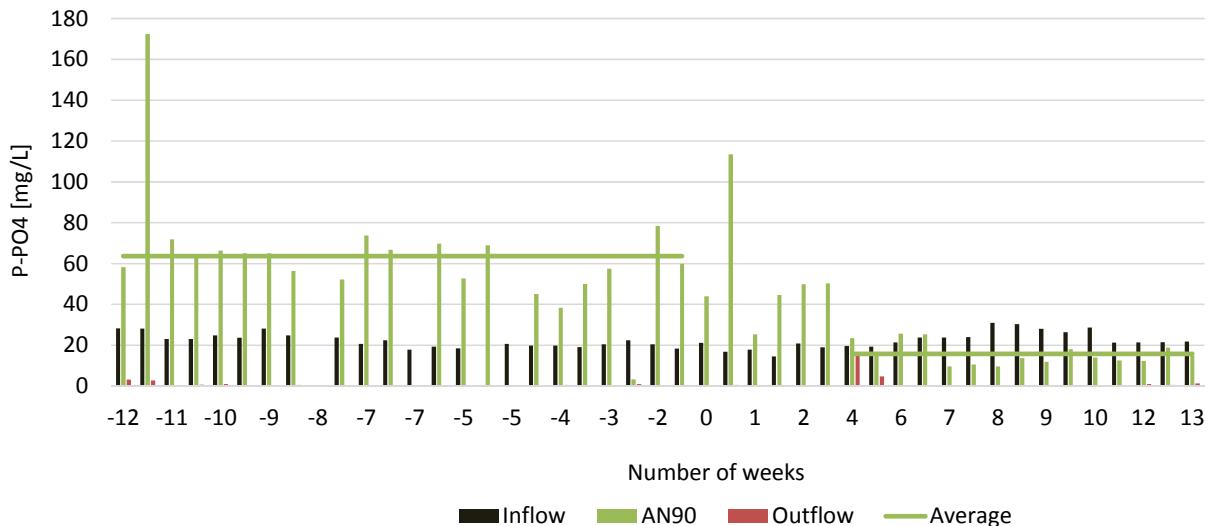


Fig. A.28: Phosphate concentration reactor RB

Figure A.29 shows the identical plot of reactor RA as comparison. The average phosphate concentration at the end of the anaerobic phase was $113 \text{ mgP} - P\text{O}_4^{-3}/\text{L}$.

