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Phosphorus Re-dissolution and Recycling from Activated Sludge in Full-Scale Wastewater Treatment Plants

Strategies, Mechanism and Pilot-Scale Implementation

Dissertation

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Annika Anders

(M.Sc.)

Aus Frankfurt am Main

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Betreuer Prof. Dr. Patrick Elter (Technische Hochschule Mittelhessen)

Prof. Dr. Harald Platen (Technische Hochschule Mittelhessen)

Gutachter/in Prof. Dr. Sylvia Schnell (Justus-Liebig-Universität Giessen)

Prof. Dr. Harald Weigand (Technische Hochschule Mittelhessen)

Angenommen vom Promotionszentrum für Ingenieurwissenschaften am Forschungscampus Mittelhessen

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I Abstract

Wastewater treatment is essential to protect the environment and human health. While traditionally the focus has been on pollutant and nutrient removal, wastewater treatment plants (WWTPs) are now evolving into facilities for the additional recovery of resources. A key target for recovery is phosphorus (P), an essential element for agriculture and critical to ensure global food security. Given the finite and geopolitically vulnerable nature of global phosphate rock reserves as well as the regulatory pressure for a circular resource use, the development of methods for a sustainable nutrient management and P recovery is becoming increasingly important. Adapting the enhanced biological P removal (EBPR) process for a biological P recovery could be a promising alternative to cost- and resource intensive chemical methods. By utilizing the metabolic capability of polyphosphate accumulating organisms (PAOs) enriched in the microbial community of activated sludge (AS), P can be released from their intracellular polyP pool under anaerobic conditions. So far, most studies are based on laboratory enriched PAO cultures, limiting the real-world applicability in full-scale systems. This thesis aimed at exploring a targeted P re-dissolution approach for non-acclimated AS of full-scale WWTPs by leveraging the P cycling ability of PAOs. Key objectives included process optimization for a rapid P re-dissolution without sludge disintegration (cell lysis), identifying an efficient carbon source, and evaluating the recovery process at pilot-scale in a full-scale WWTP. Results showed that P is effectively released from non-acclimated AS within a short period of 1–4 h by adding acetate. Laboratory batch experiments revealed that AS from pure full-scale EBPR systems exhibits the highest P re-dissolution efficiency, with up to 56% of total sludge P being released. WWTPs combining EBPR and chemical P removal (CPR) show reduced P yields (19–24%), likely due to precipitant use limiting PAO activity and polyP availability. Systematic investigation of volatile fatty acid (VFA) supplementation revealed acetate at a 200 mg/L dose as the most efficient substrate for a high, fast and consistent P re-dissolution from mixed EBPR/CPR sludge. The molar $P_{yield}/C_{consumed}$ ratio was 0.45. Other substrates, such as formate, propionate and butyrate were less effective. After full acetate consumption, ongoing P release was observed, which gave new insights into metabolic limitations within PAOs possibly due to polyP/glycogen depletion and disruption of the membrane potential. At pilot-scale, acetate-induced P re-dissolution in a mixed EBPR/CPR WWTP produced a P-rich stream suitable for fluidized bed precipitation using milk of lime. A precipitation efficiency of 99% was achieved. A P-enriched dolomite pellet with slow-release fertilizer characteristics was produced, potentially suitable for pH regulation in acidic soils and provision of the plant nutrients Mg, Ca and P. With a yield of 1.9% P, the recovery remained lower than in laboratory-scale, which was attributed to technical challenges in the extraction of the P-rich stream and biological P re-dissolution variability of the EBPR/CPR sludge. Overall results show that, particularly in pure EBPR systems, an acetate-mediated approach enables a rapid and targeted re-dissolution of P. Accordingly, the P-depleted AS can subsequently be returned to the aeration stage of the WWTP or disposed by co-incineration. Further research in pure EBPR systems and on long-term stability is recommended to enhance the recovery efficiency. Retrofitting WWTPs for an on-site EBPR based P recovery could contribute to a more circular P use, by providing a valuable recovery product. Concomitantly it may reduce sludge disposal costs and dependance on chemical precipitant usage.

II Zusammenfassung

Die Abwasserbehandlung ist für den Schutz der Umwelt und der menschlichen Gesundheit von entscheidender Bedeutung. Während der Schwerpunkt traditionell auf der Entfernung von Schad- und Nährstoffen liegt, entwickeln sich Kläranlagen nun zu Einrichtungen für die zusätzliche Rückgewinnung von Ressourcen. Als unverzichtbares Element für das Pflanzenwachstum, die Landwirtschaft und entscheidend für die Gewährleistung der globalen Ernährungssicherheit ist Phosphor (P) ein zentrales Ziel für die Rückgewinnung. Angesichts der begrenzten und geopolitisch anfälligen Verfügbarkeit der weltweiten verfügbaren Phosphaterz-Reserven sowie des regulatorischen Drucks für eine zirkuläre Ressourcennutzung, gewinnen Methoden zur P-Rückgewinnung und für ein nachhaltiges Nährstoffmanagement an Bedeutung. Die Anpassung des EBPR-Verfahrens (*enhanced biological P removal*) zur biologischen P Rückgewinnung kann eine vielversprechende Alternative zu oft kosten- und ressourcenintensiven chemischen Methoden sein. Durch Nutzung der Stoffwechselkapazität von Polyphosphat-akkumulierenden Organismen (PAOs), die in der mikrobiellen Gemeinschaft des Belebtschlammes (BS) angereichert sind, kann P unter anaeroben Bedingungen aus dem PolyP-Pool freigesetzt werden. Bislang basieren die meisten dieser Studien auf in Laborversuchen angereicherten PAO-Kulturen, was die Übertragbarkeit auf reale Systemen einschränkt. Ziel dieser Arbeit ist die Untersuchung einer gezielten P-Rücklösung aus nicht akklimatisiertem BS, indem die Fähigkeit der PAOs zur P Aufnahme und Abgabe genutzt wird. Zu den Hauptzielen gehörten die Prozessoptimierung für eine schnelle P-Rücklösung nicht akklimatisiertem BS ohne Zersetzung der Schlammstruktur (Zelllyse), die Identifizierung einer effizienten Kohlenstoffquelle und die Bewertung des Rückgewinnungsprozesses im Pilotmaßstab für eine reale Kläranlage. Die Ergebnisse zeigten, dass durch Acteatzugabe P effektiv innerhalb von 1–4 h aus nicht akklimatisertem BS freigesetzt wird. Batch-Laborversuche zeigten, dass BS aus reinen EBPR-Systemen die höchste Effizienz bei der P-Rücklösung aufweist, wobei bis zu 56% des Gesamt-P innerhalb von 4 h freigesetzt werden. Kläranlagen, die EBPR und chemische P-Entfernung (CPR) kombinieren, weisen geringere P-Ausbeuten (19–24%) auf, was auf den Fällmitteleinsatz zurückzuführen ist, der die PAO-Aktivität und die Verfügbarkeit von PolyP einschränkt. Eine systematische Untersuchung der Supplementierung von kurzkettigen Fettsäuren ergab, dass Acetat mit 200 mg/L das effizienteste Substrat ist, um aus gemischtem EBPR/CPR-Schlamm eine gleichmäßige, schnelle und hohe P-Rücklösung bei einem molarem $P_{Ausbeute}/C_{aufgenommen}$ Verhältnis von 0,45 zu erzielen. Andere Substrate wie Formiat, Propionat und Butyrat waren weniger wirksam. Nach Acetetaufnahme wurde eine weitere P-Freisetzung beobachtet, die neue Einblicke über die metabolischen Zusammenhänge innerhalb der PAOs vermuten lässt und im PolyP- und Glykogenabbau sowie dem Zusammenbruch des Membranpotenzials begründet sein kann. Im Pilotmaßstab wurde durch P-Rücklösung aus BS einer EBPR/CPR-Kläranlage ein P-reicher Strom produziert, der sich für die Wirbelbettbettfällung mittels Kalkmilch eignete und eine Fällungseffizienz von 99% erreichte. Es wurde ein P-angereichertes Dolomitpellet mit Langzeitdüngereigenschaften gewonnen, welches sich für die pH-Wert Regulation in sauren Böden sowie für die Bereitstellung der Pflanzennährstoffe Mg, Ca und P eignet. Die Rückgewinnung blieb mit einer Ausbeute von 1,9% geringer als im Labormaßstab, was auf technische Probleme bei der Abtrennung des P-reichen Stroms und sowie auf eine biologische Rücklösevariabiliät des EBPR/CPR-BS zurückzuführen ist. Die Ergebnisse zeigen, dass insbesondere in reinen EBPR Systemen ein acetatvermittelter Ansatz eine schnelle und gezielte biologische Rücklösung von P ermöglicht. Demnach kann der an P

abgereicherte BS anschließend der Belebungsstufe zurückgeführt oder durch Mitverbrennung entsorgt werden. Weitere Forschung in reinen EBPR nutzenden Kläranlagen, zur Langzeitstabilität und Prozessoptimerung werden empfohlen, um die Rückgewinnungseffizienz zu verbessern. Die Nachrüstung von Kläranlagen für eine P-Rückgewinnung vor Ort auf EBPR-Basis können zu einer zirkulären Nutzung von P beitragen, indem ein wertvolles Rückgewinnungsprodukt bereitgestellt wird, und gleichzeitig die Abhängigkeit von chemischen Fällmitteln verringert sowie die Kosten für die Schlammentsorgung gesenkt werden.

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V List of Abbreviations and Symbols

Abbreviations

Ac	acetate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AS	activated sludge
ATP	adenosine triphosphate
BOD	biological oxygen demand
Ca-P	calcium phosphate
cf.	compare with
COD	chemical oxygen demand
CPR	chemical phosphorus removal
DCP	di-calcium phosphate
DNA	deoxyribonucleic acid
DO	dissolved oxygen
Dol-P	P-enriched dolomite
e.g.	for example
EBPR	enhanced biological phosphorus removal
ESI	electronic supplementary information
FISH	fluorescence *in situ* hybridization
GAO	glycogen accumulating organism
HAP	hydroxyapatite
HRT	hydraulic retention time
i.e.	that is
MAP	magnesium ammonium phosphate

mono-AP	mono ammonium phosphate
N	nitrogen
NADH	nicotinamide adenine dinucleotide
NPK	nitrogen phosphate potassium
ORP	oxygen reduction potential
ortho-P	ortho-phosphate
P	phosphorus
PAO	polyphosphate accumulating organism
PE	population equivalent
PH2MV	poly-β-hydroxy-2-methylvalerate
PHA	polyhydroxyalkanoate
PHB	poly-β-hydroxybutyrate
PHV	poly-β-hydroxyvalerate
pmf	proton motive force
polyP	polyphosphate
ppK	polyphosphate kinase
PR	phosphate rock
RAS	return activated sludge
RNA	ribonucleic acid
rRNA	ribosomal RNA
SBR	sequencing batch reactor
SEM	scanning electron microscopy
SRT	solids retention time
SSA	sewage sludge ash
TCA cycle	tricarboxylic acid cycle
TSP	triple superphosphate
TSS	total suspended solids
VFA	volatile fatty acid

VSS	volatile suspended solids
WWTP	wastewater treatment plant
XRF	x-ray fluorescence spectroscopy

Symbols and units

%	percent
°C	degrees celsius
µ	micro
a	year
d	day
E_H	ORP referenced against standard hydrogen electrode
FNU	formazin nephelometric unit
g	gram
h	hour
L	liter
mg	milli gram
min	minute
mM	molarity, milli molar, mmol×L^{-1}
mol	mole
P/C ratio	molar ratio of phosphorus to carbon
s	second
T	temperature
w/w	weight per weight
wt%	weight percent

“I’m a molecular solid element
When burning I look grand
I was isolated from urine
By the German Henning [sic] Brand
I’m in DNA, fertilizers
Matches and much more
My molecules aren’t diatomic
I group my atoms in four […]”[1]

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[1] Excerpt from RCS escape the classroom again, Education in Chemistry, November 2019, rsc.li/34maTCe, Royal Society of Chemistry, accessed August 01, 2023.

Chapter 1

Introduction

1.1 Phosphorus and its importance

Phosphorus (P) is an essential element for all living organisms and is a key player in various biological processes. In most cells P makes up approximately 2–4% on a dry weight basis (Karl, 2000). In the form of phosphate, it is a key component in the formation of nucleotides – the building blocks of DNA and RNA, and it plays a central role in the cellular energy metabolism via adenosine triphosphate (ATP). In addition, phosphate in the form of phospholipids forms a structural component of the cell membrane and is found as calcium phosphate minerals in mammalian bones and teeth.

In 1669, P was first discovered by the German merchant and alchemist Hennig Brand in pursuit of the “philosopher’s stone” (Krafft, 1969). By heating and evaporating urine, Brand isolated white P, a highly flammable substance that glowed when exposed to oxygen (Gleason, 2007; Krafft, 1969). As a result, it was given the name “phosphorus”, which is derived from the Greek *phōsphóros* and means light-bearer. Despite having several allotropes, its two main elemental forms are white and red P (Gleason, 2007). Naturally, P does not occur as a free element but as a phosphate mineral.

Although P is the 11th most abundant element in the Earth’s crust (Cordell and White, 2011), deposits of phosphate rock[2] (PR) are geographically limited to a few countries (Figure1-1). Across the world, these PR deposits are mainly sedimentary, whereas smaller fractions are igneous deposits (El Bamiki et al., 2021; USGS, 2023). While global resources are estimated to exceed 300×10^9 tons of PR (Argus Consulting Services, 2023; USGS, 2023), PR is essentially a finite resource, as the formation of sedimentary deposits takes several million years (Cordell and White, 2011). In 2022, the world reserves of PR were estimated to be 72×10^9 tons by the U.S. Geological Survey (USGS, 2023). More than two-thirds of the world's PR reserves are located in Morocco (69.4%), followed by Egypt with 3.9% and Tunisia with 3.5% (Figure 1-1) (USGS, 2023). In Central Europe, however, there are hardly any significant PR reserves. There are large seabed deposits in the Atlantic and Pacific Oceans, but as of today they are not considered economically accessible (ESPP, n.d.; USGS, 2023).

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[2] Generalized term for natural mineral deposits (unprocessed or processed ore) of varying composition with a high concentration of phosphate minerals (Argus Consulting Services, 2023; Van Kauwenbergh, 2010).

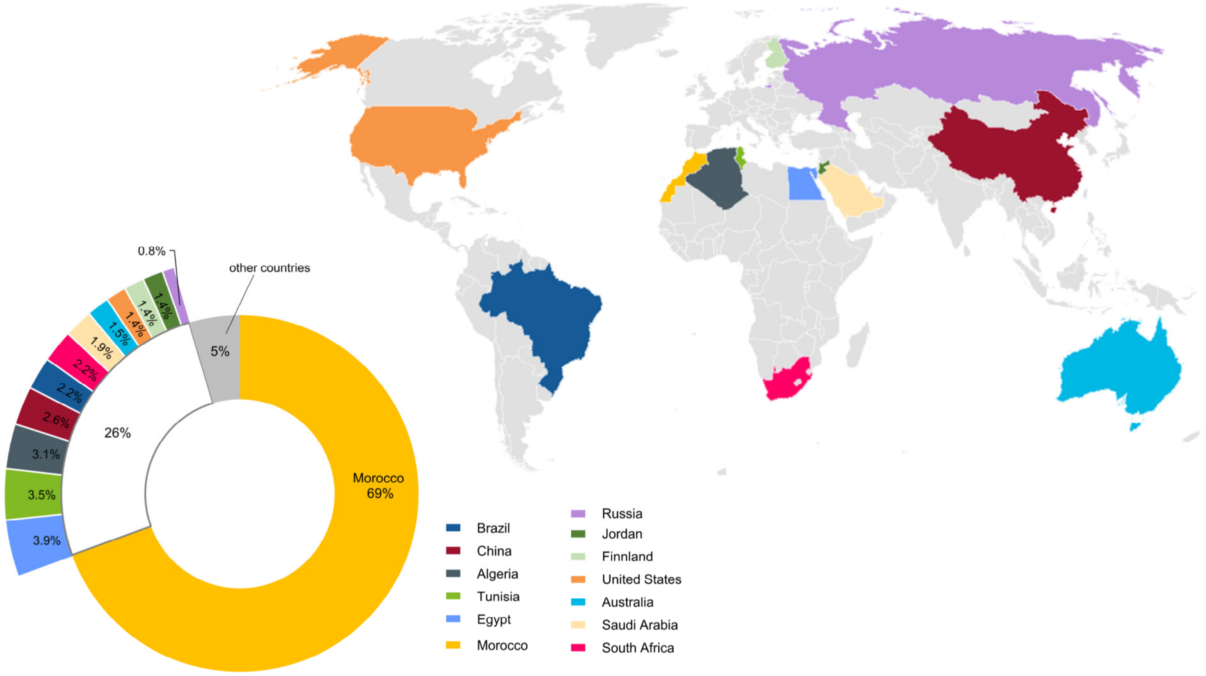

Figure 1-1| Current distribution of phosphate rock world reserves in 2022 according to the U.S. Geological Survey (USGS, 2023). Countries with phosphate rock reserves below 600 million tons have been grouped under “other countries”.

The exact quantities of available PR commodities remain uncertain, as data on PR resources and reserves are dynamic. Reserves are part of the resource pool and defined as the currently known and assessed PR deposits that are (i) accessible and (ii) can be mined at profit with the current state of technology (Wellmer and Scholz, 2017). With new exploration efforts, emerging technologies, or changes in demand and production costs, new deposits may become economically available, thus reserves would increase (Wellmer and Scholz, 2017). This is illustrated by the example of Norway, where according to an announcement by Norge Mining in 2023, new PR deposits have been discovered, with an estimated reserve size of 70×10^9 tons of mineralized PR (Simon, 2023). However, it is still debated whether these can be exploited in the near future (Knickel, 2023). The geographical disparity in PR reserves makes many countries highly dependent on P imports and thus subject to price fluctuations and decisions of global players, politics, and export policies. Because of its high economic importance and potential supply risk, PR was added to the list of critical raw materials by the European Commission in 2014 (European Commission, 2014).

In 2021, around 226×10^6 tons of PR were mined globally with the three leading producers being China (90×10^6 tons), followed by Morocco (38.1×10^6 tons) and the United States (21.6×10^6 tons) (USGS, 2023). More than 85% of the mined PR is used to produce mineral fertilizers for agriculture (European Commission, 2020). The remaining 15% is used for animal feed supplements and various industrial products, such as detergents, chemicals, food additives and batteries (European Commission, 2020).

As P is a non-substitutable nutrient for plant growth and “Life's Bottleneck” (Asimov, 1959) todays agriculture heavily rely on P-based fertilizers. With the growing world population and the need to meet crop yields for food production, the global demand for P-based fertilizers is expected to further increase (Argus Consulting Services, 2023). At the same time, high-grade PR deposits are declining (Stephen M. Jasinski, U.S. Geological Survey, personal communication, June 13, 2023), and impurities such as uranium and cadmium are increasingly present in mined ores (Chien et al., 2011; von Horn and Sartorius, 2009). This has raised concerns about the environmental impact of extensive PR mining (e.g., carbon emissions, energy, water demand, P losses), downstream processing and heavy metals in PR-derived fertilizers (IRP, 2019).

While a PR shortage in the coming years is debatable (Cordell and White, 2011; Scholz and Wellmer, 2013; Van Kauwenbergh, 2010), it is clear that P is an important feedstock. Consequently, a sustainable P management is crucial when aiming for a circular economy, achieving P security and environmental sustainability. This includes developing strategies to increase P recycling, improving efficiency in P use, and reducing P runoffs.

The investigation of alternative, secondary sources such as P-rich waste streams (e.g., wastewater) is an important starting point to recover P. It is estimated that by recovering P from wastewater 15% of the current European P imports can be met (European Commission, 2023).

1.2 Phosphorus in municipal wastewater treatment

The main sources of P in wastewater are human excreta (urine and feces), industrial and commercial discharges, and detergents (Kroiss et al., 2011). P in wastewater is primarily present as ortho-phosphate (ortho-P) and, in lower levels, present as dissolved or non-dissolved organic phosphates.

Around 1.8 g P per population equivalents (PE) is discharged daily as raw sewage (Imhoff et al., 2009). If these P loads directly enter water bodies, they can lead to eutrophication – a common cause of aquatic pollution. Eutrophication caused by the overload of nutrients is characterized by excessive proliferation of algae (algae blooms), biomass decay, depletion of oxygen and the development of “dead zones”, consequently decreasing water quality and causing environmental damage. To prevent eutrophication of the effluent-receiving water bodies and to protect the status of aquatic ecosystems, wastewater treatment plants (WWTPs) are required to remove P from the wastewater. In Europe the compliance with stringent effluent discharge limits is anchored in the European Water Framework Directive 2000/60/EG (European Commission, 2000).

Municipal wastewater typically contains around 9 mg/L of P (Imhoff et al., 2009). Depending on legal regulations, WWTPs must reduce the P levels in the effluent to 0.2–2 mg/L P. For example, the EU Urban Wastewater Treatment Directive 91/271/EEC (Council of the European Union, 1991) and the German Waste Water Ordinance (AbwV, 2020) set discharge limits for WWTPs with 10,000–100,000 PE and WWTPs >100,000 PE at 2 mg/L and 1 mg/L of total P, respectively. However, more stringent discharge limits may be imposed by regional standards (e.g., HMUKLV (2021) in Hesse, Germany) or for sensitive areas prone to eutrophication such as the Baltic Sea (Baltic Sea Action Plan, (HELCOM, 2021)).

Following these regulatory frameworks, the municipal wastewater treatment process is designed to remove nutrients, particularly P and nitrogen (N) through multiple treatment stages. The process, shown in Figure 1-2, typically consists of a primary, secondary, and sludge treatment stage. First, the main solids in the influent wastewater are removed by a screen. In the primary treatment stage, particulate matter is removed along with the primary sludge, removing approximately 11% of the total P (Cornel and Schaum, 2009). In the secondary treatment stage, N and carbon removal take place by denitrification and nitrification. P removal can be achieved by enhanced biological phosphorus removal (EBPR), chemical phosphorus removal (CPR), or a combination of both (Lopez-Vazquez et al., 2023). Thereby, approximately 78% of the P is transferred from the aqueous phase to the sludge phase, either by microbial P uptake or by chemical precipitation with metal salts such as iron or aluminium (Cornel and Schaum, 2009). While CPR is very effective it has drawbacks of high chemical costs, an increase in sludge production, and poses risks for handling and the environment (Wang et al., 2005). In contrast a biological P removal through microbial processes generally offers a more environmentally friendly and sustainable approach to P removal (Izadi et al., 2020). Afterwards, the aqueous phase is separated from the solids using a clarifier. The wastewater treatment is completed by the discharge of the surplus sludge. The aqueous phase can then be discharged as the effluent. Optionally, the effluent can undergo an advanced treatment for further purification and reduction of micropollutants such as microplastics, pathogens, pharmaceuticals. Available techniques include adsorptive methods (activated carbon), oxidative methods (UV irradiation, ozonation, chlorination) and physical methods (sand or membrane filtration). If sludge treatment is available, the primary sludge and surplus sludge are stabilized by anaerobic digestion and further processed as digested sludge. Depending on national legislations, the digested sludge can be incinerated, landfilled, or directly used in agriculture.

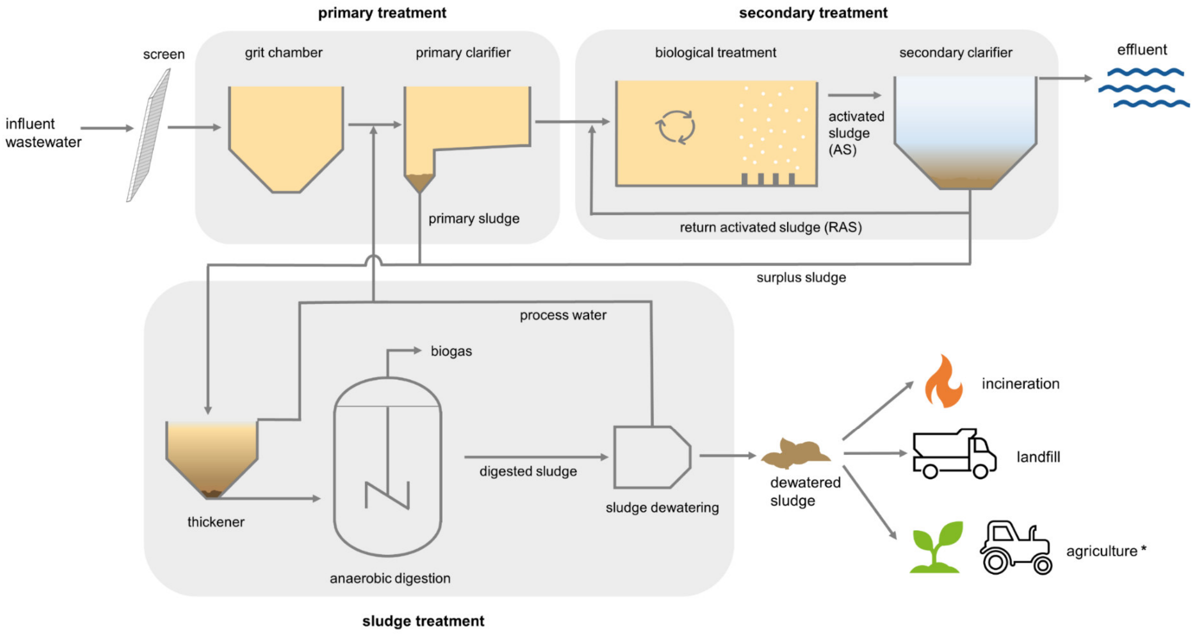

Figure 1-2| Schematic flow diagram of a municipal WWTP illustrating the different stages of water and sludge treatment. Adapted from Egle et al., (2016) and Prot, (2021).
*Due to the amendment of the German Sewage Sludge Ordinance a soil-related sewage sludge application will only be possible to a limited extent in Germany as of 2029.

Annually, 7–8 million tons of sewage sludge are produced in the EU (European Commission, 2023). Traditionally, the most straightforward option for reusing P and reintroducing it into the nutrient cycle has been the direct soil-related application of sewage sludge as a fertilizer in agriculture. Currently, approximately 40% of the European sludge is used in this manner (European Commission, 2023). However, the agricultural use of sewage sludge is subject to strict regulations, as the sludge can contain pathogens, pharmaceuticals, heavy metals, microplastics, or persistent organic trace pollutants (Ott and Rechberger, 2012; van den Berg et al., 2020). Consequently, the ongoing concern about the transfer of contaminants to crops has led to a ban of the soil-related application in several European countries, including the Netherlands and Switzerland, and prompted future bans in Germany (AbfKlärV, 2020; Bauer et al., 2020; VVEA, 2016). In addition, regional disparities between sludge availability (i.e., WWTPs) and agricultural areas with a nutrient demand (potentially seasonal) remain a major logistical challenge. This includes the transport or storage of sewage sludge as well as the associated costs and emissions (Jupp et al., 2021). Other disposal options, such as landfilling and incineration, often result in a loss of P unless the residues (i.e., incineration ash) are put to further use.

P in sewage sludge exists in several forms, often present as a mixture (Table 1-1). The three main species are polyphosphate (polyP), organic P and inorganic P present with 30–80%, 10–30% and 10–40% of total P, respectively for surplus sludge (Yu et al., 2021). The distribution of each form mainly depends on operating parameters, conditions during wastewater treatment and can differ with the treatment stage (Yu et al., 2021). The inorganic fraction is in parts derived from the influent wastewater (mainly Mg-P, Ca-P compounds) and from the CPR with Fe or Al salts as chemical precipitated P (e.g. Fe-P, Al-P compounds) (Yu et al., 2021; Wilfert et al., 2015). The exact mechanism by which P is removed during iron-based CPR is still not fully understood, but it is assumed that a mixture of iron-phosphate minerals and iron(hydr)oxide adsorped P is formed (Yu et al., 2021). Notably, as sludge treatment progresses Fe-P species may undergo changes by microbial and chemical processes (Prot et al., 2020; Wilfert et al., 2016) that may lower the solubility (Li et al., 2020). The organic and polyP fraction result from the biological treatment process, as typical microbial cell compounds and as polyP, a storage polymer of polyphosphate accumulating organisms (PAOs) involved in EBPR. In EBPR plants and WWTPs that use a combination of CPR and EBPR the biologically removed P fraction has been reported to amount to 50–70% of total P (Yu et al., 2021).

Table 1-1| P species in AS solids, including example componendts and their origin. The fraction of the individual species and types present can vary depending on the operational stage and process conditions of the WWTP.

P species	Component	Origin
inorganic	Fe-P, Al-P Ca-P, Mg-P adsorbed P	CPR and raw wastewater
organic	ATP, ADP, AMP DNA, RNA phospholipids	raw wastewater and microbial metabolism
polyP		PAO biomass (intracellular storage polymer)

1.3 Enhanced biological phosphorus removal – EBPR

The EBPR phenomenon was concurrently observed by Srinath et al. (1959) at a full-scale WWTP in India, and by Levin and Shapiro (1965), who found that sludge organisms took up excess P during aeration; a process they referred to as “luxury uptake”. In the following years more studies (Fuhs and Chen, 1975; Levin et al., 1972; Milbury et al., 1971) aimed to elucidate the mechanisms behind the biological P removal. This led to Barnard (1976) formulating the technical premise for EBPR: The sludge needed to pass through repeated anaerobic and aerobic treatment stages (Figure 1-3A). Since then, the EBPR process has become a widely adopted method in many full-scale WWTPs and is often used along CPR.

1.3.1 The classical PAO metabolism

Key for the EBPR process is a group of specialized activated sludge (AS) microorganisms known as PAOs. The behavior of these microorganisms is still being controversially discussed, but the classical PAO metabolism described below is generally most recognized in literature (Di Capua et al., 2022).

One of the most studied PAOs are members of the genus *Candidatus* Accumulibacter, which are gram negative, rod shaped bacteria, belonging to the class of *β-Proteobacteria* (Crocetti et al., 2000; Hesselmann et al., 1999). Henceforth, the genus *Candidatus* Accumulibacter and unspecified members thereof will be called *Accumulibacter.* Although *Accumulibacter* has yet been isolated as pure culture, its microbiological and biochemical physiology has been intensively studied through enrichment cultures. Here it often serves as a model organism to study the EBPR process as well as the involved intracellular storage polymers polyP, polyhydroxyalkanoates (PHA) and glycogen (Seviour et al., 2003).

Under anaerobic conditions, PAOs can take up volatile fatty acids (VFAs) as a carbon source from the aqueous phase, subsequently storing these intracellularly as PHA (Figure 1-3B). The type and composition of PHA formed can vary (Lemos et al., 1998). For instance, acetate predominantly leads to the production of poly-β-hydroxybutyrate (PHB), while propionate leads to poly-β-hydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV) (Lemos et al., 1998; Oehmen et al., 2005a; Satoh et al., 1992). The process of VFA uptake, the active transport across the cell membrane and the conversion into PHA is driven by the hydrolysis of intracellular polyP reserves (Mino et al., 1987). ATP, generated from hydrolysis of polyP, is used for the coenzyme A activation of VFAs. The conversion of ATP to ADP results in the release of ortho-P. Ortho-P is then transported out of the cell, likely via the low-affinity pit transporter, which has been found to be present in PAOs (Martín et al., 2006; Oyserman et al., 2016). This process generates a proton-motive force (pmf) that facilitates the uptake of VFAs (Burow et al., 2008; van Veen et al., 1994).

In addition, reducing equivalents are required for the formation and elongation of the PHA polymer from the coenzyme A-activated VFA building blocks (Comeau et al., 1986; Mino et al., 1998; Saunders et al., 2007). The source of reducing equivalents such as NADH+H$^+$ is still under debate. Comeau et al. (1986) and Wentzel et al. (1986) postulated that the tricarboxylic acid (TCA) cycle is involved (Comeau/Wentzel model), while other researchers suggested that reducing equivalents originate from glycolysis (Mino model) via the Embden-Meyerhof-Parnas or Entner-Doudoroff pathway (Martín et al., 2006; Mino et al., 1987). Evidence from EBPR systems suggests that both models may be valid, although the TCA cycle's contribution is likely lower (Guedes da Silva et al., 2020; Majed et al., 2012; Pijuan et al., 2008). To date, glycogen degradation via glycolysis appears to be the main pathway for generating NADH+H$^+$, especially when polyP reserves are limited (Acevedo et al., 2012; Satoh et al., 1996).

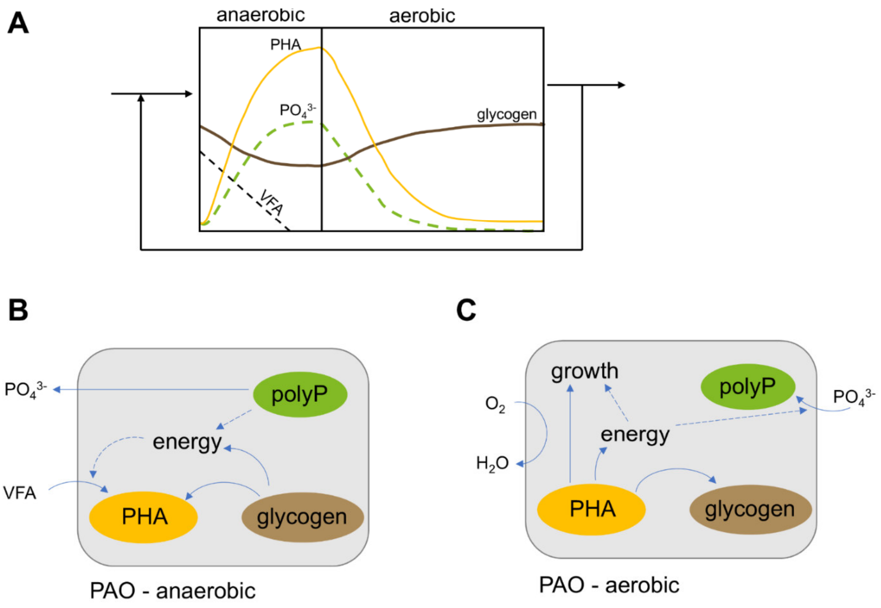

Figure 1-3| (A) Schematic representation of changes in the ortho-P and VFA concentration in the aqueous phase (dashed line) as well as intracellular PHA and glycogen concentration (solid lines) over the course of the EBPR cycle, modified from Guedes da Silva et al. (2020). (B, C) Simplified biochemical model for *Accumulibacter* under anaerobic and aerobic conditions, modified from Lopez-Vazquez et al. (2023).

Under aerobic conditions, PAOs use the previously stored PHA as a carbon and energy source for growth (biomass production) and to replenish their polyP and glycogen pools (Figure 1-3C). Ortho-P is taken up from the aqueous phase using the energy derived from PHA degradation. Part of the P is used for metabolic cell functions and cell synthesis, while excess P is used for polyP synthesis. PolyP is intracellularly stored in granules and stabilized by cations (Mg^{2+}, Ca^{2+}, K^+) (Barnard, 1976; Buchan, 1983, 1981; Levin and Shapiro, 1965; Seviour et al., 2003). As a result, under aerobic conditions, ortho-P levels in the aqueous phase decrease, whereas biomass polyP concentrations increase. Thereby the polyP concentration can reach up to 15%

of cell dry weight (Seviour et al., 2003). The amount of P that is removed from the aqueous phase is determined by the amount of intracellularly available PHA that was formed in the preceding anaerobic phase. By continuous alteration between aerobic and anaerobic phases PAOs gain a competitive advantage over ordinary heterotrophs. Unlike PAOs, ordinary heterotrophs are unable to utilize VFAs during the anaerobic phase (absence of external electron acceptors (O_2/NO_3^-)) (Lopez-Vazquez et al., 2023). This gives PAOs an advantage in the subsequent aerobic phase, as they can use their PHA pool as an energy source for cell maintenance and biomass growth rather than competing for substrates in the wastewater influent (Lopez-Vazquez et al., 2023). This leads to an enrichment of PAOs in the AS biomass. Thereby aerobic P uptake exceeds the P release resulting in a net P removal from the wastewater (Di Capua et al., 2022). The removal of AS biomass from the end of the aerobic treatment, where the internally stored polyP concentration is the highest, results in the removal of P from the WWTP with the surplus sludge.

1.3.2 Microbiology of EBPR

Due to its ecological and economic significance, EBPR is one of the most extensively researched microbiological processes. Nonetheless, the metabolic and microbial background has not yet been fully elucidated. Over the last years significant advances have been made to identify the microorganisms involved in EBPR (Fernando et al., 2019; He et al., 2010; Izadi et al., 2021; Oehmen et al., 2005b; Petriglieri et al., 2022; Singleton et al., 2022).

Initially, forty years ago, cultivation-based techniques pointed to *Acinetobacter* (a *γ-Proteobacteria*) as a putative PAO, since isolated organisms accumulated high amounts of polyP (Buchan, 1983; Fuhs and Chen, 1975). However, later studies revealed that while *Acinetobacter* may be abundant in WWTPs, they are not dominant in EBPR systems, and isolated organisms lacked the characteristic anaerobic substrate uptake linked with P release; a trait that is now recognized for PAOs in EBPR systems (Seviour et al., 2003; Tandoi et al., 1998). Subsequently, cultivation-independent methods proved essential for identifying the key microorganisms involved in EBPR. Application of modern molecular methods has led to the identification of several new genera and species as putative PAOs (Figure 1-4). Among these, *Accumulibacter* and *Tetrasphaera* spp. are considered to be particularly relevant, due to their widespread presence and abundance in full-scale WWTPs worldwide, as well as in laboratory-scale sequencing batch reactors (SBRs) designed for EBPR (Dueholm et al., 2022; Fernando et al., 2019; Oehmen et al., 2005b; Stokholm-Bjerregaard et al., 2017). Other identified PAOs include *Candidatus* Dechloromonas spp., *Candidatus* Phosphoribacter spp., *Knoellia* spp., and *Phycicoccus* spp. (Figure 1-4) (Dueholm et al., 2022; Kong et al., 2007; Petriglieri et al., 2021; Singleton et al., 2022).

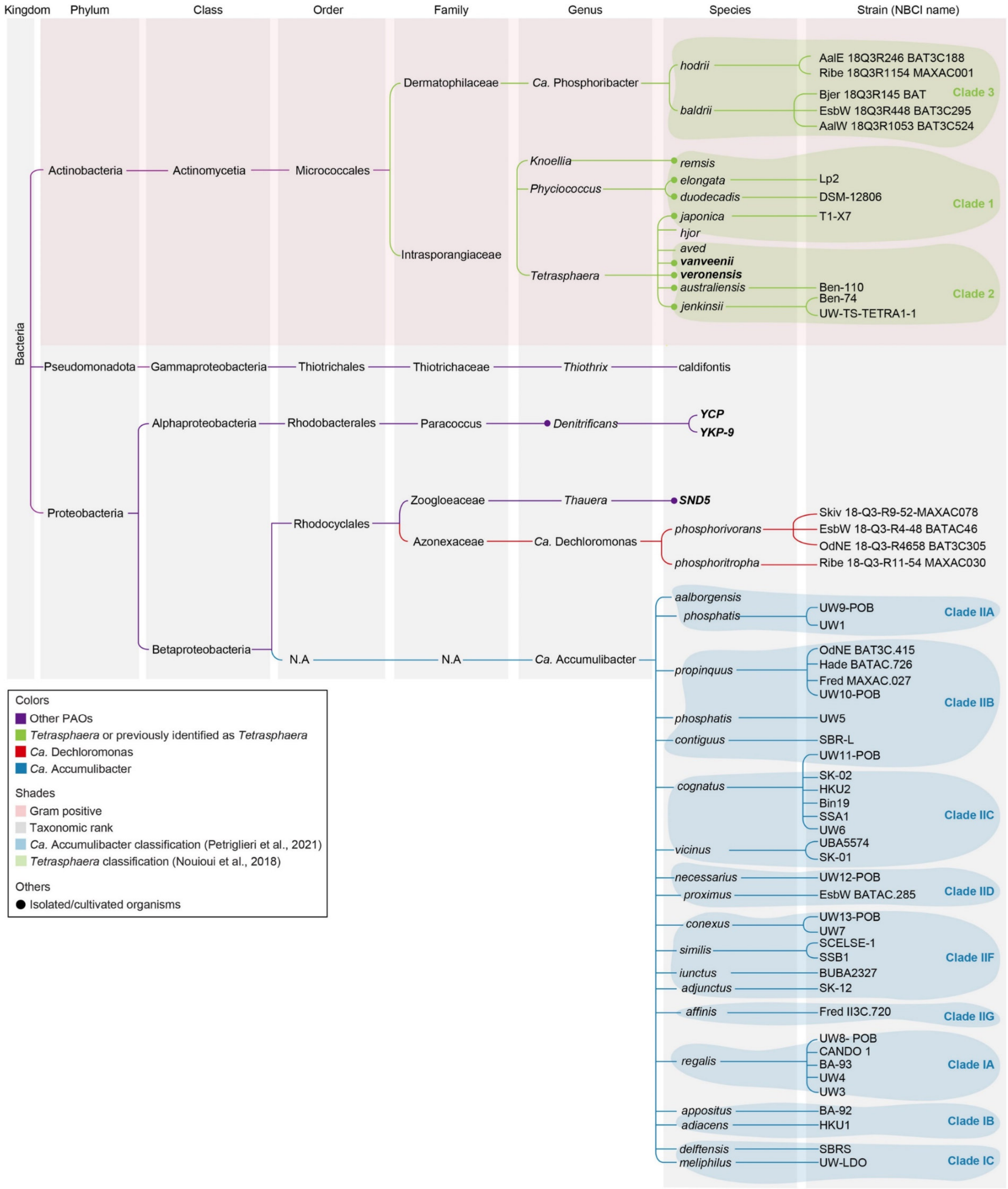

Figure 1-4| Visualization of the taxonomic classification of putative PAOs, taken from Ruiz-Haddad et al. (2024).

Candidatus Accumulibacter phosphatis was first identified through 16S rRNA sequencing (Hesselmann et al., 1999). The genome of the strain clade IIA str.-UW1 was fully sequenced by Martín et al. (2006) using shot-gun sequencing and confirmed the key features observed in experimental studies with the *Accumulibacter* genome. Although *Accumulibacter* has not yet been isolated as a pure culture, it can be enriched to over 90% in laboratory reactors (Welles et al., 2015). Consequently, many studies are using laboratory-scale enrichment cultures to investigate new lineage members, the proposed metabolism, and to link the physiology to genes.

While the 16S rRNA gene is a commonly used phylogenetic marker, its high conservation within *Accumulibacter* does not provide sufficient resolution to effectively distinguish between *Accumulibacter* species (He et al., 2007). Instead, the ppk1 gene has been identified as a more suitable marker to assess *Accumulibacter* phylogeny (Petriglieri et al., 2022) and allowed to delineate the genus into type I and II (McMahon et al., 2002) with multiple subclades (He et al., 2007; McMahon et al., 2007; Petriglieri et al., 2022) (Figure 1-4). As a single copy gene, ppk1 encodes for the polyphosphate kinase 1, which is crucial for polyP formation (McMahon et al., 2002).

Beyond *Accumulibacter*, the genus *Tetrasphaera*, has been proposed as significant in some WWTPs, but is less studied (Fernando et al., 2019). The physiology of these members deviates from the conventional concept of the *Accumulibacter*-PAO metabolism (Kristiansen et al., 2013; Nguyen et al., 2011). *Tetrasphaera* spp. has been shown to be able to accumulate polyP which is consistent with *Accumulibacter* physiology but without the formation of PHA (Kong et al., 2008; Nguyen et al., 2011). A recent genome-based phylogenetic study resulted in the reclassification of the *Tetrasphaera* lineage[3] (Nouioui et al., 2018; Singleton et al., 2022) (Figure 1-4).

This reclassification illustrates that the phylogenetic identity of putative PAOs is not yet finalized and emphasizes the ongoing efforts to not only refine phylogenetic identity but also identify new members through a combination of genome, ppk1 and 16S rRNA gene-based approaches, as well as by studying their physiological characteristics in *in situ* experiments.

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[3] The former *Tetrasphaera* species *T. elongata*, *T. duodecadis* and *T. remsis* were reassigned to the genera *Phycicoccus* (*P. elongatus, P. duodecadis)* and *Knoellia* (*K. remsis*) (Nouioui et al., 2018). The novel genus *Candidatus* Phosphoribacter was assigned (Singleton et al., 2022).

1.3.3 Metabolic diversity of PAOs

As previously mentioned, *Accumulibacter* has been intensively studied via laboratory-scale enrichment cultures using *in situ* studies and meta'omic data to verify the proposed physiology (Martín et al., 2006; Oyserman et al., 2016; Skennerton et al., 2015). In those settings the overall metabolic features are relatively well described.

In particular, the carbon and P pathway genes required for the classical PAO phenotype appear to be conserved among the *Accumulibacter* clades, and the core genome is specialized for the anaerobic utilization of VFAs (Skennerton et al., 2015). However, the specific pathways utilized remain controversial (Guedes da Silva et al., 2020). Recent studies suggest that the biochemical pathways involved in the P cycling are more diverse allowing clade members to occupy different ecological niches (Acevedo et al., 2012; Flowers et al., 2013; Majed et al., 2012; Welles et al., 2015). For instance, nitrate and nitrite utilization as electron acceptors for P uptake have been reported for some clades (Carvalho et al., 2007; Flowers et al., 2009). In response to environmental changes a flexibility in metabolic function, that adapts the P-storage metabolism (transcriptional response) has been observed (Guedes da Silva et al., 2020; Welles et al., 2015). This may explain part of the controversy in literature on *Accumulibacter's* metabolic functions when *in situ* studies are performed under different experimental conditions. For example, under anaerobic intracellular P limitation *Accumulibacter* has shown the capability to adopt the metabolism typical for of glycogen accumulating organisms (GAOs) and not release P (Acevedo et al., 2012).

For P release *Accumulibacter* exhibits a diverse carbon source utilization. Besides VFAs, amino acids, glucose, and fermentation products have been shown to be utilized by some *Accumulibacter* members (Chen et al., 2023; Kong et al., 2004; Qiu et al., 2020; Ziliani et al., 2023). Genetic data also suggests the utilization of ethanol (Skennerton et al., 2015). However, it has not yet been confirmed *in situ* studies.