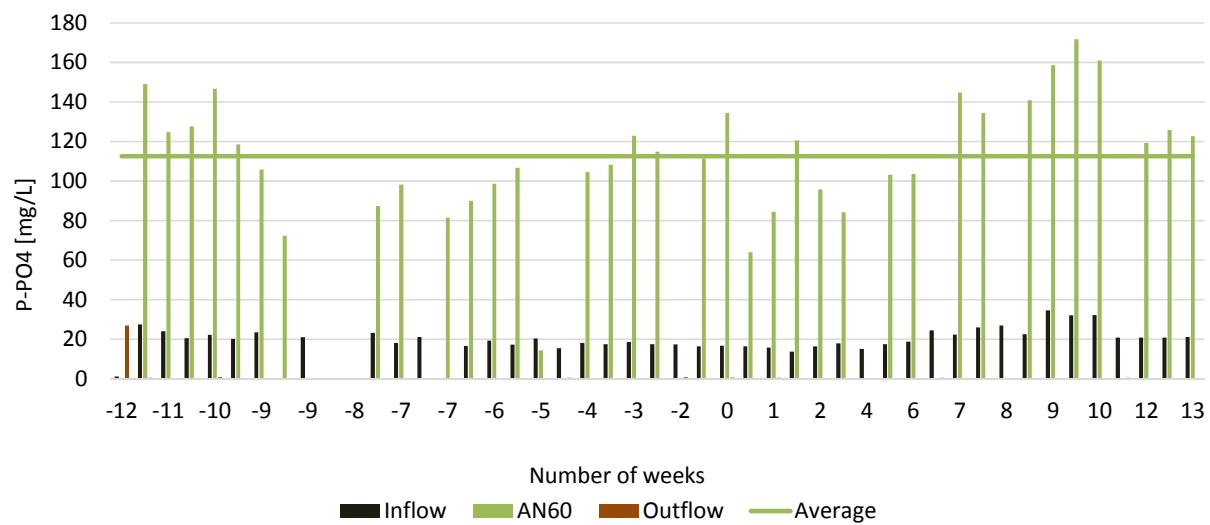


Fig. A.29: Phosphate concentration reactor RA

Figure A.30 shows the height of the biomass after 8min of settling in reactor RB during the adaption of the medium. The target height of 23 cm is plotted with dashed lines. Additionally the plot shows the settling time, which was decreased at a high level of biomass and increased at a low level.

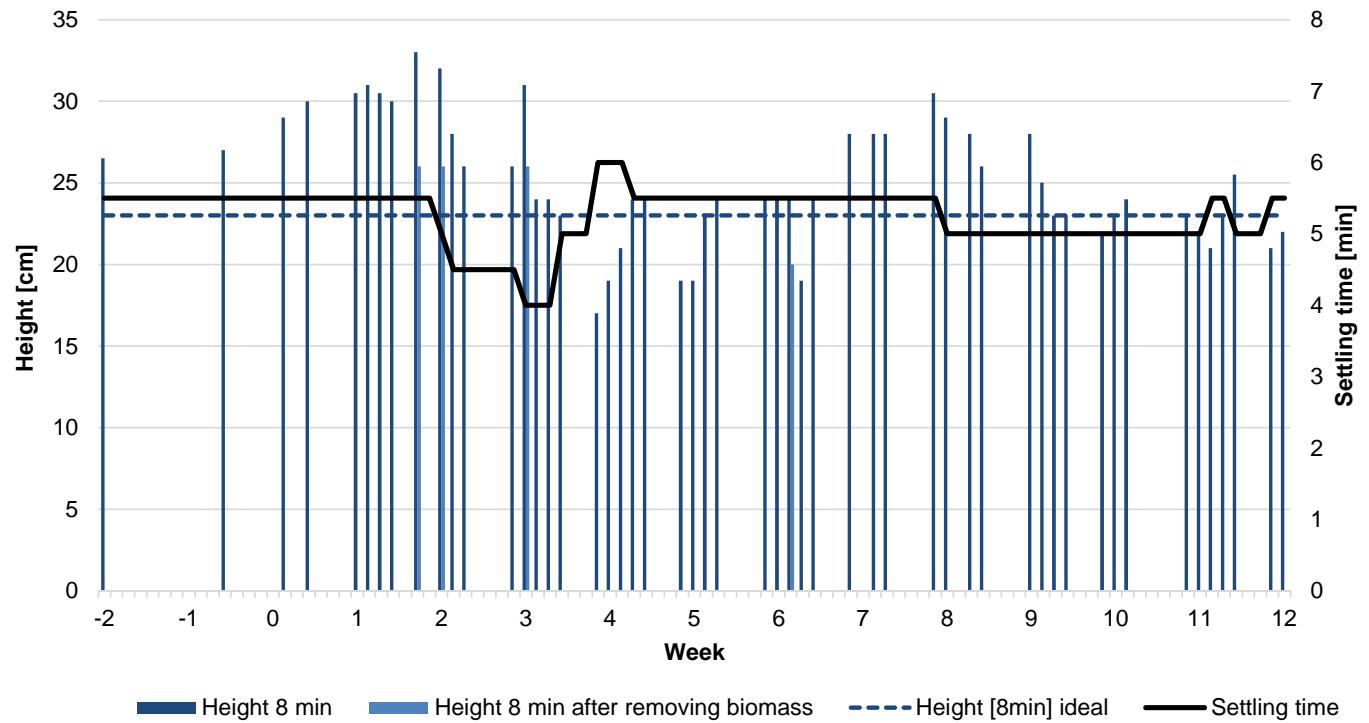


Fig. A.30: Height of biomass after 8min settling in Reactor RB after medium adaption