The anaerobic utilization of substrates in *Tetrasphaera* spp. appears to be more versatile. Both *in situ* and pure culture studies of *Tetrasphaera* spp. demonstrated assimilation of a range of substrates, including acetate, propionate, sugars and amino acids (Kong et al., 2008; Kristiansen et al., 2013). For example, Kristiansen et al. (2013) found that the genome of *Tetrasphaera* spp. contains genes for a glucose- and glutamate/aspartate-transporter, which is consistent with the *in situ* observations of glucose and amino acid utilization by Nguyen et al. (2011) and Kong et al. (2008). Genomic analysis also suggests the capability for glucose fermentation and glycogen production under anaerobic conditions (Kristiansen et al., 2013). However, the formation of glycogen in pure cultures of *T. elongata* and in *Tetrasphaera* spp. in full-scale WWTPs has not yet been observed (Fernando et al., 2019). Alongside capabilities for denitrification and fermentative growth, intracellular accumulation of fermentation by-products and amino acids has been reported (Kristiansen et al., 2013; Nguyen et al., 2015).

1.3.4 Factors affecting EBPR performance

The differences and variability in metabolic properties likely provide PAO groups with distinct ecological niches within the EBPR consortium, thereby contributing to the overall stability of the EBPR process. In full-scale WWTPs, the performance of the EBPR system depends on various factors, including microbial competition, dissolved oxygen, pH (Filipe et al., 2001a,b; Oehmen et al., 2005b), temperature (Lopez-Vazquez et al., 2009, 2008, 2007; Whang and Park, 2006), hydraulic/solids retention time (Whang and Park, 2006), and influent composition (P/C ratio, carbon source availability (Carvalheira et al., 2014; Oehmen et al., 2005a,c; Pijuan et al., 2004)), highlighting the complexity of EBPR and P cycling in full-scale WWTPs. Factors influencing EBPR structure and PAO activity have been reviewed in Oehmen et al. (2007). A selection of factors considered relevant for this thesis is briefly described below.

(i) Microbial competition

In full-scale WWTPs, the distribution and abundance of PAOs in the AS has been reported to range from 0.02% to 22% relative to the total AS population, which is much lower than in PAO enriched laboratory-scale reactors (Dueholm et al., 2022; Kong et al., 2004; Mao et al., 2015; Nierychlo et al., 2020). Most of the respective values are provided for EBPR plants located in Denmark and the USA (Dueholm et al., 2022), but there is a lack of data for other countries.

A critical factor that can affect the EBPR performance is the competition between PAOs and GAOs. Co-existing GAOs compete with PAOs for VFAs but do not store P and thus do not contribute to the P removal (Cech and Hartman, 1993; Saunders et al., 2003). Anaerobically GAOs utilize VFAs for PHA formation. The required energy and reducing equivalents are provided by the breakdown of the intracellular glycogen pool via glycolysis (Burow et al., 2007; Filipe et al., 2001a). *Candidatus* Competibacter phosphatis is a commonly GAO found both in laboratory-scale and in full-scale systems (Burow et al., 2007; Crocetti R. et al., 2002; Kong et al., 2006; Saunders et al., 2003; Stokholm-Bjerregaard et al., 2017). A moderate presence of GAOs may indicate sufficient presence of suitable organics, which can be a sign for an efficient EBPR system where VFAs are not limiting for PAOs (Nielsen et al., 2019). Additionally, it has been reported that some GAO may utilize by-products of fermentative PAOs rather than competing with classical PAOs for substrate (Stokholm-Bjerregaard et al., 2017). Therefore, GAO abundance alone can not always be a decisive factor for EBPR efficiency. Importantly, however, an excessive proliferation of GAOs can lead to out-competition of PAOs, deterioration of the P removal performance and failure of EBPR systems (Satoh et al., 1994; Saunders et al., 2003). Therefore, emphasis is often placed on designing the operating parameters of EBPR in favor of PAO proliferation.

(ii) pH and temperature

A pH range of 6.8–8.0 and temperatures of 10–20 °C have been shown to favor PAO growth (Lopez-Vazquez et al., 2009; Oehmen et al., 2005b). Lower pH values inhibit the acetate metabolism and growth of PAOs (Filipe et al., 2001b). Higher temperatures of 25–30 °C can lead to an unstable EBPR and favor GAO growth (Lopez-Vazquez et al., 2009). However, with sufficient carbon availability the system can be adapted to function well in tropical climates (Qui et al., 2019).

(iii) Solids and hydraulic retention time (SRT, HRT)

For the duration of the anaerobic and aerobic cycles SRTs of 7–20 d have been reported to promote PAOs in a practical EBPR operation (Diaz et al., 2022). SRTs below 5 d allow ordinary heterotrophs and filamentous bacteria to be more prevalent and may washout PAOs. Generally, low HRTs are recommended for the anaerobic and aerobic phases of EBPR (Diaz et al., 2022).

(iv) Influent composition

Availability of suitable carbon sources and the P/C ratio of the influent wastewater are critical for EBPR by shaping the microbial community and affecting PAO activity (Carvalheira et al., 2014). VFAs are considered to be most effective in enriching the PAO population, and stabilizing EBPR for high P removal (Oehmen et al., 2005c; Pijuan et al., 2004).

1.4 Routes and technologies for P recovery

At present, P is lost along the value chain, from mining to food to wastewater and the disposal of solid waste. Only 20% of the total mined P intended for fertilizer production is consumed (Chen and Graedel, 2016; Cordell et al., 2009). The greatest loss is associated with run-offs during crop cultivation (Cordell et al., 2009). It is estimated that less than half of the P present in waste is recycled back to the global food system (Chen and Graedel, 2016). To facilitate a circular economy, a sustainable P cycling is essential.

Policies and legislations are critical drivers for the development of P recovery technologies and the use of recovery products, which are still scarce to date. In Germany, the recovery of P from sewage sludge will become mandatory for many WWTPs due to the amendment of the German Sewage Sludge Ordinance (AbfKlärV, 2017a). WWTPs with more than 50,000 PE and 100,000 PE are required to recover P from sludge starting in 2032 and 2029, respectively. The target for recovery is to reduce the P content in the residual sludge to less than 20 g/kg_{TSS} or to achieve a recovery rate of 50%. If the sludge is mono-incinerated, at least 80% P must be recovered from the sewage sludge ash (SSA). A soil-related utilization of the sludge in agriculture is no longer allowed for these plants. WWTPs whose sludge contains less than 20 g/kg_{TSS} P and WWTPs with a size of ≤ 50,000 PE are excluded from this regulation[4] (AbfKläV, 2017a,b). In the case of Germany, 58% of the national WWTPs have a capacity greater than 50,000 PE and an average sludge P concentration of 34 g/kg_{TSS} (Destatis, 2018; Sichler et al., 2022). Thus, a large number of WWTPs are affected by this amendment.

Over the past years, such changes in legislation and a paradigm shift from “resource use” to “resource recovery” have led to intensive research on P recovery from wastewater. During wastewater treatment, P is present both in liquid (e.g., anaerobic digestion liquor, process waters) and solid streams (e.g., primary sludge, activated sludge, digested sludge, or SSA). Recovery methods can thus be classified according to the stream that is targeted:

(i) Sewage sludge ash
(ii) Aqueous phase
(iii) Sewage sludge

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[4] If the sewage sludge contains less than 20 g/kg_{TSS} P, no P recovery is required. WWTPs with 50,000 PE or less do not need to recover P and sewage sludge is permitted for soil-related utilization.

Recovery from the aqueous phase and sewage sludge will be usually integrated on-site at the WWTP, whereas recovery from the SSA is considered a downstream treatment off-site. Currently, there are over 30 different technologies for recovering P (Kabbe, 2023; Kabbe and Rinck-Pfeiffer, 2019). An overview of currently available approaches and technologies is given in Figure 1-5. The developed processes vary in terms of recovery efficiency, recovery product, and developmental stage. A comparison of exemplary P recovery technologies, their recovery steps and final recovery products is given in Table 1-2.

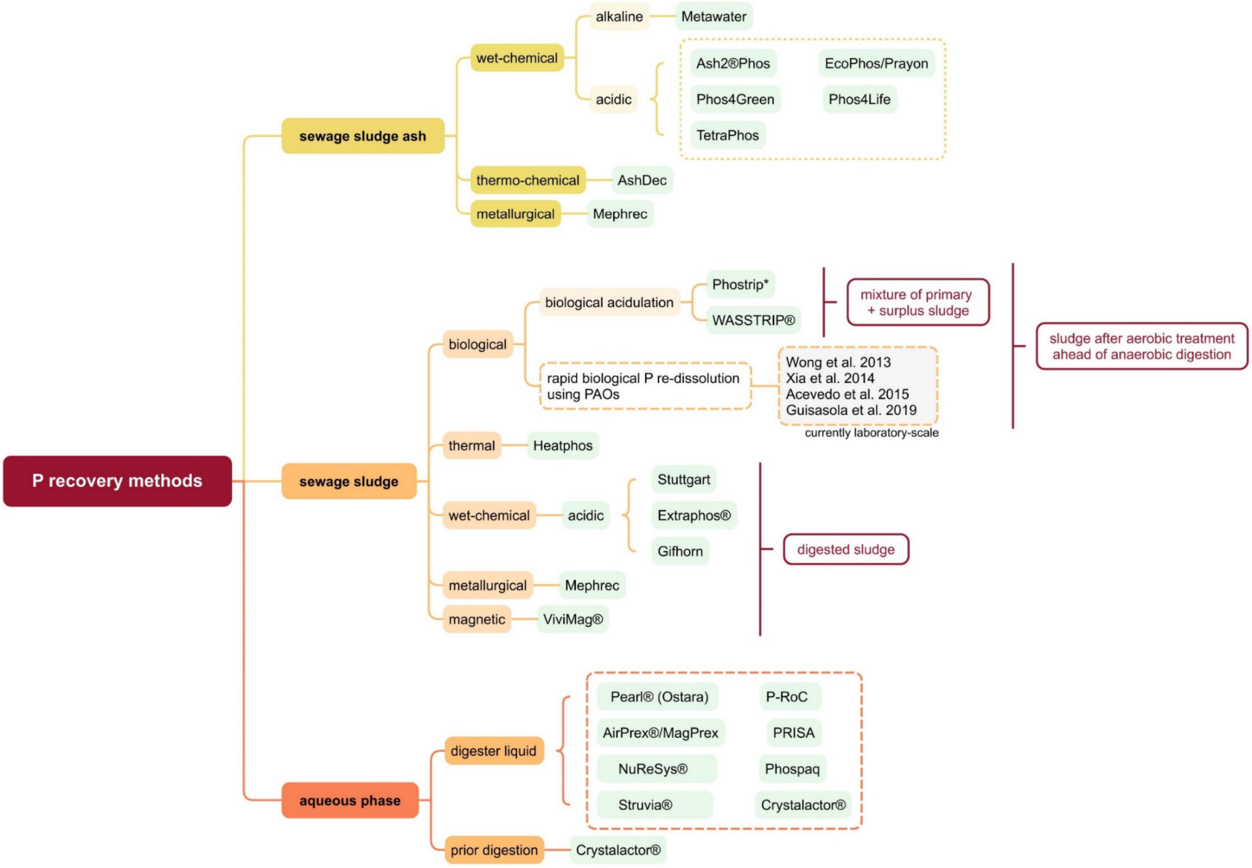

Figure 1-5| Classification of various approaches and exemplary technologies developed for P recovery from the aqueous phase, sewage sludge, or sewage sludge ash.

In the following, the state of art for P recovery from the target streams (i) SSA, (ii) aqueous phase and (iii) sewage sludge is briefly described.

(i) Sewage sludge ash (SSA)

P recovery from SSA is possible if the sewage sludge is mono-incinerated. A prerequisite for the centralized recovery from SSA are an adequate sludge transport infrastructure as well as the availability of mono-incinerators (Kabbe and Rinck-Pfeiffer, 2019).

The P content of municipal SSA is approximately 9 wt% but can reach up to 13 wt% (Krüger et al., 2014). This is comparable to low-grade PR (Liu et al., 2021). Beneficial of mono-incineration is the volume reduction of up to 90% which concentrates P. High temperatures are favorable for destruction of organic pathogens, pharmaceuticals, and bacteria. Nevertheless, heavy metals may become concentrated as well, which have raised concerns about the direct land application of SSA (Kabbe and Rinck-Pfeiffer, 2019). Therefore, recovery processes typically involve a wet- or thermo-chemical re-dissolution of P. The former is based on an acidic or alkaline treatment, while in the latter SSA is treated with chemicals at elevated temperatures. Various options have been developed, ranging from pilot-scale to full-scale implementations (Table 1-2, Figure 1-5). The most frequently investigated approach is the wet-chemical route with mineral acids, as it allows for a high cost efficiency and high potential for full-scale application (Liu et al., 2021). Here, P recovery efficiencies are high with 70–100%. Common recovery products include calcium phosphates, ammonium phosphates, and phosphoric acid (Kabbe and Rinck-Pfeiffer, 2019). As a by-product, iron or aluminum salts may be recovered. Examples include the TetraPhos® and Ash2®Phos technology, which have advanced to the point that their P recovery products are market available as RePacid® technical grade phosphoric acid (Ruscheweyh et al., 2021) and RevoCAP feed calcium phosphate for livestock farming (EasyMining Sweden AB, 2022; Presto Åkerfeldt et al., 2023), respectively. Nevertheless, acidic leachates often require additional downstream processing such as ion exchange, pH adjustment or sulfide precipitation for heavy metal removal and purification. This can be complex and cost-intensive (Liu et al., 2021). Moreover, neutralization or disposal of waste acids may be required.

(ii) Aqueous phase

Recovery from the aqueous phase targets streams such as the process water from the thickener, the sludge liquor within the digester, or the process water from the dewatering unit after the digestion. Most commonly the anaerobic digester liquor and the process water of the digestate dewatering are used (Kabbe and Rinck-Pfeiffer, 2019). To this end, various technologies directly precipitate soluble P as struvite (magnesium ammonium phosphate (MAP); $MgNH_4PO_4{\cdot}6H_2O$) or as calcium phosphates (e.g., hydroxyapatite (HAP); $Ca_5(PO_4)_3OH$) (Figure 1-5).

Naturally, struvite precipitates form under the conditions found in the anaerobic digester. During anaerobic digestion, P and ammonium are released from the organic matter by polyP hydrolysis, cell lysis, and degradation of proteins and nitrogen compounds, respectively. This can create a P- and ammonium-rich liquor. In the presence of magnesium and pH >8 struvite crystals form (Martí et al., 2010). Since anaerobic digestion processes show an increase in pH, uncontrolled struvite scaling frequently causes pipe blockages in anaerobic digesters and downstream equipment, especially in EBPR-plants (Marti et al., 2008). A controlled struvite precipitation and recovery from the digester liquid in a designated reactor often by addition of commercial Mg salts and NaOH aims to reduce the issue. This can lower maintenance and operating costs, achieving a better sludge dewaterability, and a reduced P backflow to the WWTP influent (Egle et al., 2016). As a result, P recovery from the aqueous phase has been commercially implemented in some WWTPs in Europe, Japan, China, and North America, with systems like AirPrex®, Pearl®, NuReSys®, or Phospaq^{TM} (Table 1-2). Two struvite recovery products are marketed under Berliner Pflanze (AirPrex®) or CrystalGreen® (Pearl®) as a slow-release fertilizer and a source of P, N, and Mg (Li et al., 2023).

In general, the chemical precipitation processes can achieve a high P removal efficiency (up to 98%) once the P is in soluble form. Typical P concentrations of the aqueous phase deemed suitable are at least 50–100 mg P/L (Cornel and Schaum, 2009; Di Capua et al., 2022; Ghosh et al., 2019). In rarer cases, lower concentrations of 20 mg P/L may be technically feasible but may result in a lower P removal efficiency (Reinout Vreugdenhil, product manager Crystalactor®, Royal HaskoningDHV, personal communication, September 26, 2023). The requirement of high initial P concentration often limits these recovery options to WWTPs that rely on EBPR and have an anaerobic digestion unit available. The processes are characterized by a recovery efficiency of 10–40% relative to the total P load of the wastewater influent but may incur high operating costs (Cornel and Schaum, 2009; Egle et al., 2016; Ghosh et al., 2019).

(iii) Sewage sludge

Alternatively, P can be recovered from the sewage sludge by enhancing the re-dissolution of P through wet-chemical, thermo-chemical or biological methods to produce a P-rich stream. The subsequent processes to recover the re-dissolved P from the P-rich stream are mostly based on crystallization or precipitation similar to the recovery from the aqueous phase.

(iia) Wet-chemical approaches

Similar to SSA, P can be re-dissolved from the sludge by wet-chemical (acidic/alkaline) and thermo-chemical treatment. Current approaches predominantly rely on wet-chemical acid leaching from digested sludge (Stuttgart, Extraphos®, Gifhorn) for CPR-based WWTPs (Table 1-2). For example, at pilot-scale the Stuttgart process has achieved high recovery yields of up to 67% of total sludge P with a leaching pH < 3.5 (Meyer et al., 2018). It was noted that the efficiency of P re-dissolution is heavily dependent on the applied pH conditions and the sludge characteristics (Meyer et al. 2019). Due to the low pH, metal ions and heavy metals are concomitantly released with P and need to be removed at additional cost. In fact, citric acid, which is added to complex metal ions prior to P precipitation, accounts for up to 70% of the operating costs (Meyer et al., 2018). As of now the process has a high chemical consumption and waste acids may need further treatment for disposal.

The recovery potential of wet-chemical approaches is typically 40–60% P (Egle et al., 2016). However, the recovery yield must be evaluated against the chemical consumption and compared to wet-chemical approaches that target the SSA (Kabbe and Rinck-Pfeiffer, 2019). Since the treatment of sewage sludge has a lower P recovery but high chemical costs and a greater sludge-water volume, in this case, targeting the SSA via wet-chemical route may be favored over sewage sludge.

The economic feasibility of the Stuttgart and Extraphos® technology at full-scale is still uncertain, as these technologies have only been implemented at pilot-scale. The Gifhorn technology, has since been discontinued at the German WWTP Gifhorn due to insufficient benefits for the plant (Hans-Oskar Baron, WWTP manager, Abwasser- und Straßenreinigungsbetrieb Stadt Gifhorn ASG, personal communication, October 27, 2023).

(iiib) Physical approaches

Recently, the recovery of P as vivianite has been investigated. Wilfert et al. (2016) found that vivianite is increasingly present in anaerobically digested sludge from iron-based CPR-WWTPs. Later, Prot et al. (2020, 2019) demonstrated that vivianite can be recovered from digested sludge by magnetic separation (ViviMag® technology, Kemira). While the direct use of vivianite as a fertilizer may be limited to iron-deficient soils, it could be used as a raw material for industrial processes (Prot et al., 2019; Wilfert et al., 2018).

(iiic) Biological approaches for P recovery from activated sludge prior to anaerobic digestion

Due to the high levels of P in the sludge after the aerobic treatment, targeting the main treatment line ahead of the anaerobic digestion unit has recently been proposed as a promising option to recover high levels of biomass P while concomitantly preventing unwanted struvite scaling issues in the downstream equipment (Melia et al. 2017; Zhang et al., 2022).

Here, biological technologies to re-dissolve P from AS and subsequent recover P have gained interest as a more environmentally friendly alternative to cost- and resource-intensive chemical methods that create additional waste streams.

P accumulated as polyP can be re-dissolved from the sludge biomass according to the principles of EBPR (cf. chapter 1.3). In an active microbial process, PAOs enriched in the microbial community of AS release P from intracellular polyP into the aqueous phase by uptake of readily available carbon. It has been reported that in AS, polyP is the dominant P species, accounting for an average of 58% of total P, even with dual CPR usage (Yu et al., 2021). In laboratory settings without CPR, the polyP fraction can even reach up to 95% of total P (Zhang et al., 2013). This again highlights the significance of the polyP pool of AS as a target for recovery.

The Phostrip process is one of the few existing, but mostly discontinued, full-scale processes that is based on biological P re-dissolution by relying on prolonged anaerobic storage (up to

20 h) of the sludge for internal production of VFAs from the organic matter and release of P (Kaschka and Weyrer, 1999; Kim et al. 2000; Levin and Della Sala, 1987). However, such extended anaerobic storage can alter the AS structure and the biotic community, promote cell lysis and is likely to affect PAO activity (Chen et al., 2003; Wilen et al., 2000). A suitable redesign of this approach for a rapid P re-dissolution from the AS could enable a simple process design for on-site recovery at WWTPs. The majority of such previous works have been based on laboratory-scale SBRs with highly PAO enriched cultures to recover a P-rich stream during the anaerobic phase (Acevedo et al., 2015; Guisasola et al., 2019; Wong et al., 2013; Xia et al., 2014).

For example, Wong et al. (2013) proposed the use of a PAO enriched biofilm for P recovery involving repeated P uptake and release cycles. In a two-step process, the authors exposed the biofilm to synthetic wastewater for N removal and P uptake. Subsequent P was released by supplementation of an external carbon, which resulted in a cumulative P release four times higher than the influent. However, the supplemented carbon was in large parts not efficiently use for P release. By repeated cycling of the biofilm through both stages a P-rich recovery stream with 100 mg P/L was produced (Wong et al., 2013).

Acevedo et al. (2015) conducted a study with AS highly enriched in *Accumulibacter* (85%) in a laboratory-scale SBR-EBPR configuration. By supplementation of up to 350 mg/L acetate and repeated P uptake/release cycles a P-rich stream with 114–119 mg P/L was obtained. In a five-week trial the authors were able to recover up to 81% P of the synthetic influent, but long-term effects were not investigated. While a strong decrease of the polyP inside the *Accumulibacter* cells was observed, the ability of the organisms to take up P was not affected. However, Acevedo et al. (2015) noted a shift in the metabolic behavior of *Accumulibacter*, from polyP degradation towards glycogen degradation. It was concluded that this may have been due to extensive extraction of polyP.

In a similar approach, Xia et al. (2014) demonstrated that 79% of the influent P could be re-dissolved from AS that was highly enriched in PAOs from a laboratory-scale SBR-EBPR reactor. For P release the AS was supplemented with carbon at 10% of the influent COD amount. After filtration, P was recovered as $Mg_3(PO_4)_2$ by chemical precipitation. P re-dissolution cycles were performed once per day and treated sludge was cycled back to the EBPR reactor. The system was operated for a period of 24 days, but the effect of repeated re-dissolution cycles on the process efficiency is not known.

Interestingly, Guisasola et al. (2019) recently showed that a long-term operation of a laboratory-scale EBPR system for P release is possible by optimizing the influent C/P ratio and volume of the P-rich stream. In the study up to 60% of the influent P was released without adverse effects on the EBPR performance (Guisasola et al., 2019).

These studies highlight the potential for a controlled and fast P re-dissolution by targeting the P cycling capability of PAOs as an opportunity for enhancing resource recovery. However, there is limited understanding of how these laboratory observations translate to the complex and heterogeneous conditions in full-scale WWTPs. Therefore, more knowledge is needed to optimize this strategy for real-world application beyond the laboratory-scale.
Challenges and gaps that need to be addressed include:

(1) **Operational variability in full-scale WWTP systems**
Although EBPR is widely applied, the process typically does not utilize PAOs with optimal efficiency and is often combined with additional CPR to meet effluent limits. Chemical precipitant dosing and site-specific variables, such as influent composition, seasonal variations, and prevailing environmental conditions may affect sludge characteristics and P re-dissolution differently. A deeper understanding of how these variations affect the recovery process is needed. The integration of biological recovery with existing treatment plant operations, particularly those employing mixed chemical and biological P removal strategies, remains underexplored.

(2) **PAO abundance and metabolic variability**
Unlike enriched laboratory SBRs, PAOs are typically less abundant in full-scale WWTPs (Anders et al., 2021; Bertanza et al., 2020). The complex composition of AS with interactions of PAOs, competing organisms and other sludge constituents can make it challenging to predict the microbial behavior. Moreover, within the PAO group, metabolic heterogeneity and transcriptional responses to environmental conditions have been observed (Majed et al., 2012). These challenges highlight the need to study non-acclimated AS for the P recovery process.

(3) **Site-specific evaluation of an optimal substrate supplementation strategy and cost-benefit state**
Evaluating the optimal carbon source type and supplementation level for P re-dissolution is important to ensure cost-efficient resource use. The specific substrate requirements and cost-effectiveness of supplementation may need to be assessed for each WWTP. The optimal carbon source and supplementation level for the diverse sludge characteristics across WWTPs has not been comprehesively studied.

(4) **Pilot-scale validation for real-world implementation**
Pilot-scale trials are essential to bridge the gap between laboratory-scale studies and full-scale implementation. These trials should focus on up-scaling the process, assessing their economic and operational viability, including the development of value-added P recovery products.

Table 1-2| Examples of currently available P recovery technologies and their recovery products classified by their target stream. References: Driessen et al., 2018; EasyMining Sweden AB, 2022; Egle et al., 2016; ESPP European Sustainable Phosphorus Platform, 2023; Ghosh et al., 2019; Giesen, 1999; Gysin et al., 2018; Kabbe, 2023; Kabbe and Rinck-Pfeiffer, 2019; Lebek et al., 2018; Meyer et al., 2019, 2018; Ortwein, 2018; Paques, n.d.; Ruscheweyh et al., 2021; Schnee and Opitz, 2019; van Houwelingen and Piekema, 2018. For a more extensive overview of currently available technologies please see Kabbe, 2023 and Kabbe and Rinck-Pfeiffer, 2019.

Target stream	Route	Technology (provider)	Short description	Products	Developmental stage
SSA	wet-chemical	**TetraPhos®** (Remondis)	P is leached with H_3PO_4. Sulfide and H_2SO_4 are added for heavy metal depletion and Ca precipitation, respectively. Leachate is purified by ion exchange. Energy consumption is high.	75% H_3PO_4 (RePacid®), Fe/Al-salts, gypsum	pilot-scale; Hamburg (DE) full-scale; Hamburg, Köhlbrandhöft (DE)
		Ash2®Phos (EasyMining Sweden AB)	SSA is dissolved in HCl. P, Fe, Al are separated by precipitation (use of lime). Heavy metal removal is >96% and 90–95% of P is recovered.	Ca-P (RevoCAP feed phosphate), upcycling to mono-AP/DAP with HCl & ammonia possible, Fe/Al- salts	pilot-scale; Uppsala & Helsingborg (SE) full-scale; Helsingborg (SE) (permit-process), chemical park Schkopau (DE) (under construction)
		EcoPhos/Prayon (Prayon)	SSA is leached with H_3PO_4. Leachate is purified by ion exchange and treated for heavy metal depletion.	DCP, H_3PO_4	pilot-scale; Varna (BG)
		Phos4Green Glatt (Serplant GmbH)	SSA is dissolved in H_3PO_4, P is recovered by fluidized-bed spray granulation.	custom P or NPK compound fertilizers	full-scale; SERAPLANT, Haldensleben (DE) (closed, declared insolvency in summer 2022)
		Metawater (METAWATER Corp. Ltd.)	SSA is subjected to alkaline leaching of P with NaOH at 50–70 °C for 90 min. After filtration P is precipitated with $Ca(OH)_2$. Leaching of P is restricted to 30% to limit leaching of heavy metals. Treated residual ash is used as a construction material.	HAP (registered as a fertilizer in Japan; 2009)	full-scale; Gifu, Tottori (JP)
	thermo-chemical	**AshDec** (Metso Outotec)	SSA reacts with Na_2SO_4 at 850–900 °C. Product is treated for partial heavy metal removal.	$CaNaPO_4$	pilot-scale; Leoben (AUT)

mono-AP=mono-ammonium phosphate, DCP=di-calcium phosphate, NPK=nitrogen, phosphorus and potassium

Table 1-2 continued |

aqueous phase	**Pearl®** (Ostara Nutrient Recovery Technology Inc.)	P-rich liquid (dewatering of digested sludge) is treated in a fluidized-bed reactor and internally recirculated. NaOH and $MgCl_2$ are dosed for pH adjustment and as a source of Mg, respectively. P concentration of > 100 mg/L of incoming aqueous feed is recommended. P removal efficiency from liquid is >85%.	struvite (Crystal Green®)	full-scale; in > 20 WWTPs worldwide (USA, CDN, NL)
	AirPrex®/MagPrex^{TM} (CNP CYCLES GmbH)	Digested sludge is stripped of CO_2 by addition of air (pH increase). Mg-salts are added to form struvite crystals.	struvite (Berliner Pflanze®)	full-scale; in >15 WWTPs in DE, USA, NL; e.g., MG-Neuwerk, Niersverband (DE); Wassmannsdorf, Berliner Wasserbetriebe (DE)
	Crystalactor® (Royal HaskoningDHV)	P-rich liquid (>20 mg P/L) is fed into a fluidized-bed reactor and internally recirculated. Initially, a seed material (e.g., sand) is added to aid crystallization. Mg-salts or lime is dosed. Settled pellets are harvested. Sequential use of reactors is possible. Effluent filtration may be needed. High operational costs.	struvite or Ca-P	full-scale; Nanjing (CN), WWTP Geestmerambracht from 1994–2010 (NL)
	NuReSys® (Nutrients Recovery Systems)	Comprises two units (air stripper & crystallization reactor). Air is added to strip CO_2. NaOH and $MgCl_2$ are added to the stirred crystallization reactor for pH adjustment and as Mg source. Can be used for digested sludge liquid or post-dewatering liquor.	struvite (Biostru®)	full-scale; e.g., Nieuwkerke, Leuven AQUAFIN (BE); RWZI Apeldoorn (NL), Tres Rios Water Reclamation Facility (USA)
	Phospaq^{TM} (Paques Technology B.V.)	In a side stream liquor (dewatering of digested sludge) is fed into an airlift reactor (provides mixing and stripping of CO_2). Addition of MgO or $Mg(OH)_2$ leads to struvite formation. Produced struvite can be harvested from the bottom of the reactor.	struvite	full-scale in some WWTPs in NL, CN, UK, (often food processing wastewaters)

Ca-P=calcium phosphate

Table 1-2 continued |

sewage sludge	wet-chemical	**Stuttgart** (MSE GmbH)	Digested sludge is leached with H_2SO_4. After solid/liquid separation and ultrafiltration P is precipitated as struvite from the liquid by addition of MgO and NaOH (pH adjustment). Citric acid is used for the complexation of metal ions.	struvite	pilot-scale; Offenburg (DE)
		Extraphos® (Buddenheim KG)	Digested sludge is fed into a pressurized reactor (10 bar) and CO_2 is added (carbonic acid formation) to re-dissolve P. After solid/liquid separation lime is added to the liquid for precipitation of P. Precipitated P is recovered with filter press.	DCP	pilot-scale; Mainz-Mombach (DE), Itzehoe (DE)
		Gifhorn (Seaborne EPM AG)	Digested sludge is leached with H_2SO_4. Heavy metals are precipitated by dosing Na_2S at pH 5.6. pH is adjusted with NaOH. After solid/liquid separation $Mg(OH)_2$ and NaOH are added for precipitation at pH 9. Precipitated P is recovered by solid/liquid separation.	mix of struvite and HAP	full-scale; Gifhorn (DE) (shut-down)
	magnetic	**ViviMag®** (Kemira)	Vivianite that was formed during anaerobic digestion is recovered by magnetic separation. Applicable for digested sludge from WWTPs that use iron salts, if during anaerobic digestion Fe(III) is reduced to Fe(II) and vivianite is formed.	vivianite	pilot-scale; Nieuwveer (NL), Schönebeck (DE)

DCP=di-calcium phosphate, HAP=hydroxyapatite

Chapter 2

Scope of the thesis

2.1 Research project Re-BioP-Cycle

As previously outlined, there is growing interest in developing technically and economically sustainable strategies for recovery of P to counteract the pressure on current PR reserves, to reduce national dependence on imports and to close the anthropogenic P cycle.

The research project Re-BioP-Cycle[5] “Recycling of phosphorus from municipal WWTPs by coupled bioP re-dissolution and crystallization in a fluidized bed reactor” (AiF-No. 19746 N) (Proposal Re-BioP-Cycle, 2017), aimed to investigate a two-stage process to recover P from the AS in full-scale municipal WWTPs. As shown in Figure 2-1, AS is withdrawn as non-acclimated AS at the end of the aerobic treatment stage of the WWTP and transferred to a side stream unit in which ortho-P is re-dissolved from microbial polyP of PAOs. Subsequently, the re-dissolved P can be recovered as a precipitate such as a calcium phosphate product to be used in agriculture. The process of P precipitation is performed in a fluidized bed reactor with finely dispersed milk of lime and dolomite. By keeping the re-dissolution times short the P-depleted sludge may be reintroduced to the biological treatment stage of the WWTP for new P uptake or may be transferred to the sludge treatment stage.

Within the framework of the Re-Bio-P cycle project, the overall objective of this thesis was to investigate a targeted, bio-based P re-dissolution approach based on EBPR, by focusing on understanding the variability in P re-dissolution efficiency across WWTPs, optimizing key operational parameters and evaluating scalability through pilot-scale testing. This aimed to develop a process that combines on-site P recovery with wastewater treatment. As a decentralized process, this may offer the possibility to deplete the sludge of P by creating a valuable recovery product and concomitantly reducing sludge volume, transport costs or unwanted struvite scaling.

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[5] Funded by the Federal Ministry for Economic Affairs and Energy through the German Federation of Industrial Research Associations e.V. (AiF). Research partners involved: Forschungsgemeinschaft Kalk und Mörtel e.V., Competence Centre for Sustainable Engineering and Environmental Systems – Technische Hochschule Mittelhessen, Ingenieurbüro ALWIN EPPLER GmbH & Co. KG and WWTP Giessen – Mittelhessische Wasserbetriebe.

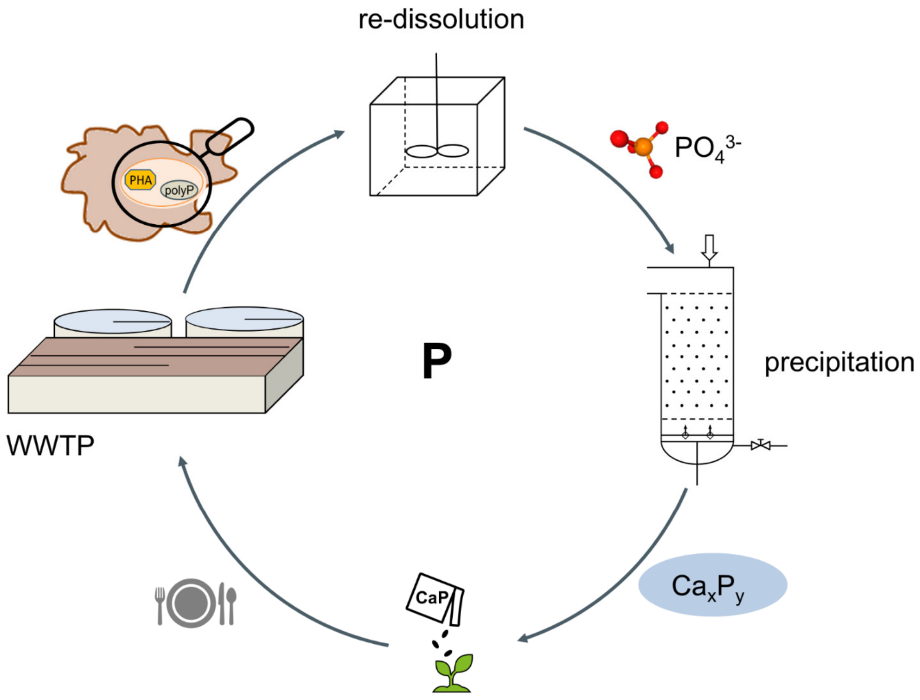

Figure 2-1| Idealized P reycling scheme from AS of municipal WWTPs and hypothesized application of the recovered P as a calcium phosphate product for agriculture.

So far, most on-site applications have been based on the use of anaerobic digestion units thereby facilitating polyP hydrolysis and degradation of biomass. However, with P, trace metals, anthropogenic organic chemicals and pharmaceuticals may be desorpted from the sludge and may end up in the recovery product (Azizan et al., 2021; Grabic et al., 2022; Selling et al., 2008). Few studies target the AS at an earlier point in the wastewater treatment process. Specifically, after the aerobic phase of biological treatment the AS contains the highest concentration of biomass P. As part of the EBPR, it has been known that the re-dissolution of P from the polyP storage in PAOs occurs upon addition of a carbon source without the presence of oxygen. Previous research has demonstrated that a significant P re-dissolution can be achieved during anaerobiosis (Acevedo et al., 2015; Guisasola et al., 2019; Wong et al., 2013; Xia et al., 2014) but the biological recovery of P from AS through enhanced re-dissolution remains limited to laboratory-scale investigations with acclimated AS (cf. Chapter 1.4). This limits real implementation at scale and further investigations under real-world conditions are needed. EBPR, as a biological system, greatly depends on microbiological and operational conditions. For example, recently, it has been shown that transferred microbial populations between WWTPs do not persist at full-scale, and the original WWTP population is maintained, likely due to each's plants specific influent characteristics (Dottorini et al., 2023).

In turn, the ability of the AS for P re-dissolution may be determined by the microbial community and the distinctive characteristics and composition of the AS may account for variations in P release. Therefore, it is important to investigate the behavior of real/non-acclimated AS and its suitability for a bio-based P recovery by re-dissolution.

To address the aforentioned gaps the research was divided into two main research tasks. The first task was to maximize the P re-dissolution from non-acclimated AS and identify optimal process conditions since a high concentration P in the recovery stream has been reported as essential for efficient precipitation (Cornel and Schaum, 2009). During the studies appropriate process conditions for the re-dissolution of P were assessed. Short-term re-dissolution times were sought to avoid irreversible damage to PAOs, and to avoid deleterious effects on EBPR activity. The second task was up-scaling the re-dissolution and recovery process. A pilot plant was constructed at the WWTP Giessen and used to evaluate a potential implementation of the recycling process into the wastewater treatment process (prove of principle). This included the design and construction, as well as the testing of the operating parameters previously selected in laboratory-scale experiments and the final assessment of the recover product.

Based on the objective the following research questions were pursuit in this thesis:

1. Can P be re-dissolved from from PAOs present in the full-scale municipal WWTP Giessen? (Review of the re-dissolution process for non-acclimated AS).
2. How does the carbon source type and supplementation level affect the P re-dissolution? What is the kinetic of P re-dissolution and carbon uptake?
3. How does the maximum P re-dissolution yield differ from WWTPs that employ different P removal strategies?
4. Is it possible to up-scale the process for treatment of AS in a side stream unit and recover P as a calcium phosphate product? What are the main limitations or biological chemical and technical bottlenecks?

2.2 Thesis Outline

This thesis includes six chapters, describing the work developed during this thesis project. **Chapter 1** provides the background of the research by discussing the relevance of P in wastewater and as a critical resource as well as the EBPR process. It provides an overview of current P recovery technologies with a focus on emerging biological approaches. The current **chapter 2** introduces the main objectives of the thesis.

Chapter 3 comparatively investigates the acetate-mediated P re-dissolution from non-acclimated AS, to reflect real-world conditions. Three municipal WWTPs which differed in operation mode (EBPR/CPR) and sludge characteristics were investigated for the potential to re-dissolve P. Substrate uptake and P release kinetics were obtained by analysing soluble ortho-P, total sludge P and aqueous substrate concentrations. The abundance of PAOs and GAOs within the microbial community was analyzed by fluorescence *in situ* hybridization (FISH).

Focussing on a single WWTP, **chapter 4** revisited the effect of different carbon sources (formate, acetate, propionate, butyrate) on P re-dissolution in more deteail. The optimal carbon supplementation level for enhancing the P re-dissolution was identified by evaluating the substrate uptake and P release.

Chapter 5 presents the results of testing the P recovery approach at pilot-scale based at the WWTP Giessen. The chapter evaluates the P re-dissolution and fluidized bed precipitation for recovery of a P-enriched dolomite product. Based on the investigations performed in chapter 4, acetate was chosen as the optimal carbon source to induce re-dissolution under short-term anaerobic treatment. Findings highlight practical challenges and opportunities for real-world implementation.

This is followed by a comprehensive summary of the most important results (**chapter 6**). Implications as well as emerging bottlenecks and limitations are discussed in light of a practical WWTP implementation. Finally, an outlook to the future of recovering and recycling P for a circular economy is given.

Chapter 3

Anders et al. (2021) *J. Environ. Chem. Eng. Vol. 9 Issue 6*

Phosphorus recycling from activated sludge of full-scale wastewater treatment plants by fast inversion of the biological phosphorus elimination mechanism

This chapter[6] has been published in the *Journal of Environmental Chemical Engineering*, Volume 9, Issue 6 in December 2021 as a full research article entitled:

“Phosphorus recycling from activated sludge of full-scale wastewater treatment plants by fast inversion of the biological phosphorus elimination mechanism”
https://doi.org/10.1016/j.jece.2021.106403

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[6] For consistency, the formatting and orthography, including citation style of the following article have been adapted to the dissertation style.

Full research article:

Phosphorus recycling from activated sludge of full-scale wastewater treatment plants by fast inversion of the biological phosphorus elimination mechanism

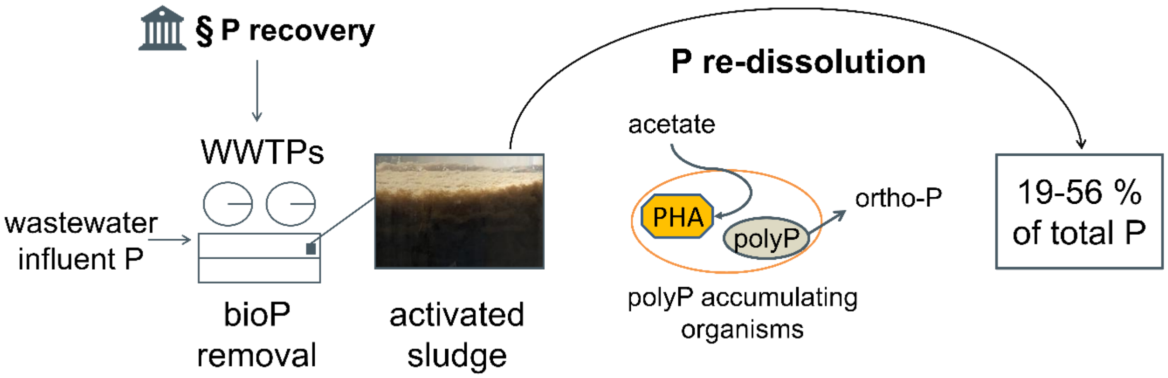
Annika Anders[a], Harald Weigand[a], Harun Cakir[b], Ulrich Kornhaas[c], Harald Platen[a]

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

[b] *Forschungsgemeinschaft Kalk & Mörtel e.V., Annastr. 67-71, D-50968 Cologne, Germany*

[c] *Ingenieurbüro Alwin Eppler GmbH & Co. KG, Gartenstr. 9, D-72280 Dornstetten, Germany*

Graphical Abstract



Keywords

Activated sludge, Enhanced biological phosphorus removal (EBPR), Phosphorus re-dissolution, Phosphorus recovery, Polyphosphate accumulating organisms (PAO), Wastewater

Abstract

Enhanced biological phosphorus removal (EBPR) is often applied by wastewater treatment plants (WWTPs) whereby phosphorus (P) is accumulated in the activated sludge (AS) biomass. The German Sewage Sludge Ordinance demands that large WWTPs recycle P from their process streams by 2029. This study aimed at evaluating the potential of an acetate-mediated P re-dissolution from non-acclimated AS of three full-scale WWTPs. All AS exhibited enhanced P re-dissolution during acetate uptake and followed kinetics typical of EBPR systems. Re-dissolution efficiency differed depending on the WWTP operation mode. The highest P re-dissolution yield (56% of total P) was obtained for pure EBPR sludge. Lower re-dissolution rates of 19–22% were observed in the AS from WWTPs that employed additional chemical P removal, probably due to shortage of intracellular polyphosphate. Pure EBPR systems may be suited for the implementation of a re-dissolution based strategy for P recycling from AS where P-depleted AS could potentially be returned to the biological treatment stage.

3.1 Introduction

Phosphorus (P) is an essential nutrient and indispensable for food and feed production. In 2018, 249 Mt of phosphate rock (PR) were mined worldwide and primarily used to produce fertilizers for agriculture (USGS, 2020). However, PR reserves are limited. In 2014 the European Union has listed PR among the top 20 critical raw materials (European Commission, 2014). This accounts for the concentration of mineable deposits in only a few countries and Europe's almost complete dependency on P imports. In addition to the economic and geopolitical concerns, sedimentary PR deposits are increasingly contaminated with heavy metals such as uranium and cadmium which may partially end up in fertilizer products and may be released into the environment (Chien et al., 2011). Considering the global population growth, it is expected that mining of PR will increase to meet agricultural demands. Hence, it is clear that an efficient and sustainable recovery route of P from secondary resources is essential.

On a global scale around 1.3 Mt of sewage-borne P are annually treated in wastewater treatment plants (WWTPs) (Li and Li, 2017). To prevent eutrophication of effluent receiving water bodies, P is most commonly removed by the enhanced biological phosphorus removal (EBPR) or by the chemical P removal (CPR) using iron or aluminum salts. In some cases, EBPR is combined with CPR to meet effluent standards. By this, about 75–90% of the influent P is transferred to the sewage sludge (Cornel and Schaum, 2009). Therefore, sewage sludge is considered a secondary P resource, which could substitute up to 20% of the worldwide PR demand (Yuan et al., 2012). As a consequence, the 2017 amendment of the German Sewage Sludge Ordinance demands that large WWTPs recover P from process streams by 2029 and restricts a direct agricultural application of dewatered sludge (AbfKlärV, 2017).

Depending on their access point, P recycling technologies can be classified as downstream recovery or as WWTP-integrated recovery (Kabbe and Rinck-Pfeiffer, 2019). The former typically address P retained in the ash after sewage sludge mono-incineration. They often rely on intensive use of energy for drying and chemicals for the ash treatment (Cornel and Schaum, 2009) unless the sludge is incinerated in dewatered state and the ash is directly applied to farmlands. The WWTP-integrated processes aim at a P recovery from the sludge or the sludge water phase. Re-dissolution from the solid fraction may be accomplished chemically (e.g. Stuttgart process) or by thermal treatment (e.g. Lysotherm process and Heatphos process) (Kabbe and Rinck-Pfeiffer, 2019; Takiguchi et al., 2003). Re-dissolution also takes place during anaerobic sludge digestion due to sludge disintegration and cell lysis (Li and Li, 2017). Alternative concepts could include a sludge treatment step where biomass P is dissolved biologically.