Table A.9 shows: the total solids concentration of the sieving (500 TS, 250 TS, F TS), their average (TS Siev.), total solids values of the whole biomass as reference (TS 1, TS 2, TS 3) and the SVI after 30 minutes. The medium was adapted on 21.05.2018 (see Schedule A.14) in week 13 of the whole working time of this master thesis, which is week zero of the medium adaption. The first three measurements were done 9 weeks before (RA -9 and RB -9), resp. at the adaption of the medium (RA 0).

Tab. A.9: TS of sieving and total biomass, SVI

Week	500 TS [mg/L]	250 TS [mg/L]	F TS [mg/L]	TS Siev.	TS 1 [mg/L]	TS 2 [mg/L]	TS 3 [mg/L]	TS Av. [mg/L]	SV30 30min [ml/ml]	SVI 30min [mL/g]
RA -9					7379	5125	3440	5315	0.27	51
RB -9					2925	2940	2780	2882	0.19	67
RA 0	3509	357	882	4748	3196	2781	3964	3314		
RA 11	3392	558	353	4303	5078	5852	5043	5324	0.24	45
-2	3669	1064	919	5652	5096	5314	5132	5180	0.29	55
-1										
0										
1	1556	635	1870	4061	4155	4004	3806	3988		
2	894	586	1818	3298	4044	3928	4028	4000	0.28	70
3	684	335	2046	3065	3480	4032	4645	4053	0.25	62
4	660	145	1584	2389	2404	3081	3048	2844	0.18	62
5	288	84	1576	1948	2236	2452	2162	2283	0.19	85
6	384	103	1695	2182	2448	2575	2609	2544	0.19	76
7	230	63	2022	2315	2741	2757		2749	0.27	98
8	163	55	2107	2325	2693	2758	2846	2766	0.33	121
9	247	82	2159	2488	2776	2816	2993	2862	0.27	96
10	168	13	1889	2070	2270	2200	2410	2294	0.23	99
11	161	31	1976	2168	2539	2506	2419	2488	0.22	87
12	137	26	1761	1924	2468	2474	2537	2493	0.19	78
Av.	711	248	1802	2760	3027	3146	3220	3119	0.24	82

Table A.10 shows the volatile solids according to the total solids of Table A.9.

Tab. A.10: VS of sieving and total biomass

Week	500 VS [mg/L]	250 VS [mg/L]	F VS [mg/L]	VS sieving [mg/L]	VS 1 [mg/L]	VS 2 [mg/L]	VS 3 [mg/L]	VS Av. [mg/L]	VS/TS
RA -9					3953	2419	847	2406	0.45
RB -9					-600	-850	184	-422	-0.15
RA 0	2661	240	542	3443	2233	1843	2776	2284	0.69
RA 11	2570	428	249	3247	3770	4360	3789	3973	0.75
-2	2353	816	661	3830	3416	3455	3418	3430	0.66
-1									
0									
1	1087	414	1265	2766	2910	2729	2666	2768	0.69
2	630	409	1217	2256	2762	2742	2776	2760	0.69
3	538	258	1445	2241	2489	2894	3427	2937	0.72
4	554	122	1350	2026	2008	2600	2522	2376	0.84
5	201	68	1241	1510	1724	1932	1728	1795	0.79
6	289	78	1227	1594	1766	1830	1824	1807	0.71
7	178	42	1374	1594	1909	1836		1872	0.68
8	85	1	1397	1483	1730	1758	1825	1771	0.64
9	179	59	1448	1686	1862	1905	2081	1950	0.68
10	119	12	1291	1422	1556	1537	1679	1591	0.69
11	121	23	1207	1351	1726	1703	1619	1683	0.68
11	114	35	1227	1376	1727	1756	1797	1760	0.71
Av.	496	180	1258	1933	2122	2206	2280	2192	0.71

Table A.11 shows: TS Reactor, which is the biomass in the reactor in total solids calculated as the average of three TS analysis each week. TS Clarifier resp. VS Clarifier describe the amount of biomass caught in the clarifier. TS and V manually represent the mass resp. the volume of mixed biomass removed from the reactor for sieving and TS analysis. V add. manually is the additional volume of biomass removed for example for DNA analysis. Biomass caught in the filter for water analysis was not taken into account. This additional volume is taken into account to calculate the TS removed:

$$TS_{removed}[mg] = \frac{TS_{manually} * (V_{manually}[mL] + V_{add.manually}[mL])}{V_{manually}[mL]} \quad (A.7)$$

TS clarifier plus TS removed divided by the number of cycles and the amount of COD per cycle leads to the TS/COD removed ratio.

The medium was adapted at 21.05.2018 (see Schedule A.14) in week 13 of the whole working time of this master thesis, which is week zero of the medium adaption. The first three measurements were done 9 weeks before (RA -9 and RB -9), resp. at the adaption of the medium (RA 0).

The ratio of VS/TS of the biomass in the clarifier is also on average around 72 % (for further information see excel "SVL_TS_VS" in the digital appendix).

Tab. A.11: TS in reactor, clarifier and removed, SRT

Week	TS Reactor [mg]	TS Clarifier [mg]	VS Clarifier [mg]	TS manually [mg]	V manually [mL]	V add. manually [mL]	TS removed [mg]	TS / COD removed	SRT [days]	TS removed [kg/kg]
RA -9	12755									
RB -9	6916									
RA 0	7953									
RA 11	12778									
-2	12433	1359	991	859	156.5	15	941	0.13	38	96
-1		2864	1964			62	340	0.19	27	133
0		2590	1793			12	66	0.15	33	111
1	9572	1907	1309	2128	539	12	2175	0.24	16	170
2	9600	2916	2000	756	207	12	800	0.22	18	155
3	9726	4254	3322	612	176	12	654	0.28	14	205
4	6826	2540	2174	452	175	12	483	0.17	16	126
5	5480	3110	2284	393	187	112	629	0.22	10	156
6	6106	3400	1375	427	182	12	455	0.22	11	161
7	6597	4101	2794	457	182	12	487	0.27	10	191
8	6637	4747	3184	473	187	12	503	0.30	9	219
9	6868	6042	4095	500	188	14	537	0.38	7	274
10	5505	6300	4991	373	172	14	404	0.39	6	279
11	5972									
12	5982									
Av.	7485	3548	2483	675	214	24	652	0.24	17	175

A.5 Schedule

Table A.12 shows an overview of the schedule of this master thesis. The period of the anaerobic batch tests is light gray and the period after the medium adaption in RB is dark gray.

Tables A.13 to A.16 show the daily schedule with the order and time of each experiment.