In an EBPR process, P removal is achieved by cycling the activated sludge (AS) through anaerobic and aerobic stages, thereby promoting an enrichment of polyphosphate accumulating organisms (PAOs). Under anaerobic conditions, PAOs can take up carbon sources such as volatile fatty acids (VFAs) and convert them to polyhydroxyalkanoates (PHAs) (Comeau et al., 1986; Mino et al., 1998). Energy for this uptake is provided by hydrolysis of stored polyphosphate (polyP) and consequently, ortho-phosphate (ortho-P) is released into the liquid phase. Under aerobic conditions, PHAs are degraded to accumulate excess P from wastewater. Intracellular storage of polyP results in a net P removal from the liquid phase (Fuhs and Chen, 1975; Mino et al., 1998). Glycogen accumulating organisms (GAOs) in AS can also metabolize VFAs under anaerobic conditions but do not contribute to the aerobic P removal as no polyP is accumulated in the cells (Cech and Hartman, 1993; Saunders et al., 2003). Thus, a predominance of GAOs may decrease EBPR efficiency (Cech and Hartman, 1993; Satoh et al., 1994).

This has motivated research on how to provide PAOs a competitive advantage over GAOs and a series of influencing factors have been recognized. These include pH (Filipe et al., 2001; Oehmen et al., 2005a), carbon source type (Carvalheira et al., 2014; Oehmen et al., 2005b), temperature (Lopez-Vazquez et al., 2009, 2007; Whang and Park, 2006), sludge retention time (Whang and Park, 2006) as well as the influent P/C ratio (Liu et al., 1997; Welles et al., 2017). Current understanding of EBPR processes may be used to develop novel AS-based P recovery approaches. Re-dissolution of P from highly enriched PAO cultures has been previously shown (Acevedo et al., 2015; Xia et al., 2014). The authors (Acevedo et al., 2015; Xia et al., 2014) used a sequencing batch reactor (SBR) supplemented with a carbon source. Acevedo et al. (2015) demonstrated that stored polyP could be reduced by 99%. PAOs were able to resist under these extreme conditions suggesting that the P-depleted sludge may be returned to the WWTP. So far, most studies have been carried out with lab-scale SBRs operated with PAO-enriched or GAO-enriched sludge and only few studies have used non-acclimated sludge from full-scale WWTPs. Studies using non-acclimated sludge have mainly focused on the identification and contribution of EBPR-involved microorganisms in substrate uptake, the PHA production as well as P accumulation rather than a P recovery (Fernando et al., 2019; Kong et al., 2004; Lemos et al., 1998; López-Vázquez et al., 2008; Nguyen et al., 2011). AS is a dynamic and complex system exposed to changing environmental and operational conditions. Therefore, studies with real-world sludge are particularly important for the development of scalable sludge-based P recovery processes.

Here we evaluated the potential of an acetate-mediated P re-dissolution employing AS collected at three full-scale WWTPs with regard to future P recycling. Given the plant size (served population) and the P levels of the digested sludge, by 2029, all three WWTPs fall under the ban of the conventional direct agricultural re-use according to the German Sewage Sludge Ordinance. This means that alternative routes of P recovery are mandatory and pertinent reporting obligations need to be met by 2023 (AbfKlärV, 2017). The investigations aimed at inverting the EBPR process by supplementing the AS with acetate to accelerate P re-dissolution from AS. Complementarily, fluorescence *in situ* hybridization (FISH) was employed to characterize the sludge in terms of PAO and GAO abundance. To avoid alteration of the biotic community, the treatment time was limited to a maximum of four hours. This accounted for the fact that in perspective the AS would be returned to the wastewater treatment after P harvesting.

3.2 Materials and methods

3.2.1 Phosphorus re-dissolution experiments

The AS of three full-scale German WWTPs treating municipal wastewater was investigated in terms of P re-dissolution upon acetate supplementation. WWTP1, WWTP2 and WWTP3 vary in size and process design and are located in Hesse, Bavaria and Lower Saxony, respectively. All three plants employ the EBPR process but WWTP1 and WWTP2 additionally use chemical precipitation agents for further P elimination.

The main operational features and selected influent wastewater parameters of the plants are given in Table 3-1. The AS was sampled at the end of the aerated biological treatment before the sludge is transferred into settling tanks. Sampling was performed during spring and summer ensuring comparable ambient conditions by excluding extreme weather events such as drought periods or extreme precipitation. For WWTP1, biological replicates were investigated with AS independently sampled at the same location at five different dates. Sampling at WWTP2 and WWTP3 was performed once. All sludge samples were processed on-site within less than one hour to prevent changes due to bacterial activity. Re-dissolution studies were performed under anoxic conditions over four hours with AS from WWTP1 and WWTP3. Due to access restrictions imposed by the Covid pandemic, the on-site incubation at WWTP2 was limited to one hour. The initial pH of AS was 7.0 ± 0.1 (Table 3-1). In view of a practical application, the pH was not adjusted in the re-dissolution experiments. The batches were intermittently agitated by hand at room temperature (~22 °C). To accelerate P re-dissolution 1 L of each AS was initially spiked with sodium acetate trihydrate (p.a. ≥99%, Merck KGaA, Darmstadt, Germany). The spike level was 7% (w/w) acetate per sludge dry weight based on preliminary tests. All experiments were conducted against a non-supplemented control. Frequent aliquots of the suspension were taken to acquire time profiles of P release and acetate uptake. Total phosphorus, iron and aluminum were determined in the sludge according to analytical methods described below.

Table 3-1| Operational features of the full-scale wastewater treatment plants WWTP1, WWTP2, WWTP3 and characteristics of activated sludge used in the P re-dissolution experiments. The latter are given in terms of the whole sludge suspension as well as its solid phase (biomass) and liquid phase.

Parameter	**WWTP1**[b]	**WWTP2**[c]	**WWTP3**[c]
plant capacity [PE]	300,000	445,000	240,000
served population [PE]	286,000	400,000	146,000
type of P elimination	EBPR + CPR	EBPR + CPR	EBPR
CPR precipitant	ferrous chloride $Fe(II)Cl_2$ (during biological treatment)	sodium aluminate (after biological treatment)	none
influent wastewater[a]			
COD [kg/d]	42,347	43,900	10,635
BOD5 [kg/d]	22,761	23,400	3,930
activated sludge suspension			
TSS [g/L]	6.3 ± 0.5	3.0	2.7
pH	6.9–7.0	7.1	7.0
activated sludge biomass			
P [mg/g_{TSS}]	37.59 ± 7.11	24.81 ± 0.83	36.83 ± 0.40
Fe [mg/g_{TSS}]	40.39 ± 9.55	7.45 ± 0.24	9.45 ± 0.18
Al [mg/g_{TSS}]	9.01 ± 0.36	27.53 ± 0.24	15.42 ± 2.55
activated sludge liquid phase			
Nitrate [mg N/L]	4.28 ± 1.31	5.15 ± 0.08	0.91 ± 0.12
Ortho-P [mg P/L]	0.07 ± 0.04	0.25 ± 0.02	0.06 ± 0.00

BOD biological oxygen demand, COD chemical oxygen demand, PE population equivalent

[a] Average annual values supplied by plant staff.

[b] Repeated sludge sampling during the years 2019–2020, mean values represent biological replicates ± standard deviation, n = 5.

[c] Single sludge sampling during field study (n = 1), mean values represent technical triplicates ± standard deviation.

3.2.2 Analytical methods

Solution phase samples were processed by 0.45 µm membrane filtration. Dissolved ortho-P was determined photometrically at 880 nm using the molybdenum blue method (APHA, 1999). For VFA and nitrate determination, samples were additionally filtered through a 0.2 µm membrane filter and analyzed using an ion chromatograph (Metrohm 861 Advanced Compact IC) with a conductivity detector. Sample splits of 20 µL were injected on a Metrosep A Supp 5 column (150 mm × 4.0 mm, particle size 5 µm, Metrohm, Herisau, Switzerland) and separated with 1.0 mmol/L $NaHCO_3$/3.2 mmol/L Na_2CO_3 eluent. Runs had a duration of 20 min at an eluent flow rate of 0.7 mL/min. Detection took place after chemical suppression by measuring conductivity. The samples' conductivity signals were aligned with those of external standards. Technical triplicates were analyzed, throughout.

Sludge biomass was processed by drying at 105 °C for 24 h and subsequent grinding in a ball mill. Aluminum was determined by X-ray fluorescence spectroscopy (XRF) analysis of dried sludge samples using an energy-dispersive X-ray fluorescence spectrometer (Spectroscout, Spectro, Ametek, Kleve, Germany) with sample rotation and helium sparging. Biomass-borne phosphate and iron analysis were carried out after aqua regia digestion of the AS according to European standard procedure DIN EN 16174:2012-11 (CEN, 2012). Phosphate content was calculated from the ortho-P concentration determined in the digestates according to the molybdenum blue method. Iron in aqua regia digestates was determined by atomic absorption spectroscopy (acetylene/air-flame, AAnalyst 100, Perkin Elmer, Waltham, MA, USA).

Total suspended solids (TSS) concentration of the sampled sludge was determined according to standard methods (APHA, 1999) and used to calculate the specific P release and substrate usage.

Community FISH analyses were performed on AS samples from the biological treatment to assess the abundance of PAOs and GAOs prior to re-dissolution experiments. The AS samples were stabilized by addition of ethanol (98%, 1:1, v/v) and kept at 4 °C until analyzed at vermicon AG, Munich, Germany, employing VIT® gene probe technology. Abundance of PAO and GAO was determined using fluorescence-labeled specific oligonucleotide gene probes targeting the 16S rRNA in corresponding target cells. This method allows specific labeling of intact and viable cells by hybridization. VIT®PAO probe mix and VIT®GAO probe mix was used targeting the genera *Candidatus* Accumulibacter (henceforth called *Accumulibacter)* and *Candidatus* Competibacter (henceforth called *Competibacter),* respectively. EUB338 probe mix (Daims et al., 1999) was used to target the total bacterial population. Abundance of specific communities was estimated by analyzing fluorescence signals of 20 separate pictures (randomly chosen microscopic fields at 1000× magnification using an epifluorescence microscope) and expressed as the percentage of PAOs

(*Accumulibacter*) and GAOs (*Competibacter*) of the total viable bacteria population. The limit of detection equaled 1000 cells/mL. Statistical analysis (simple ANOVA) of relative *Accumulibacter* abundance was performed to determine whether the WWTPs differed significantly with respect to the microbial consortia composition.

3.2.3 Data evaluation

Yield of re-dissolved P (Y_P [%]) was calculated according to Eq. (3-1) P_t [mg/L] denotes the concentration of ortho-P at the sampling time, P_0 [mg/L] is the concentration of ortho-P in the aqueous phase at t = 0, P_{AS} [mg/g_{TSS}] is the total phosphorus content of the sludge at t = 0 and TSS [g/L] is the total suspended solids concentration of the AS suspension at t = 0.

$$Y_P = \frac{P_t - P_0}{TSS \cdot P_{AS}} \cdot 100\% \tag{3-1}$$

P release curves were fitted using a zero-order kinetic model (Eq. (3-2)) or a modified version of the Gompertz model (Gompertz, 1825; Zwietering et al., 1990) (Eq. (3-3)). The (modified) Gompertz model has been widely used to fit sigmoidal growth curves (Zwietering et al., 1990) as well as bacterial transformation processes (Li et al., 2005), hydrogen or methane production kinetics (Mu et al., 2006; Wang and Wan, 2009; Ware and Power, 2017) and drug dissolution processes (Easton, 2002; Ramteke et al., 2014). Here, it was used to describe the changes in the P release rate and the approach to a saturation value. The term A_{max} [mg P/g_{TSS}] represents the upper asymptote (saturation value), μ [mg P/(g_{TSS}×min)] is the maximum release rate, λ [min] is the lag phase, and t [min] is time. Fits were performed with the MS Excel SOLVER add-in by minimizing the sum of squared residues between observed and model-predicted values. Quality of the model-fit was quantified by using the coefficient of determination (R^2).

$$P_{i,rel}(t) = \mu \cdot t \tag{3-2}$$

$$P_{i,rel}(t) = A_{max} \cdot \exp\left\{-\exp\left[\frac{\mu \cdot \exp(1)}{A_{max}}(\lambda - t) + 1\right]\right\} \tag{3-3}$$

3.3 Results and discussion

3.3.1 Operational, chemical and microbial WWTP characteristics

Three full-scale WWTPs were studied regarding the feasibility of inverting the biological P elimination in the context of a P recycling strategy. Table 3-1 summarizes important plant properties and characteristics of the AS used in the batch experiments expressed in terms of the whole suspension, the solid biomass and the liquid phase. The plants differ with respect to size, influent wastewater quality, and particularly, with respect to the P elimination strategy. While WWTP1 and WWTP2 employ EBPR in combination with CPR using iron and aluminum salts, respectively, WWTP3 relies on EBPR, solely. High total P levels were identified in the sludge biomass of all plants but iron and aluminum levels differed depending on the precipitant type employed in CPR. Consistent with the accumulation in the sludge, the liquid sludge phase was low in nutrient levels with ortho-P ranging from 0.06 mg P/L to 0.25 mg P/L and nitrate ranging from 0.9 mg N/L to 5.2 mg N/L. Consequently, high total P contents were detected in the solid phase of the AS. Total P content in WWTP2 sludge biomass was 1.5 times lower than in WWTP1 and WWTP3.

Microscopic examination and FISH analysis revealed distinct PAO cell clusters in the AS from WWTP1 through WWTP3, denoted as AS1, AS2 and AS3 in the following. Representative microscopic images of *Accumulibacter* and *Competibacter* cells of each AS are shown in Figure 3-1. In general, the observed PAO and GAO abundance was within the range of literature values from different studies investigating full-scale plants (Bertanza et al., 2020; Kong et al., 2004; Saunders et al., 2003).

The average *Accumulibacter*-related PAO abundance was between 9% and 19% of the total bacteria population of the different AS (Figure 3-2). *Accumulibacter* abundance differed significantly among the WWTPs as indicated by simple ANOVA ($p = 0.95$). Preconditions for ANOVA were met according to the results of the Kolmogorov-Smirnov and Levene tests. Cells exhibited the typical arrangement of coccoid cells in clusters as described by Buchan, 1981; Crocetti et al., 2000; Fuhs and Chen, 1975 and Nielsen et al., 2009. However, density and distribution of clusters varied. Especially AS1 showed a heterogeneous and more diffuse distribution of *Accumulibacter* (Figure 3-2; median < mean; broad spread of upper and lower frame of box) with few highly concentrated clusters. Generally, *Accumulibacter* population was least abundant in AS1 which had a densely packed floc structure, likely caused by the ferrous chloride addition during the aerobic treatment (Oikonomidis et al., 2010). While the sludge structure was less dense in AS2, compact *Accumulibacter* clusters were more abundant. Strong fluorescence signals indicated that *Accumulibacter* cells were concentrated in tight clusters for AS3. *Competibacter*-related GAOs showed low abundance in AS1 and were absent in AS2 (Figure 3-2), suggesting suboptimal growth conditions. A greater *Competibacter* population was detected in AS3.

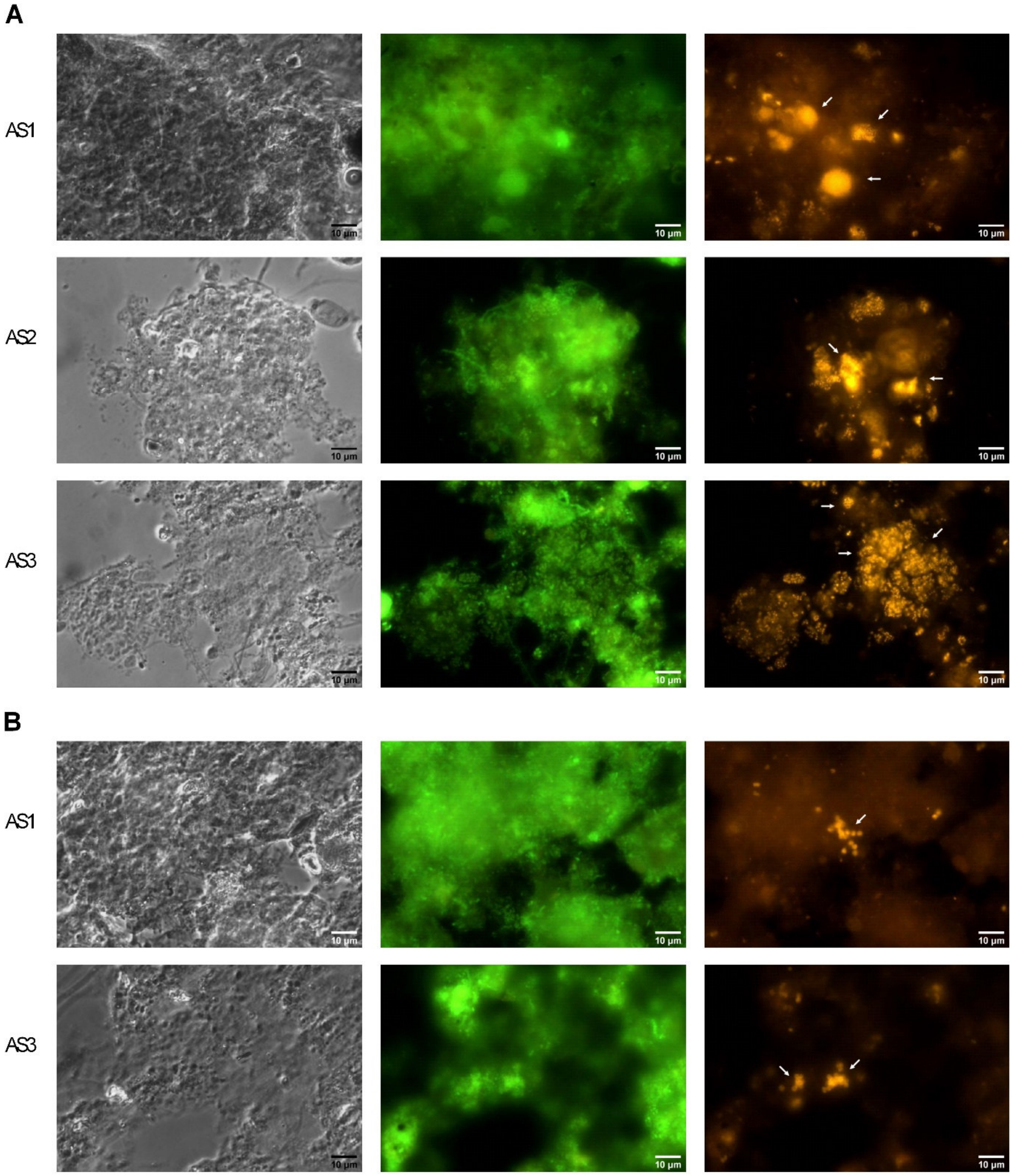


Figure 3-1| Phase contrast microscopic visualization (column 1) and FISH analysis (columns 2 and 3) of activated sludge samples AS1, AS2, and AS3. Green fluorescence signals of total bacteria hybridized with EUB338 probe mix are shown in column 2. *Accumulibacter* PAOs (A) identified by specific VIT®PAO probe mix appear yellow (column 3). *Competibacter* GAOs (B) identified by specific VIT®GAO probe mix appear yellow (column 3). Arrows mark examples of cell clusters exhibiting strong fluorescence signals.

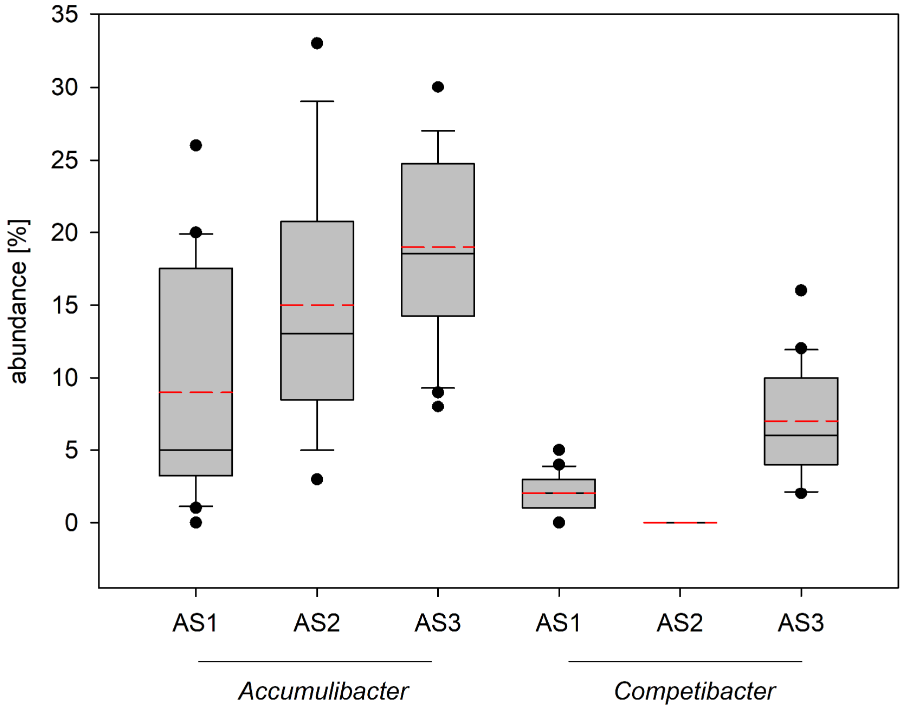


Figure 3-2| Abundance of *Accumulibacter* PAO (left) and *Competibacter* GAO (right) in activated sludge samples AS1, AS2 and AS3 as determined by FISH analysis. Abundance is expressed as percentage of total bacteria population (EUB338 probe mix). The upper and lower frame of boxes represent the 25th and 75th percentiles, the black bar is the median, the red dashed bar is the arithmetic mean and the whiskers denote the 10th and 90th percentiles, outliers are shown as dots.

3.3.2 Phosphorus re-dissolution experiments

The kinetics of P release from the three ASs and the corresponding P yields were examined in batch experiments upon acetate supplementation. For comparison, results were normalized to the TSS concentration thereby accounting for the different TSS levels observed at the three WWTPs. The P release started immediately after supplementation but proceeded at WWTP-specific rates (Figure 3-3A). Regardless of the AS, control experiments without acetate supplementation showed negligible P release during 240 min (data not shown), reinforcing that the observed P release was induced by the acetate supplementation. The P release characteristics of AS1 and AS3 could be satisfactorily modeled using the modified Gompertz model (Eq. (3-3)), whereas AS2 was adequately described by a linear function (Eq. (3-2)). Parameters obtained by minimizing the sum of squared residues between measured data and model prediction are shown in Table 3-2. The rate of maximum P release decreased in the order AS3 (0.20 mg P/(g_{TSS}×min)) > AS2 (0.08 mg P/(g_{TSS}×min)) > AS1 (0.06 mg P/(g_{TSS}×min)). Over a period of 240 min, AS1 released a total of 7.8 mg P/g_{TSS} into the aqueous phase. A higher P release was observed in AS3 with a total of 20.7 mg P/g_{TSS}. No maximum

P release could be determined for AS2 within the experimental period (Figure 3-3A). After the re-dissolution experiments, the P levels of the solid phase were reduced to 31.6 ± 4.2, 21.7 ± 0.6, 17.7 ± 0.4 mg P/g_{TSS} for AS1 through AS3, respectively. Thus, P release to the aqueous phase was quantitatively mirrored by reduction of AS-borne P. In all batches, acetate concentration started to decrease immediately after supplementation indicating uptake by the sludge biomass (Figure 3-3B). Around 32% of the supplemented acetate was consumed by AS1 biomass. During the initial 40 min the uptake was approximately linear at a rate of 0.41 mg/(g_{TSS}×min). Acetate uptake by AS2 and AS3 was higher in terms of total consumption and the uptake was faster. In AS3, acetate uptake proceeded over the whole investigation period. As indicated by a virtually zero concentration the supplemented acetate was fully consumed after 240 min.

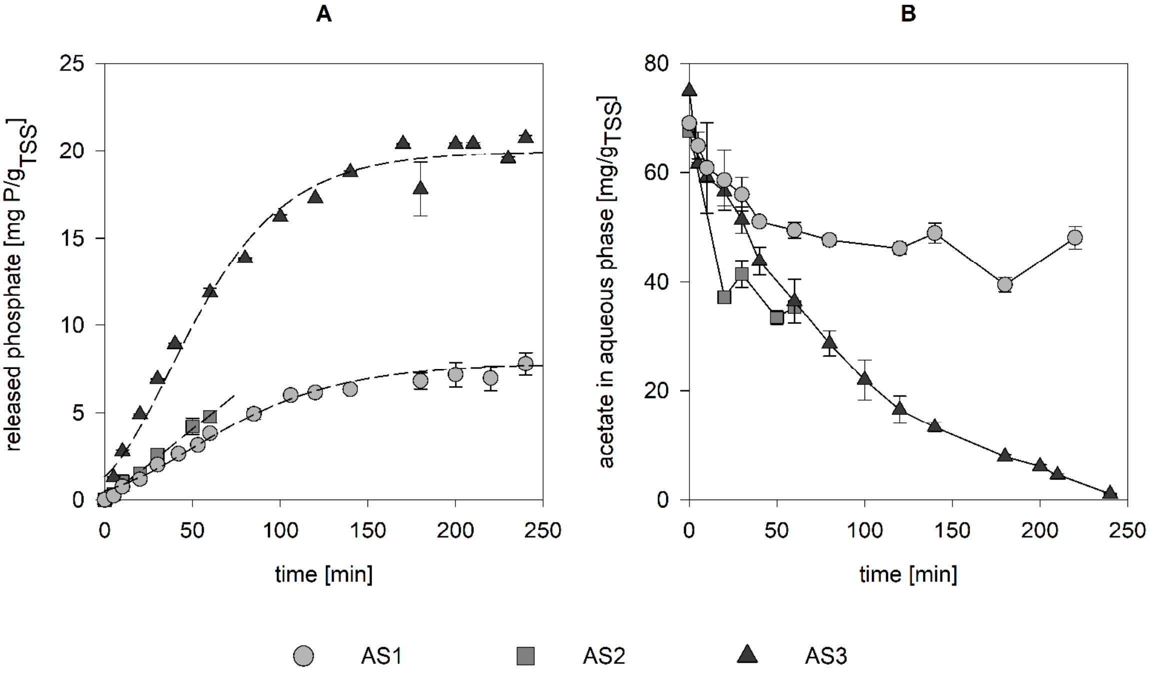


Figure 3-3| P release (A) and acetate uptake kinetics (B) of acetate-supplemented activated sludge sampled at different WWTPs. Measured data are represented by symbols (mean ± standard deviation). Modeled P release is represented by dashed line. AS1: biological replicates n = 5. AS2 and AS3: single sludge sampling, analyzed in technical triplicates.

The P release from either AS was associated with acetate uptake. Under anaerobic conditions, energy for acetate uptake is supplied by degrading the internal polyP storage (Mino et al., 1998). It has been shown, that the energy demand for VFA uptake and the resulting P release depends on the pH (Oehmen et al., 2005a). In this study, the initial pH of the AS suspension was similar among the batches (Table 3-1) and was rather constant during the incubation period (6.9–7.1). Therefore, pH effects on P release and acetate uptake can be ruled out. In our experiments, both, yield of re-dissolved P and P release rate increased with

Accumulibacter abundance in the AS (Figure 3-4). *Accumulibacter* represent an important genus within the PAO in EBPR systems and greater abundance has been linked with an improved P removal (Crocetti et al., 2000; Kong et al., 2004). Hence, polyP storage can be assumed to increase with *Accumulibacter* abundance and should result in higher P re-dissolution under anoxic conditions after VFA supplementation. This is largely supported by the experimental findings summarized in Figure 3-4, as re-dissolved P levels and maximum P release rate, increased with *Accumulibacter* abundance in the AS. However, correlation of re-dissolved P and maximum P release rate after supplementation was not linear against *Accumulibacter* abundance indicating the relevance of further factors such as the WWTP operation mode. In addition, not all *Accumulibacter* cells may participate in polyP storage as previously reported for other full-scale plants (Kong et al., 2004).

Acetate uptake increased with the *Accumulibacter* and *Competibacter* abundance in the sludge biomass. This is in accordance with the fact that acetate can be readily metabolized by both PAOs and GAOs (Oehmen et al., 2010) and at similar rates by some groups (López-Vázquez et al., 2007; Zeng et al., 2003). Therefore, acetate supplementation may induce competition for VFA uptake between microbial communities and reduce P re-dissolution efficiency (Saunders et al., 2003). Table 3-2 shows the stoichiometric parameters for P release and acetate uptake observed in this study.

Table 3-2| Yield of re-dissolved P (Y_P), molar conversion ratio, and best-fit kinetic parameters of the P re-dissolution studies with acetate-supplemented activated sludge (AS) sampled at different WWTPs. Curve fitting was done with the modified Gompertz model, I[0 min;240 min] for AS1 and AS3 and a linear function $P_{i,rel} = \mu \times t$, I[0 min;60 min] for AS2.

	AS1	**AS2**	**AS3**
Y_P (60 min) [%]	11	19	32
Y_P (240 min) [%]	22	n.a.[a]	56
$P_{release}$/$acetate_{uptake}$ ratio [mol P/mol C][b]	0.20	0.14	0.30
Best-fit parameters			
A_{max} [mg P/g_{TSS}]	7.84	-	19.96
μ [mg P/(g_{TSS}×min)]	0.06	0.08	0.20
λ [min]	0.44	-	0.00
R^2	0.99	0.99	0.99

[a] n.a.: not available

[b] operational value for t = 60 min after acetate supplementation

The $P_{release}$/$acetate_{uptake}$ ratio was highest for AS3, followed by AS1 and AS2. Compared to literature, these values are rather low. While Smolders et al. (1994) proposed a theoretical stoichiometric ratio of 0.48 mol P/mol C for the anaerobic PAO metabolism at neutral pH, other studies show that the $P_{release}$/$acetate_{uptake}$ ratio can vary between 0.29 and 0.51 mol P/mol C for full-scale plants (López-Vázquez et al., 2007; Saunders et al., 2003). The low $P_{release}$/$acetate_{uptake}$ ratios in AS1 and AS2 cannot be explained by the abundance of GAO according to *Competibacter* FISH results. Rather, they may be explained by polyP limitation. Since anaerobic conditions do not permit growth of *Accumulibacter* PAOs, intracellular pools are finite (Kong et al., 2004). Moreover, PAOs can employ different metabolic pathways depending on the availability of internal storage compounds and external environmental conditions (Majed et al., 2012). Under polyP limitation, PAOs can shift to a GAO-like metabolism and use glycogen as the energy source for VFA uptake thereby coping with P starvation in periods of cell maintenance (Acevedo et al., 2017, 2012; Welles et al., 2017). Therefore, P re-dissolution efficiency may vary significantly with metabolic states and relative activity of individual PAO cells as well as with the share of PAO and GAO groups and sub-groups (Fernando et al., 2019; Majed et al., 2012; Nguyen et al., 2011; Oehmen et al., 2010). In this study, only *Accumulibacter* were targeted as PAOs in the FISH analyses although other putative PAO groups, such as *Tetrasphaera* spp. may have been present in the respective AS.

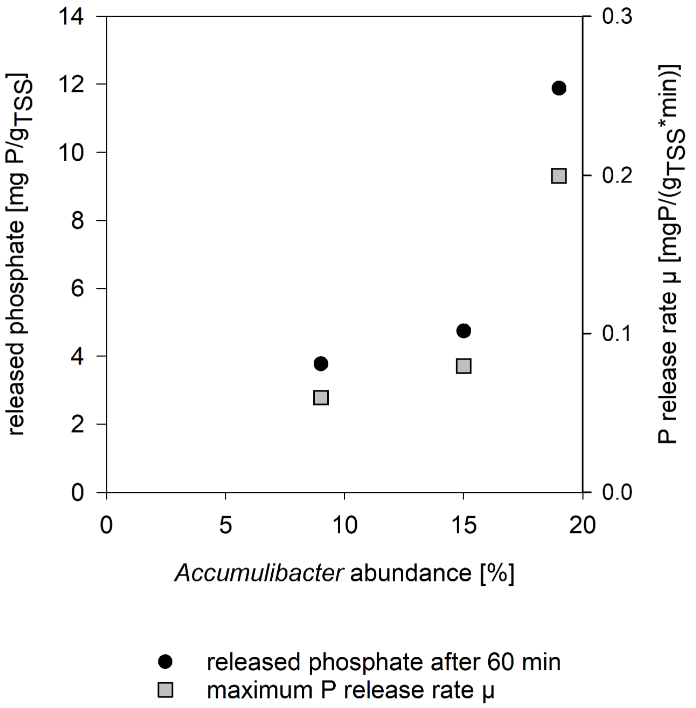


Figure 3-4| Mean *Accumulibacter* abundance (percentage of total bacteria population; EUB338 probe mix) against P release 60 min after acetate supplementation (left Y-axis) and maximum P release rate determined with the modified Gompertz model (right Y-axis).

In AS1, the low P yield was not acetate-limited (Figure 3-3B). Low P re-dissolution may thus have resulted from saturation of the intracellular PHA storage. Alternatively, the available polyP may have been limiting; possibly as a result of CPR employing ferrous chloride as bacteria and the chemical elimination processes compete for influent P. The additional CPR employed at this WWTP may have been the cause of the low *Accumulibacter* abundance in AS1. This is suggested by previous findings on a reduced biological P removal upon precipitant addition (Bertanza et al., 2020; Liu et al., 2011; Röske and Schönborn, 1994). At WWTP1 iron dosage has been employed for several years and accumulation of metal salts in sludge biomass likely had additional interfering effects on microbial P cycling and substrate transport as suggested by Liu et al. (2011). In WWTP2 the additional CPR is based on sodium aluminate after the biological treatment stage. In this case, carry-over by the return sludge may have contributed to a lower aerobic P uptake activity by PAOs (Liu et al., 2011), thereby explaining the rather low P yield from AS2. The high P yield from AS3 and the relatively high $P_{release}$/$acetate_{uptake}$ ratio (Table 3-2) agree with the high *Accumulibacter* abundance (Figure 3-2). WWTP3 meets the legal discharge limits by stable EBPR operation without the need for additional chemical precipitants. A high PAO activity is in line with the effective uptake of influent P as well as with the efficient hydrolysis of polyP upon acetate supplementation. Welles et al. (2017) and Acevedo et al. (2015) showed that the $P_{release}$/$acetate_{uptake}$ ratio was higher in enriched PAO cultures when the P content of biomass increased. Inferred from that, we hypothesize that AS3 contained a higher level of polyP in sludge biomass that was available for acetate-mediated P re-dissolution (compared to AS1 and AS2). For technical reasons the batch experiments with AS2 were restricted to 60 min, re-dissolving 19% of the total P. During this time, no maximum in P re-dissolution was reached (Figure 3-3A). Thus, P yield can only be directly compared between AS1 and AS3, which also had comparable initial P contents. Over 240 min AS1 yielded 22% while for AS3 56% of the total P were re-dissolved (Table 3-2) According to literature values, polyP accounts for 22–70% of total P at WWTPs employing EBPR and occasional low iron dosage (Fernando et al., 2019; Petriglieri et al., 2021; Staal et al., 2019). Our P yields fall within this range.

3.3.3 Implications for P recovery and envisaged process integration

Here we studied the re-dissolution of AS-borne P upon acetate supplementation over treatment times between 1 and 4 h. Li and Li (2017) reported a re-dissolution of around 45% during a four-day acidogenic co-fermentation in a side stream system at low pH. Shorter treatment times are a clear benefit of our system in terms of a possible implementation as a P recovery strategy. Overall, we could re-dissolve 19–56% of total P. Higher re-dissolution rates of up to 83% were obtained with lab-enriched PAO sludge in a SBR with synthetic wastewater and carbon source supplementation (Acevedo et al., 2015; Xia et al., 2014). Practical re-dissolution rates at full-scale WWPTs range from 40% to 60% (Kabbe and Rinck-Pfeiffer, 2019). Such rates were obtained upon a (thermo)hydrolytical sludge treatment or after extended anaerobic treatment which provide a disintegration of the sludge. The process output can thus not reasonably be returned to the wastewater treatment but needs to be disposed of. In line with literature data, our P yields were higher for pure EBPR sludge (AS3) than for sludge from the chemically enhanced EBPR processes (AS1, AS2). As shown for AS3, re-dissolution may even meet the 50% recovery target stated by the German Sewage Sludge Ordinance (AbfKlärV, 2017). These findings suggest that pure EBPR systems may be suited for the implementation of a P recovery strategy that makes use of the fact that acetate supplementation of the AS triggers re-dissolution of P within a reasonable response time. The process can potentially be implemented as a side stream system after the aerobic treatment and allows for a cycling of sludge after re-dissolution as shown in Figure 3-5. Downstream processing of the re-dissolved P would have to include a phase separation (e.g. by sedimentation) followed by precipitation (crystallization e.g. as calcium phosphates by lime addition).

The P-depleted sludge could potentially be cycled back to the anaerobic/aerobic treatment of the WWTP and excess sludge can be transferred to the anaerobic digestion. Based on principles of EBPR we hypothesize that repeated recovery cycles may positively affect the aerobic P uptake as well as anaerobic PHA accumulation. By returning the AS to the wastewater treatment in subsequent aerobic conditions, previously stored PHAs are used by PAOs for growth, glycogen production and accumulation of P from the influent to replenish the depleted polyP pool. Consequently, if a higher amount of intracellular PHA is available to provide energy, P uptake is expected to increase, which may gradually lead to a more efficient biological P removal.

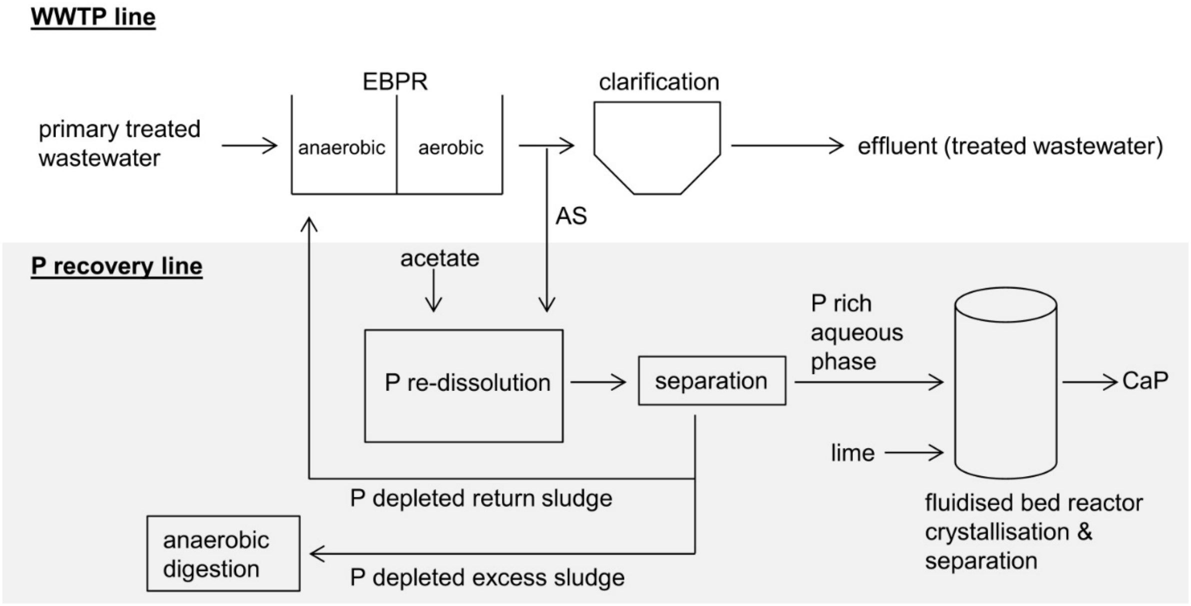


Figure 3-5| Schematic of the envisaged on-site integration in the wastewater treatment process. P re-dissolution from activated sludge (AS) and subsequent P recovery by crystallization of P as calcium phosphate (CaP) in a fluidized bed.

Further, improved EBPR efficiency could reduce the usage of chemical precipitants in WWTPs. This would not only be beneficial in terms of precipitant costs, sludge volume and disposal costs but would also improve P recovery potential. As a side benefit a reduced nutrient backflow with digester supernatant can be expected, thereby reducing the risk of un-controlled struvite precipitation A cost estimate for the re-dissolution process was derived from the P yield observed in batch AS3 and the corresponding consumption of acetate. Using the price for technical grade sodium acetate trihydrate (1,3 €/kg; Möller Chemie GmbH & Co. KG, Germany), treatment costs for one tonne AS (dry matter) amount to 210 € leading to a P yield of 21 kg P/$t_{AS\ TSS}$. When employing Ph. Eur., USP grade sodium acetate trihydrate (3 €/kg; WHC GmbH, Germany) costs would increase to 484 € for one tonne AS (dry matter). Additional costs will occur for the recovery of the re-dissolved P from the aqueous phase. Although the process seems uneconomical against the conventional manufacturing of P fertilizers it could allow for the recycling of P as demanded by the German Sewage Sludge Ordinance.

3.4 Conclusions

The AS of three German municipal WWTPs was investigated in terms of P re-dissolution upon acetate supplementation. Results show that it was possible to re-dissolve P within four hours from all AS, but the process efficiency depended on the plant operation mode.

The P yield increased with *Accumulibacter* abundance. Low P yields in CPR enhanced sludge (AS1) were likely the effect of a low polyP content. This suggests that microbial P re-dissolution was limited by prior application of chemical precipitants. Best re-dissolution results were obtained with pure EBPR sludge (AS3). Therefore, in such systems, P re-dissolution by a short-term anoxic/anaerobic acetate treatment of AS may be an environmentally friendly P recycling strategy.

Long-term effects of repeated P re-dissolution cycles on the stability of the EBPR process remain to be investigated in future studies. Ongoing work focuses on the implementation and operation of a pilot plant at a full-scale municipal WWTP.

CRediT authorship contribution statement

Annika Anders: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing | **Harald Weigand:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition | **Harun Cakir:** Project administration, Funding acquisition | **Ulrich Kornhaas:** Methodology, Resources | **Harald Platen:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 4

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

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[7] For consistency, the formatting and orthography, including citation style, of the following article have been adapted to the dissertation style.

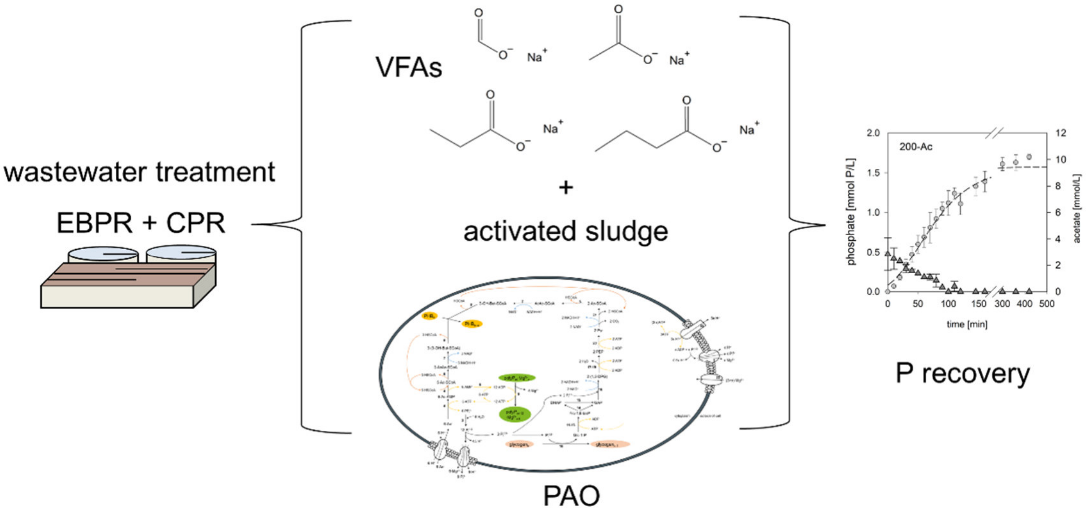
Full research article:

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

Annika Anders[a,], Harald Weigand[a], Harald Platen[a]

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

Graphical Abstract



Keywords

Activated sludge, enhanced biological phosphorus removal (EBPR), phosphorus re-dissolution, phosphorus recovery, polyphosphate accumulating organisms (PAO), volatile fatty acids (VFA), wastewater

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† Electronic supplementary information (ESI) available.

Abstract

Municipal sewage sludge is a sink for wastewater-borne phosphorus (P) and a source for P recovery. Many wastewater treatment plants (WWTPs) employ the enhanced biological P removal (EBPR) which relies on the ability of polyphosphate accumulating organisms (PAOs) to store P in the biomass. Inversion of EBPR may provide a tool for on-site P recovery from activated sludge (AS). Key features of anaerobic P release and the metabolism of acclimated PAOs are well known from laboratory experiments. However, uncertainty persists regarding the behavior of non-acclimated sludge which hampers the practical implementation of P recovery. In this light, we revisited the effects of volatile fatty acid supplementation (formate, acetate, propionate, and butyrate) on the anaerobic P re-dissolution from non-acclimated AS of a full-scale WWTP. All supplementations induced P re-dissolution but the highest re-dissolution was observed with acetate (1.54–1.68 mmol P/L) with a $P_{yield}/VFA_{consumed}$ ratio of 0.45. For AS with 6.3 g_{TSS}/L, a supplementation level of 200 mg/L acetate was most efficient. Recovery amounted to 21–24% of total P within 300 min. Surprisingly, P re-dissolution continued even after acetate had been fully consumed. From the energetic viewpoint, this seems contradictory. Therefore, we integrated the process stoichiometry with known metabolic pathways accounting for the main electron, energy, carbon and P flows for the acetate-induced P re-dissolution. Results show that induction of anaerobiosis in AS from the EBPR process is, indeed, a viable technical option for P recovery. Yet, efficiency needs to be improved since P re-dissolution was either limited by acetate uptake capacity or by available polyphosphate.

Water impact

Sustainable nutrient recovery from wastewater is crucial and the German Sewage Sludge Ordinance enforces phosphorus recovery by 2029. Therefore, we investigated the potential of a biological phosphorus re-dissolution from activated sludge by carbon supplementation for crystallization of a phosphorus fertilizer in a fluidized bed reactor. Results highlight persisting limitations as well as perspectives for full-scale technology implementation.