Tab. A.12: Schedule overview

Begin	End	Week	RB	RA
26.02.2018	04.03.2018	1	Read into the topic, lab introduction	
05.03.2018	11.03.2018	2	Lab introduction, plan batch tests	
12.03.2018	18.03.2018	3	Plan batch tests, write introduction, do regular measurements	
19.03.2018	25.03.2018	4	First batch tests, improve text, TS /VS	
26.03.2018	01.04.2018	5		Batch Tests: Ac, Pr
02.04.2018	08.04.2018	6	Batch Test: Medium 7	Batch Tests: Gl, AA, St
09.04.2018	15.04.2018	7	Batch Tests: Ac, Pr, Gl	
16.04.2018	22.04.2018	8	1st meeting; Batch tests: AA, St, Pe	Batch Tests: Pe, None
23.04.2018	29.04.2018	9	Batch tests RB: Gly, Pe (2)	Batch Test: Gly
30.04.2018	06.05.2018	10	Review results, plan adaption of RB	
07.05.2018	13.05.2018	11	TS / VS, Sieving, SVI	
14.05.2018	20.05.2018	12		Batch Tests: Ac (pH), Gly 1/2
21.05.2018	27.05.2018	13	Change to Medium 4	TS / VS, Sieving
28.05.2018	03.06.2018	14	TS / VS, Sieving	Batch Tests: Ac (pH = 7.2)
04.06.2018	10.06.2018	15	2nd meeting, TS/VS, Sieving, SVI	Gly 1/2 2.
11.06.2018	17.06.2018	16	TS / VS, Sieving, SVI	Gly 2.
18.06.2018	24.06.2018	17	TS / VS, Sieving, SVI	Gly 1/4
25.06.2018	01.07.2018	18	TS / VS, Sieving, SVI	Gly 1/4 2., Asp. Acid
02.07.2018	08.07.2018	19	TS / VS, Sieving, SVI	Glut. Acid, Alanine
09.07.2018	15.07.2018	20	TS / VS, Sieving, SVI	Arginine, Leucine
16.07.2018	22.07.2018	21	TS / VS, Sieving, SVI	Proline, Glycine 3.
23.07.2018	29.07.2018	22	TS / VS, Sieving, SVI	
30.07.2018	05.08.2018	23	TS / VS, Sieving, SVI	
06.08.2018	12.08.2018	24	TS / VS, Sieving, SVI	Gly 1/2 3., Gly 1/2, Gly 1/8
13.08.2018	19.08.2018	25	TS / VS, Sieving, SVI	TS / VS, Sieving, SVI
20.08.2018	26.08.2018	26		
27.08.2018	02.09.2018	27		
03.09.2018	09.09.2018	28	Final presentation (03.09.2018)	
10.09.2018				

Tab. A.13: Schedule part 1

		RB	RA
Week 4	19.03.2018		
	20.03.2018		
	21.03.2018		
	22.03.2018		09:44 Ac + Pr
	23.03.2018	TS / VS, SVI	TS / VS, SVI
Week 5	26.03.2018		
	27.03.2018		11:30 Acetate
	28.03.2018		10:50 Propionate
	29.03.2018		
	30.03.2018		
Week 6	02.04.2018		
	03.04.2018		14:00 Glucose
	04.04.2018		16:00 Amino Acids
	05.04.2018		13:30 Starch
	06.04.2018	09:10 Mix7	
Week 7	09.04.2018	11:15	Acetate
	10.04.2018	11:15	Propionate
	11.04.2018	11:15	Glucose
	12.04.2018		
	13.04.2018		
Week 8	16.04.2018	11:00	Amino Acids (failed)
	17.04.2018	14:30	Amino Acids
	18.04.2018		1. Intermediate Meeting
	19.04.2018	10:30	10:30 Peptone
	20.04.2018	10:30	14:00 None
Week 9	23.04.2018		
	24.04.2018		
	25.04.2018	14:30	Glycine
	26.04.2018		
	27.04.2018	11:00	Peptone (2.)
Week 10	30.04.2018		
	01.05.2018		
	02.05.2018		
	03.05.2018		
	04.05.2018		

Tab. A.14: Schedule part 2

Week 11	07.05.2018 08.05.2018 09.05.2018 10.05.2018 11.05.2018	TS / VS, Sieving, SVI	
Week 12	14.05.2018 15.05.2018 16.05.2018 17.05.2018 18.05.2018		10:00 Acetate fixed pH 14:00 Glycine 1/2
Week 13	21.05.2018 22.05.2018 23.05.2018 24.05.2018 25.05.2018	Change to Medium 4	TS / VS, Sieving
Week 14	28.05.2018 29.05.2018 30.05.2018 31.05.2018 01.06.2018	TS / VS, Sieving	10:00 Acetate pH 7.2
Week 15	04.06.2018 05.06.2018 06.06.2018 07.06.2018 08.06.2018	TS / VS, Sieving, SVI (Medium 4)	2. Intermediate Meeting 13:00 Glycine 1/2 2.
Week 16	11.06.2018 12.06.2018 13.06.2018 14.06.2018 15.06.2018	TS / VS, Sieving, SVI	10:00 Glycine 2.
Week 17	18.06.2018 19.06.2018 20.06.2018 21.06.2018 22.06.2018	TS / VS, Sieving, SVI (Medium 4)	08:30 Glycine 1/4