4.1 Introduction

On a global basis, each year around 1.3 Mt of sewage-borne phosphorus (P) are removed in wastewater treatment plants (WWTPs) (Li and Li, 2017). To avoid eutrophication of water bodies and to comply with regulatory P discharge values, many WWTPs have either implemented a chemical P precipitation (CPR, chemical phosphorus removal) or an enhanced biological phosphorus removal (EBPR) process. Thereby, influent P is removed from the aqueous phase and incorporated in the solid sludge.

In the EBPR process, P removal is achieved by alternatingly cycling the activated sludge (AS) through aerobic and anaerobic zones. Ortho-phosphate (ortho-P) from the influent wastewater is used by sludge-borne microorganisms for metabolism and growth. According to the classical understanding of the EBPR process, polyphosphate accumulating organisms (PAOs) intracellularly store excess P as polyphosphate (polyP) under aerobic conditions (Fuhs and Chen, 1975; Mino et al., 1998). Energy for P uptake is provided by oxidation of carbon sources from the wastewater or by degradation of stored polyhydroxyalkanoates (PHAs). Under anaerobic conditions, PAOs can hydrolyze polyP as an energy source and ortho-P is released into the aqueous phase. Simultaneously, volatile fatty acids (VFAs), such as acetate, are taken up for PHA replenishment (Comeau et al., 1986; Mino et al., 1998). The reducing equivalents required for PHA formation may be obtained from glycolysis or from the tricarboxylic acid (TCA) cycle (Comeau et al., 1986; Martín et al., 2006; Mino et al., 1987). Repeated cycling of the AS through aerobic/anaerobic conditions leads to an enrichment of PAOs in the sludge providing the basis for an efficient EBPR process.

Candidatus Accumulibacter and *Tetrasphaera* are two important genera of PAOs in full-scale EBPR systems (Fernando et al., 2019; Stokholm-Bjerregaard et al., 2017). Both accumulate polyP, yet based on individual metabolic properties they likely occupy different ecological niches within the EBPR consortium (Kong et al., 2004; Kristiansen et al., 2013). In the case of *Tetrasphaera* spp. several authors have observed an additional fermentation and denitrification capability and a more versatile substrate utilization without PHA storage (Kong et al., 2005; Kristiansen et al., 2013; Nguyen et al., 2011). This differs from the classical PAO metabolism of *Candidatus* Accumulibacter spp. (henceforth called *Accumulibacter*) described above. Moreover, the metabolic versatility of PAOs has been shown to depend on operational and physiological conditions, such as pH, availability of influent P, source of reducing power as well as biomass polyP and glycogen content (Acevedo et al., 2017; Oyserman et al., 2016; Welles et al., 2017). A broad range of carbon sources (VFAs, amino acids, sugars), as well as the accumulation of storage polymers such as polyP, glycogen, PHA and yet unidentified storage reserves, can play a role in metabolic behavior (Kristiansen et al., 2013; Lemos et al., 1998). This illustrates the complexity of metabolic processes taking place in the AS.

As P is eliminated from the sewage by incorporation into the solid phase, the AS could be used as a gateway for P recovery instead of directly applying it to farmland. In Germany, such sustainable P recovery strategies have gained importance since the 2017 amendment of the Sewage Sludge Ordinance. The regulation obliges large WWTPs to recycle P from their process streams as of 2029 and bans the direct application of AS to farmland by WWTPs serving >50,000 population equivalents (AbfKlärV, 2017). Current approaches focus either on the ash derived from the mono-incineration of sewage sludge or aim at a P re-dissolution and selective precipitation after chemical or thermal hydrolysis of the sludge (Kabbe and Rinck-Pfeiffer, 2019). Compliance with the mandatory P recovery rates of 80% and 50%, respectively, requires a relatively high energy input and consumption of additional chemical compounds (Amann et al., 2018; Kabbe and Rinck-Pfeiffer, 2019). Thus, sustainability and cost-efficiency of such processes may be questioned. In this light, the fundamentals of EBPR may be used in an alternative engineering approach where the biological P uptake process is simply inverted to provide a P-rich aqueous phase for further processing. Acevedo et al. (2015) and Xia et al. (2014) have shown that it is possible to re-dissolve high amounts of intracellularly stored P from laboratory PAO-enriched sludge by supplementation of a carbon source. Based on the consumption of acetate, in a previous study, we delineated treatment costs for such a process in the range of 210–484 € per tonne of AS dry matter (Anders et al., 2021).

Advantages include the easy on-site integration of the re-dissolution process at WWTPs, reduction of transport costs, recycling of P-depleted AS into the aerobic stage to re-new aerobic P uptake, and chemical precipitant savings. Further, reduced sludge volume may help to increase storage capacities and cut disposal costs. Most importantly, in accordance with the German Sewage Ordinance the P-depleted sludge can be disposed of by co-fueling (e.g. in cement kilns) and thus thermal disposal is no longer bound to mono-incineration with posterior P recovery from the ash.

Using the biological P re-dissolution as a strategy for P recovery at full-scale WWTPs entails the identification of a suitable carbon source. P re-dissolution and VFA utilization are interdependent and PAOs may synthesize different PHAs from individual VFAs (Lemos et al., 1998). It is well known that P can be re-dissolved from the intracellular polyP pool of *Accumulibacter* by supplementation of acetate (Fernando et al., 2019; Kong et al., 2004). However, uptake capacity and preference for certain carbon sources often depend on the habitat of the AS organisms and prevailing environmental conditions. Therefore, substrates may not be universally suitable for an extensive P re-dissolution in full-scale WWTPs. As opposed to laboratory-acclimated PAO-sludge, in full-scale EBPR processes, microbial competition and interaction within the heterogenic AS community play an important role and will likely affect P re-dissolution efficiency. Specifically, glycogen accumulating organisms (GAOs), often abundant in AS derived from full-scale systems, compete with PAOs for anaerobic VFA uptake without contributing to aerobic P removal as they do not accumulate polyP (Cech and Hartman, 1993; Saunders et al., 2003).

So far, intensive research focused on the mechanisms of EBPR activity and the role of the bacterial communities in P removal. Factors such as pH (Oehmen et al., 2005a), carbon source type (Carvalheira et al., 2014; Oehmen et al., 2005b), temperature (Lopez-Vazquez et al., 2009) and influent composition (Welles et al., 2017) have been studied in the light of EBPR optimization. By contrast, the potential benefits of inverting the process in a sustainable and biological P recovery strategy that can be implemented along the wastewater treatment line remain largely unexplored. A previous study has shown high efficiency of P re-dissolution from AS from pure EBPR systems with acetate supplementation (Anders et al., 2021). However, in full-scale WWTPs operators often secure P removal by additional CPR, thereby potentially affecting activity and behavior of the microbial population (Liu et al., 2011; Röske and Schönborn, 1994). Especially, the usage of iron-based precipitation agents has been recognized to lower the P recovery potential (Wilfert et al., 2015). Due to the strict effluent standards, WWTP operators often have not yet been able to refrain from the addition of chemical precipitants. Thus, investigation of AS biomass from full-scale systems that rely on combined EBPR and CPR is of importance to advance a future implementation of a sludge-based P recovery strategy that makes use of the EBPR anaerobiosis induced by the addition of a carbon source. Because of the high diversity and versatile metabolism of PAOs, different carbon sources may be suitable for a biological P re-dissolution. In a recent publication, Zhang et al. (2022) outlined that full-scale mainstream P recovery from EBPR sludge could be a promising approach for on-site implementation on the medium term. The authors highlighted that future works should among other aspects focus on real municipal wastewater and pointed out the additional costs when EBPR sludge is supplemented with external carbon sources. In terms of costs, it is important not only to determine the effect of carbon source type but also to quantify the required supplementation level. To this end, we revisited the re-dissolution of P from real municipal AS (non-acclimated AS) of a full-scale WWTP under varied carbon supplementation. Formate, acetate, propionate, and butyrate were evaluated as these are economical carbon sources that can be present in the WWTP influent or can be produced by hydrolysis and pre-fermentation processes. A series of batch tests with freshly sampled EBPR-CPR sludge served to study the effect of the carbon source type on P recovery and to identify its optimal dosage.

4.2 Materials and methods

Laboratory studies regarding the P re-dissolution upon VFA supplementation were conducted with AS collected at a WWTP located in central Germany. The plant has a capacity of 300,000 population equivalents and uses the EBPR process. To comply with the strict discharge limits for P set out by the European Water Framework Directive as well as the regional effluent standards (HMUKLV, 2015) of 0.4 mg/L total P, the plant has additionally implemented a CPR step where ferrous chloride is added to the aeration tanks. All AS samples were taken as a mixed liquor AS suspension at the outlet of the aerated biological treatment stage before the sludge is transferred into the settling tanks. Biological replicates were obtained by sampling AS at different dates (on eight occasions) during the months of August, October and November in 2019 and 2020 thereby accounting for sludge variability. The *in situ* temperature of the sampled AS was 18–19 °C, throughout. Periods of heavy rainfall or drought and out-of-line events of WWTP operation were excluded from sampling. The re-dissolution experiments were performed within one hour after sampling to prevent changes in bacterial activity and composition during storage.

All samples were characterized in terms of total suspended solids (TSS) as well as total P, iron, calcium, potassium and magnesium content according to analytical methods described below. Volatile suspended solids (VSS) and chemical oxygen demand (COD) values were reported by the plant staff.

4.2.1 Effect of carbon source on P re-dissolution

Four different VFAs were tested for their impact on P release from AS. A defined volume (5 mL) of VFA stock solution (20 g/L) was added to 500 mL of the AS to obtain a spike concentration of 200 mg/L formate, acetate, propionate, and butyrate, respectively. All VFA were added as sodium salts (purity ≥98–99%) purchased from Merck KGaA, Darmstadt, Germany and Carl Roth GmbH & Co. KG, Karlsruhe, Germany. Experiments were carried out in closed 500 mL Erlenmeyer flasks. Biological triplicates were conducted with independently samples AS and were accompanied by a non-supplemented control. The batches were kept at room temperature for 220 min and were stirred at 100 rpm using a magnetic stirrer to maintain the AS in suspension. The pH was not adjusted. Aliquots were sampled with a syringe for time-resolved determination of P release and VFA uptake.

4.2.2 Effect of acetate supplementation level on P re-dissolution

To investigate a potential effect of the carbon supplementation level, AS was supplemented with 100–600 mg/L acetate added as sodium acetate trihydrate (p.a., purity ≥99%, Merck KGaA, Darmstadt, Germany) employing the setup described in section 5.2.1 but using a volume of 1 L AS. Acetate was added to the AS as a concentrated stock solution (20 g/L). Additionally, the pH, redox potential (E_H) and dissolved oxygen (DO) concentration were monitored using WTW Multi 3630 IDS (WTW, Xylem Analytics, Weilheim, Germany) equipped with SenTix®940, SenTix®ORP-T900 and FDO®925 sensors. The ortho-P and acetate concentrations in the aqueous phase were followed over time. Experiments were performed as five biological replicates with acetate concentrations of 100–400 mg/L and in biological triplicates with 600 mg/L acetate to check for reproducibility. AS without acetate supplementation served as a control.

4.2.3 Analytical methods

Ortho-P was determined in 0.45 µm filtered samples using the molybdenum blue method (APHA, 1999). The VFA concentration was determined using an ion chromatograph (Metrohm 861 Advanced Compact IC). Sample splits of 20 µL obtained after 0.2 µm membrane filtration were separated with a 1.0 mmol/L $NaHCO_3$/3.2 mmol/L Na_2CO_3 eluent on a Metrosep A Supp 5 column (150 mm x 4.0 mm, particle size 5 µm, Metrohm, Herisau, Switzerland). Runs were conducted at a flow rate of 0.7 mL/min and had a duration of 20 min. Detection took place after chemical suppression by measuring conductivity. The samples' signals were aligned according to the retention times of external standards. TSS was determined according to the APHA standard method (APHA, 1999). Homogenized sludge biomass was dried at 105 °C for 24 h and ground with a ball mill prior to aqua regia digestion according to the European standard procedure DIN EN 16174:2012-11 (CEN, 2012).

Total phosphate in the digestates was determined as ortho-P as described above. The iron, calcium, potassium and magnesium concentration in the digestates was determined by atomic absorption spectroscopy (acetylene/air-flame, AAnalyst 100, Perkin Elmer, Waltham, MA, USA).

4.2.4 Data evaluation

A modified version of the Gompertz model (Zwietering et al., 1990) was fitted to the P release curves to describe the changes in the P release rate (Eq. (4-1)). The model has been previously used to fit sigmoidal kinetics in different research areas from bacterial growth to microbial product formation and re-dissolution processes (Easton, 2002; Li et al., 2005; Wang and Wan, 2009; Zwietering et al., 1990). In Eq. 4-1, the term A_{max} [mmol P/L] represents the upper asymptote of P release (saturation value), µ [mmol P/(L×min)] the maximum release rate, λ [min] the lag phase, and t [min] the time after supplementation. Model-fits were obtained by minimizing the sum of squared residues between observed and model-predicted values using the MS Excel SOLVER add-in. Quality of the model-fit was assessed using the coefficient of determination (R^2).

$$P_{i,rel}(t) = A_{max} \cdot \exp\left\{-\exp\left[\frac{\mu \cdot \exp(1)}{A_{max}}(\lambda - t) + 1\right]\right\} \tag{4-1}$$

4.3 Results and discussion

In the investigated WWTP, P is removed from the influent in an EBPR process supported by the additional dosage of ferrous chloride to meet the strict effluent standards. The P content in the final stabilized sewage sludge exceeds 20 mg P/g_{TSS}. Thus, the WWTP falls under the obligation to recover P as of 2029 (AbfKlärV, 2017).

During the sampling period, the mixed liquor AS suspension from the outlet of the aerated biological treatment stage had a stable TSS concentration of (6.3 ± 0.5) g/L and the VSS/TSS ratio was 0.65. The COD (determined in the supernatant of WWTP settling tanks after aerobic treatment) was around 17 mg/L. The dissolved ortho-P concentration of the aqueous phase of AS was (0.10 ± 0.03) mg P/L. Acetate was not detected in the aqueous phase. Total P, Fe, Ca, K and Mg content in the AS dry matter was (36.21 ± 3.39) mg P/g_{TSS}, (40.83 ± 10.84) mg Fe/g_{TSS}, (16.14 ± 5.89) mg K/g_{TSS}, (15.11 ± 0.95) mg Ca/g_{TSS}, and (6.60 ± 2.77) mg Mg/g_{TSS}, respectively.

4.3.1 Effect of carbon source on P re-dissolution

We examined the short-term effects of different VFAs on the P re-dissolution from AS under laboratory conditions. VFAs were supplied to the AS batches as 200 mg/L spikes. The pH of the AS suspension was around 7.05 ± 0.08 at all times.

The P re-dissolution kinetics observed upon supplementation of the different carbon sources are shown in Figure 4-1A and the parameters of the Gompertz model are summarized in ESI† Table S4-3. The error bars indicate only small variation among the biological replicates. The non-supplemented control showed a minimal P release of 0.03 mmol P/L within the experimental period and no VFAs (formate, acetate, propionate or butyrate) were detected. By contrast, all VFA-spiked batches released P in the form of ortho-P. The P re-dissolution decreased in the order acetate > propionate > butyrate > formate. In the acetate-supplemented batches, P release started immediately after spiking and exhibited a high release rate of 17.20×10^{-3} mmol P/(L×min) (see values of μ in ESI† Table S4-3). For propionate, butyrate and formate spiking resulted in lower P release rates of 4.29×10^{-3}, 2.58×10^{-3} and 1.41×10^{-3} mmol P/(L×min), respectively. The onset of P release was delayed by a 15–30 min lag phase (see values of λ in ESI† Table S4-3). Substrate consumption varied with the type of carbon source (Figure 4-1B). While acetate was rapidly and entirely consumed, the cumulative uptake of propionate, butyrate and formate amounted to 55%, 34%, and 15% of the supplementation level, respectively. Complete acetate consumption suggested that under anaerobic conditions all of the carbon source was utilized by PAOs or the competing GAO population. This is in accordance with literature reporting on acetate utilization during anaerobic cell maintenance of *Accumulibacter* (Nielsen et al., 2010; Oehmen et al., 2010) and supports the findings of Lemos et al. (1998) on the suitability of acetate for PHA production. High P re-dissolution upon acetate supplementation agrees with the abundance of this VFA in the influent of WWTPs (López-Vázquez et al., 2007; Oehmen et al., 2005b) whereby sludge organisms are likely adapted to utilize this carbon source.

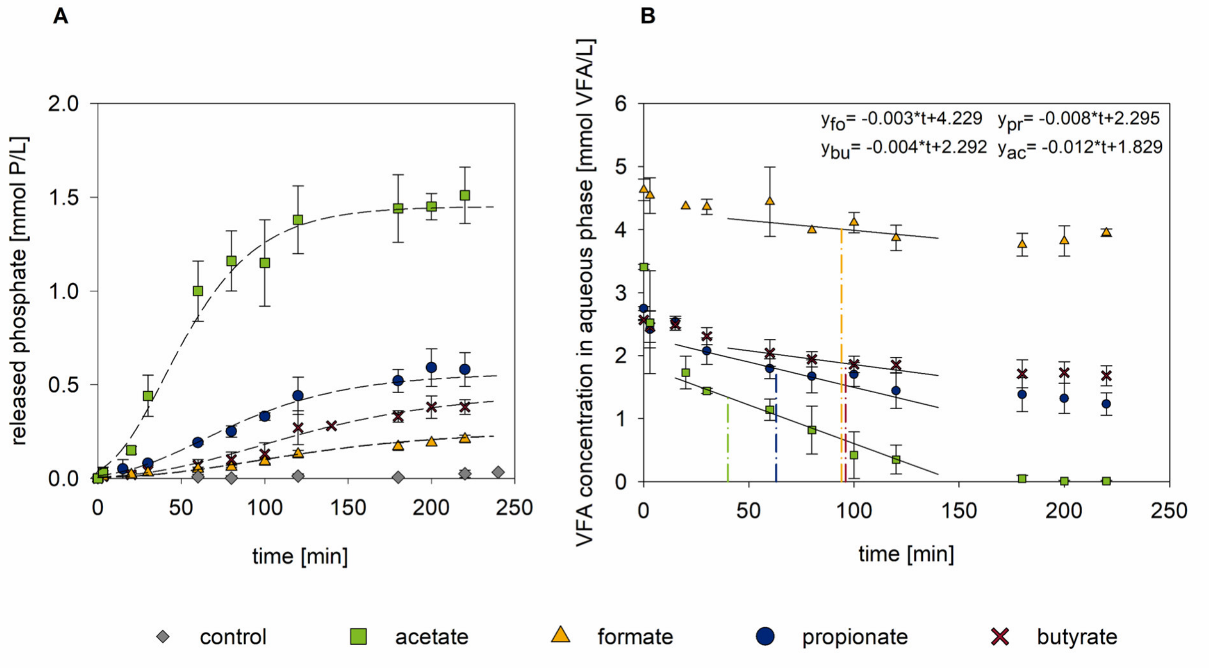

Figure 4-1| Kinetics of P re-dissolution (A) and VFA uptake (B) from activated sludge individually supplemented with 200 mg/L formate, acetate, propionate and butyrate, against a non-supplemented control. Measured data are represented as symbols (mean ± standard deviation; biological triplicates). (A) Modeled P release is represented by the dashed line. (B) Dash-dotted drop-down lines mark the time of maximum P release rate μ deduced from the P re-dissolution kinetics. The corresponding VFA concentration used to derive the $P_{release}/C_{uptake}$ ratios was obtained from the intersection of the drop-down lines with the linearly approximated VFA time profile (fo: formate, ac: acetate, pr: propionate, bu: butyrate).

Performance of P re-dissolution in the inverted EBPR system

Ratios of P release and substrate utilization are common indicators in P re-dissolution studies that characterize the EBPR performance and relative significance of the polyP- and glycogen-based metabolisms. Yet, in many cases the calculation of the indicators is unclear. Here, we compared three different performance indicators (Table 4-1). On the one hand, the molar ratio of P release to carbon uptake ($P_{release}/C_{uptake}$) was evaluated at the timing of the maximum P release rate μ (see Figure 4-1B and ESI† Table S4-3). The indicator expresses the apparent P re-dissolution stoichiometry. On the other hand, the spike efficiency was evaluated as the ratio of the cumulative P yield to the initial VFA spike level (P_{yield}/VFA_{spike}) as well as to the total VFA consumption ($P_{yield}/VFA_{consumed}$). The latter ratios are indicative of the cost-benefit state. For a P recovery process, it would be economically beneficial to release high levels of P from the AS within a short time and at maximal utilization of the available substrate. Detailed information on indicator calculation can be found in the ESI†.

Table 4-1| Indicators of the P re-dissolution performance with different VFA supplementations.

Supplemented VFA	**$P_{release}/C_{uptake}$ [mol P/mol C]$^{a, b}$**	**P_{yield}/VFA_{spike} [mol P/mol VFA]^{b}**	**$P_{yield}/VFA_{consumed}$ [mol P/mol VFA]^{b}**
formate	0.15	0.05	0.31
acetate	0.13	0.45	0.45
propionate	0.07	0.21	0.39
butyrate	0.06	0.15	0.43

[a] calculated at the timing of maximum P release rate μ (model-fit) obtained with the second derivative

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

[b] For further information on indicator calculation please refer to the ESI †.

The $P_{release}/C_{uptake}$ ratio was higher with formate (0.15 mol P/mol C) and acetate (0.13 mol P/mol C) than with propionate (0.07 mol P/mol C) and butyrate (0.06 mol P/mol C).

In previous studies on full-scale acetate-fed EBPR systems, the $P_{release}/C_{uptake}$ ratio was in the range of 0.29–0.66 mol P/mol C (López-Vázquez et al., 2007; Qiu et al., 2019; Saunders et al., 2003). Compared to this, our $P_{release}/C_{uptake}$ ratio with acetate was lower. This could be due to a higher abundance of GAOs competing with PAOs for available substrate, as members of both lineages have similar acetate uptake rates (López-Vázquez et al., 2007; Oehmen et al., 2005b). Alternatively, carbon consumption by other groups of microorganisms is also possible.

Predominance of GAO over PAO is unlikely given that the abundance of *Candidatus* Competibacter spp. amounts to 2% of the total bacterial population which is clearly below the PAO *Accumulibacter* abundance (9% of the total bacterial population) (Anders et al., 2021). More importantly, acetate uptake in combination with glycogen catabolism for PHA formation is not exclusive to GAOs, but also PAOs can utilize the glycogen pool depending on their metabolic state and energy storage pool (Acevedo et al., 2015; Kolakovic et al., 2021; Majed et al., 2012; Welles et al., 2017). Therefore, in our case, the $P_{release}/C_{uptake}$ may have been limited by low polyP hydrolysis and a mixed polyP-/glycogen-based metabolism of PAOs rather than by the competing GAOs themselves. Generally, the $P_{release}/C_{uptake}$ indicator is suited to characterize within-study effects of substrate utilization rather than for comparisons among different studies. In the latter case, comparability may be particularly hampered if microbial community data are lacking.

With propionate, the $P_{release}/C_{uptake}$ ratio was only half that of acetate and the rate of propionate uptake was lower (acetate: 42.73 µmol C/(L×min), propionate: 26.26 µmol C/(L×min) during the first 2 h). The reduced rate of propionate utilization is in line with the inability of *Candidatus* Competibacter spp. to take up propionate at standard conditions (20 °C, pH 7) (Carvalheira et al., 2014; Oehmen et al., 2005b), while *Accumulibacter* PAOs possess a similar preference towards uptake of both acetate and propionate (Oehmen et al., 2005c).