Tab. A.15: Schedule part 3

Week 18	25.06.2018	TS / VS, Sieving, SVI	12:00	Glycine 1/4 2.
	26.06.2018		13:00	Aspartic Acid
	27.06.2018			
	28.06.2018			
	29.06.2018			
Week 19	02.07.2018	TS / VS, Sieving, SVI		
	03.07.2018		15:00	Glutamic Acid
	04.07.2018			
	05.07.2018		09:00	Alanine
	06.07.2018			
Week 20	09.07.2018	(Medium 4)		
	10.07.2018			
	11.07.2018		12:30	Arginine
	12.07.2018			
	13.07.2018		11:00	Leucine
Week 21	16.07.2018	TS / VS, Sieving, SVI	11:00	Proline
	17.07.2018			
	18.07.2018		09:00	Glycine 3.
	19.07.2018			
	20.07.2018			
Week 22	23.07.2018	TS / VS, Sieving, SVI Remove Idle Phase (Medium 4)		
	24.07.2018			
	25.07.2018			
	26.07.2018			
	27.07.2018			
Week 23	30.07.2018	TS / VS, Sieving, SVI		
	31.07.2018			
	01.08.2018			
	02.08.2018			
	03.08.2018			
Week 24	06.08.2018	TS / VS, Sieving, SVI (Medium 4)	15:30	Glycine 1/2 3.
	07.08.2018			
	08.08.2018		09:00	Glycine 1/2 Nitrate
	09.08.2018			
	10.08.2018		08:30	Glycine 1/8

Tab. A.16: Schedule part 4

Week 25	13.08.2018 14.08.2018 15.08.2018 16.08.2018 17.08.2018	TS / VS, Sieving, SVI	TS / VS, Sieving, SVI
Week 26	20.08.2018 21.08.2018 22.08.2018 23.08.2018 24.08.2018		
Week 27	27.08.2018 28.08.2018 29.08.2018 30.08.2018 31.08.2018		
Week 28	03.09.2018 04.09.2018 05.09.2018 06.09.2018 07.09.2018	Final Presentation	
Week 29	10.09.2018		Hand in report

B Digital Appendix

- Batch_Tests.xlsx
- Schedule.xlsx
- SVL_TS_VS.xlsx
- Pictures_SVI
- Media for the reactors
 - Complex Monomeric Medium.docx
 - Complex Polymeric Medium.xlsx
 - Simple Monomeric Medium
 - Complex Polymeric, concentrated, 30.04.2018.xlsx
- Protocol
 - Protocol Biomass collection.docx
 - Protocol Sieving.docx
 - Protocol Petridish.docx
 - Protocol TS, VS and SVI.docx
 - Protocol_ImageJ.pdf
- Water test
 - Absorbance glucose, 26.03.2018.xlsx
 - Feuille de prélèvement jeudi 15.03.2018.docx
 - Feuille de prélèvement lundi 15.03.2018.docx
 - Protocole analytique pour boue granulaire 020318.docx
 - Valeurs idéales 020318.docx
 - RA_results_C1_29.08.2018
 - RB_results_C2_29.08.2018
 - IC_27.08.2018

Matlab Code

- Figures of oxygen saturation, conductivity and pH
 - Acquisition.m
 - AcquisitionpH.m
 - Acquisitionplotyyy.m
 - plotyyyAq.m
 - SchlaufeAcq.m
- Figures of phosphate, nitrate and COD
 - Anaerobic_batch.m
 - plotyyy.m
 - Schlaufe.m
- ImageJ
 - ImageJ.m
 - Schlaufe_ImageJ.m