The P release rate was further reduced in the case of supplementation with formate and butyrate, with similar profiles throughout the experiment. Only few studies have examined the effect of formate on P re-dissolution (Abu-Ghararah and Randall, 1991; Burow et al., 2007; Kong et al., 2004) and anaerobic formate utilization by PAOs has so far been ruled out (Kong et al., 2004). In our study, uptake of formate went along with moderate P re-dissolution, indicating PAO activity. Direct formate utilization for polyhydroxybutyrate (PHB) synthesis by PAOs is unlikely since formate would need to undergo carboxylation to form acetyl-CoA as a precursor (Abu-Ghararah and Randall, 1991). We hypothesize that formate was metabolized to PHA via the glycine-serine interconversion through glycine and acetyl-CoA. The reductive glycine pathway is a possible route for carbon fixation in formate assimilating organisms (Bar-Even, 2016). Recently, it was shown that glycine can induce P release from PAOs (Nguyen et al., 2015; Oyserman et al., 2016; Tian et al., 2022). *Accumulibacter* (Oyserman et al., 2016) and *Tetrasphaera* spp. (Nguyen et al., 2015) were able to consume glycine with and without PHA synthesis, respectively. Oyserman et al. (2016) found that acetate induced the expression of genes involved in the anaerobic glycine metabolism in *Accumulibacter.* In our case, these genes may have been readily active at the time of AS sampling and may have allowed formate utilization. Otherwise, formate itself may have induced gene expression. Low uptake of formate might be explained by feedback inhibition to avoid high intracellular concentrations of substrate or conversion products.

For butyrate, an initial adaptation period (lag-phase) preceded P re-dissolution. While the butyrate concentration decreased at a rate of 25.5 µmol C/(L×min) during the first 2 h, P release started 30 min after supplementation. Butyrate may directly be used for PHB formation or indirectly via intermediates produced by *Tetrasphaera* spp. or other sludge bacteria (Kristiansen et al., 2013). In any case, concomitant P re-dissolution can be expected. Dependence of the involved lineages may also explain contradictory results regarding the utilization of butyrate in previous literature (Kong et al., 2004; Lemos et al., 1998). In our study, the batches of the butyrate supplementation were devoid of other VFA, which may indicate that potential by-products like acetate were immediately taken up. Since the acetate uptake rate was most likely higher than its production from butyrate, the lack of observable VFA formation within the experimental time frame of 220 min is reasonable.

Literature suggests that substrate uptake rates and utilization efficiencies differ not only due to PAO-GAO competition but also within specific groups and lineages within either type of organism (Oehmen et al., 2010). This precludes the elucidation of metabolic pathways when real and non-acclimated AS is studied, as was the case in our study. Under these conditions the $P_{release}/C_{uptake}$ ratio may be ambiguous. Regarding the cost-benefit state of a full-scale process implementation, the P_{yield}/VFA_{spike} as well as the total $P_{yield}/VFA_{consumed}$ may be useful indicators. Spike efficiencies of formate, acetate, propionate and butyrate were 0.05, 0.45, 0.21 and 0.15 mol P/mol VFA_{spike}, respectively (Table 4-1). Compared to acetate, spike efficiency was reduced by 53–89% for the other tested substrates. The ratio of $P_{yield}/VFA_{consumed}$ decreased in the order acetate > butyrate > propionate > formate. For acetate and propionate, the values of P_{yield}/VFA_{spike} and $P_{yield}/VFA_{consumed}$ were close. We propose that this could be used as an indication for P re-dissolution combined with efficient substrate usage. Identical values for acetate confirm the superiority of this VFA in terms of P release and substrate utilization. From an engineering perspective and regarding process economics we suggest the use of P_{yield}/VFA_{spike} and $P_{yield}/VFA_{consumed}$ in future studies on the optimization of P recovery from AS.

4.3.2 Effect of acetate supplementation level on P re-dissolution

Having seen that supplementation with acetate yielded the highest P re-dissolution from AS, we studied the effects of acetate dosage by varying the spike levels between 100 and 600 mg/L, henceforth referred to as 100-Ac, 200-Ac, 400-Ac and 600-Ac. Irrespective of the initial acetate spike level, the pH and DO concentration were comparable among the batches and were broadly constant over time. The pH was between 6.8 and 7.1 and the DO was virtually zero. The E_H dropped from 320–200 mV (values reported against standard hydrogen electrode) to below 50 mV within 60 min of incubation indicating that, ultimately, anaerobic conditions prevailed in all batches.

All spike levels induced a re-dissolution of intracellular P into the aqueous phase (Figure 4-2). Variation among the biological replicates of the same spike level was small, demonstrating that independent samples collected during the normal WWTP operation responded reproducibly. This is important in terms of a potential full-scale implementation as a P recovery scheme. The control batch without acetate supplementation exhibited no P release over a period of 300 min but showed low concentrations of dissolved P towards the end of the experiment (Control, Figure 4-2). The latter may have resulted from cell decay. In agreement with previous studies (Acevedo et al., 2015; Oehmen et al., 2005b), we observed sigmoidal P re-dissolution curves (Figure 4-2). Again, P release kinetics could be satisfactorily described using the modified Gompertz model (Eq. (4-1)). The best-fit parameters are shown in Table 4-2. The modeled maximum P release rate μ was 0.02 mmol P/(L×min) for 100-Ac and 0.01 mmol P/(L×min) for 200-Ac through 600-Ac. As opposed to the sigmoidal P re-dissolution, the acetate uptake in 100-Ac and 200-Ac was broadly linear over time and led to a full consumption of the spike at a rate of 26 μmol acetate/(L×min). Full acetate consumption occurred within 60 min and 100 min, respectively. In 100-Ac the fast depletion resulted in a short-term P release from AS. Increasing the acetate spike from 100 mg/L to 200 mg/L led to a prolonged and two times higher P re-dissolution. The maximum P yield of (1.54 ± 0.09) mmol P/L was reached after 300 min of incubation. Increasing the acetate spike beyond 200 mg/L (400-Ac and 600-Ac, Figure 4-2) did not increase the P yield further nor did it result in faster release kinetics. In the respective batches, more than 50% of the supplemented acetate remained in the aqueous phase at the end of the experiment (no substrate limitation). Inhibitory effects due to undissociated acetic acid are unlikely since the circumneutral pH of the batches was above its pK_a of 4.76.

Table 4-2| Best-fit parameters (I [0 min; 420 min]) of the modified Gompertz model for acetate dosage-dependent P re-dissolution from activated sludge.

	100-Ac	**200-Ac**	**400-Ac**	**600-Ac**	**Control**
A_{max} [mmol P/L]	0.73	1.54	1.68	1.63	0.92
μ [mmol P/(L×min)]	0.02	0.01	0.01	0.01	0.002
λ [min]	7.72	5.76	0.98	0.88	309.80
R^2	0.997	0.989	0.993	0.985	0.982

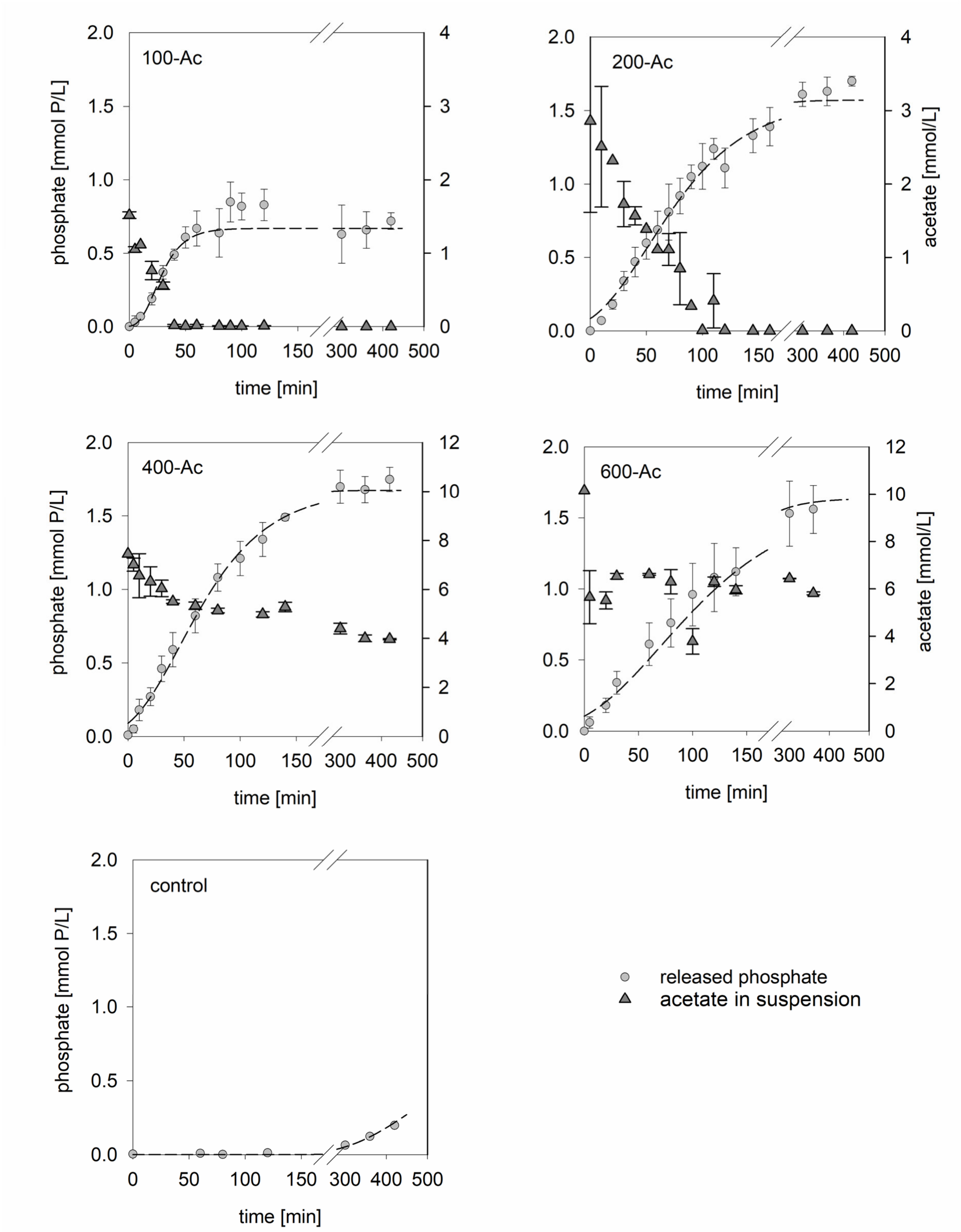

Figure 4-2| Time profiles of P re-dissolution and acetate uptake after supplementation of activated sludge with different acetate spike levels. Activated sludge batches spiked with 100–600 mg/L acetate labeled as 100-Ac, 200-Ac, 400-Ac and 600-Ac. Activated sludge without acetate supplementation is labeled as control. Measured ortho-P (left Y-axis) and acetate concentration (right Y-axis) in the aqueous phase are represented by symbols (values of biological replicates ± standard deviation; n=5 for 100-Ac, 200-Ac, 400-Ac and control; n=3 for 600-Ac). Modeled P release is represented by the dashed line.

Overall, results show, that P re-dissolution was influenced by the acetate spike level but was also limited by the ability of AS to completely take up acetate at higher concentrations. Substrate limiting conditions may have prevailed in 100-Ac. The final P release was very similar for acetate levels equal to or greater than 200-Ac. In 400-Ac and 600-Ac, P re-dissolution may have been limited by acetate uptake capacity or by available polyP content of the cells. In 400-Ac and 600-Ac, acetate uptake leveled off with increasing P re-dissolution. This is in line with previous observations on saturation of uptake and storage capacity for acetate in PAOs after 2–3 h (Kong et al., 2004; Nguyen et al., 2011) and reduction of PHA synthesis at high PHA concentrations (Wang et al., 2016). In total the uptake of around 3 mol acetate yielded 1.5–1.7 mol P. It is likely that the ratio of P release and acetate uptake was influenced by the ratio of PAO and GAO levels in the sewage sludge, as both groups of organisms use acetate but only PAOs release P (Cech and Hartman, 1993; Oehmen et al., 2005b; Saunders et al., 2003). Please note in Figure 4-2 that P re-dissolution and substrate uptake were not synchronous. Specifically, P release continued after acetate uptake ceased or acetate was fully consumed. A similar phenomenon was also reported by Pijuan et al. (2004) and Puig et al. (2008), who observed a secondary P release rate. The authors hypothesized that this was caused by additional polyP hydrolysis to cover energy demands of cellular maintenance. However, in our case this explanation is not valid since the control batch exhibited increasing levels of P release after 300 min, only. Therefore, we propose the following model.

Model of metabolite flows during anaerobic P re-dissolution

It seems counter-intuitive that P re-dissolution extended beyond the uptake of acetate (Figure 4-2). To explain this observation, we revisited the interplay of known metabolic pathways in *Accumulibacter* and integrated a detailed stoichiometric balance of carbon, P, reducing equivalents and ATP flows (Figure 4-3). The physiological pH of the bacterial cell and medium was assumed to be around 7. Based on the coexistence of $H_2PO_4^-$ and HPO_4^{2-} this results in an average P_i charge of -1.5. However, the current understanding of the low affinity inorganic phosphate transporter (pit) demands that HPO_4^{2-} (P_i^{2-}) is excreted from the cell (Martín et al., 2006; Oyserman et al., 2016; van Veen et al., 1994). In supplementary experiments (data not shown), we observed an increase in Mg^{2+} levels in the aqueous phase during acetate-induced P re-dissolution. Concomitantly Mg^{2+} concentration in the AS dry matter decreased. Therefore, the negative charge is counterbalanced by co-excretion of polyP-borne Mg^{2+}.

The observed P re-dissolution is primarily a result of ATP/AMP conversion during acetate assimilation and acetyl-CoA formation. Therein, each mol acetate consumes 1 mol ATP, obtained by cleavage of polyP units. The by-product pyrophosphate is hydrolyzed to P_i, which is released from the cell or used for glycogen degradation. Acetate uptake occurs through an acetate permease (ActP) and is driven by the proton motive force (pmf) (Burow et al., 2008;

Saunders et al., 2007). It has been suggested that pmf can be generated by proton efflux with transport of P_i and counter cations (e.g. Mg^{2+}) via the low affinity inorganic phosphate transporter (pit) (Martín et al., 2006; Oyserman et al., 2016; van Veen et al., 1994). We suggest that, per mol P, 0.5 mol Mg^{2+} can be obtained from polyP (see ESI†). Figure 4-3 shows that Mg requirements for efflux of P_i cannot be compensated through polyP hydrolysis alone but may require extracellular Mg uptake.

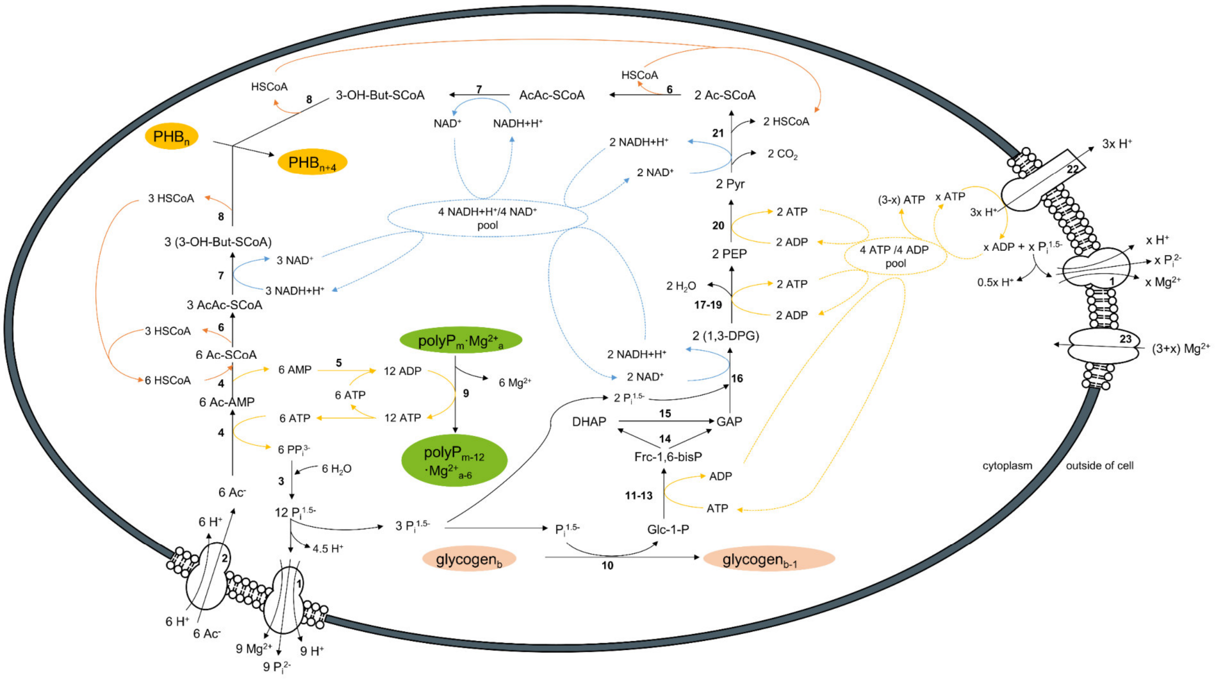

Figure 4-3| Conceptual model and integrated balance for the main flows of carbon, P, reducing equivalents and energy during polyP hydrolysis and glycogen degradation for PHB synthesis during acetate-induced P re-dissolution in PAOs. Carbon and P routes are presented in black, flows of NAD^+/NADH + H^+ and AMP/ADP/ATP are presented in blue and yellow, respectively. Numbers indicate involved enzymes and transporter proteins (see ESI† Table S4-4). Abbreviations: 3-hydroxybutyryl-CoA (3-OH-But-SCoA), 1,3-bisphosphoglycerate (1,3-DPG), acetate (Ac), acetoacetyl-CoA (AcAc-SCoA), acetyl-AMP (Ac-AMP), acetyl-CoA (Ac-SCoA), coenzyme A (HSCoA), dihydroxyacetone phosphate (DHAP), fructose-1,6-bis-phosphate (Frc-1,6-bisP), glucose-1-phosphate (Glc-1-P), glyceraldehyde 3-phosphate (GAP), phosphoenolpyruvate (PEP), pyruvate (Pyr).

Reducing equivalents are required for 3-Hydroxybutyryl-CoA formation from 2 mol acetyl-CoA and elongation of one unit of the PHB polymer. These could potentially originate from the TCA cycle and/or from glycolysis (Comeau et al., 1986; Martín et al., 2006; Mino et al., 1987). Maintenance of the TCA cycle under anaerobic conditions requires a re-oxidation of quinone, e.g. by a proposed novel cytochrome complex (Martín et al., 2006). Alternatively, here we focused on glycogen degradation as a source for reducing equivalents. Therein, degradation of 1 mol of glucose produces 3 mol of NADH + H^+. These are available for PHB formation from assimilated acetate. During glycogen degradation to pyruvate by the Embden-Meyerhof-Parnas pathway 3 mol ATP are provided if glucose and glyerinealdehyde-3-phosphate are phosphorylated with P_i originating from polyP degradation. A maximum of 3 ATP is available for cellular maintenance such as generating pmf by reverse H^+-ATPase activity and additional P release. Intracellular accumulation of ATP may eventually lead to inhibition of glycogen degradation.

We propose the following chemical equation (4-2) for the conversion of educts

$$6Ac^-_{(out)} + 6H^+_{(out)} + polyP_m{\cdot}Mg^{2+}_{a(in)} + glycogen_{b(in)} + PHB_{n(in)} \rightarrow (9+x)P_i^{2-}{}_{(out)} + 6Mg^{2+}_{(out)} + (9+4x)\,H^+_{(out)} + polyP_{m-12}{\cdot}Mg^{2+}_{a-6(in)} + glycogen_{b-1(in)} + PHB_{n+4(in)} \quad (4\text{-}2)$$

where x denotes mol of ATP consumed for proton efflux and 0 ≤ x ≤ 3, indices $_{in}$ and $_{out}$ denoted inside the cell/cytoplasm and outside the cell, respectively.

With Ac^-/H^+ uptake, 6 mol positive charge equivalents are transported into the cell and with P_i^{2-} release 18 to 24 mol negative and 21 to 33 mol positive (H^+ and Mg^{2+}) charge equivalents are transported to the outside, depending on ATPase activity. Compared to the cytoplasm, the outer cell will become positively charged which disrupts cell potential and halts the metabolism. As glycogen degradation is repressed, internal P_i previously used for the induction of glycogen cleavage (Cifuente et al., 2019) can be released thus explaining the P re-dissolution after cessation of acetate uptake. As an alternative to disruption of pmf, the metabolism may also be halted by (i) polyP limitation and subsiding NADH requirement, (ii) glycogen limitation or (iii) ATP accumulation. We propose the above metabolic model as a hypothesis that may explain why P release continued after full substrate consumption or after cessation of substrate uptake. To validate the model, future experiments should include pertinent analyses (e.g. Mg^{2+}, Ca^{2+}, intracellular glycogen concentration, enzyme activity). Possibly, validation also would need to be performed in less buffered systems to verify the proposed net proton release.

4.4 Implications and conclusion

EBPR-CPR sludge from a full-scale WWTP was investigated in terms of P re-dissolution under supplementation with various carbon sources. Results show that acetate had the highest efficiency for a fast P re-dissolution. An acetate spike level of 200 mg/L proved best in terms of substrate utilization and P yield, while higher acetate levels seemed to exhaust the P re-dissolution capacity. For all spike levels, P re-dissolution extended beyond the uptake of acetate. Known metabolic pathways of PAO were integrated into a conceptual model that accounts for the stoichiometric balance of carbon, P, reducing equivalents and ATP flows. The model suggests that the extended P re-dissolution may be explained by the halting of glycogen degradation.

Although additional CPR is believed to hamper P recovery from AS it still allowed for a partial microbial P re-dissolution. Acetate treatment recovered 7.6–8.6 mg P/g_{TSS} or 11.7–13.2 mg P/g_{VSS} which equaled 21–24% of the total sludge P content. With the applied strategy of acetate-induced P re-dissolution, yields are limited to the recycling of P from the intracellular polyP pool at maximum. Our previous results showed, that also with pure EBPR sludge from full-scale plants (excluding the influence of CPR) a complete re-dissolution of sludge P was not possible (Anders et al., 2021). By supplementation of 0.07 g/g_{TSS} (Anders et al., 2021) up to 0.23 g/g_{TSS} (unpublished data) acetate the re-dissolved P yield was 56–60% of total sludge P. This limitation may be a protective mechanism to avoid extensive PAO activity loss. Thus, EBPR performance may be sustained by recycling leached AS into the aerobic stage to re-new aerobic P uptake. A maximum P recovery value of 60% of influent P has been proposed by Zhang et al. (2022) allowing for stable PAO activity and EBPR operation. We hypothesize that repeated re-dissolution cycles may also improve adaption of PAOs to ultimately meet the regulatory P recovery limits, provided the use of chemical precipitants is reduced. This may suggest that in the future WWTP operators reduce the use of chemical precipitants or implement a post-EBPR precipitation step to allow higher P re-dissolution efficiencies and practical application of the process in WWTPs. In terms of substrate availability, a biological re-dissolution approach with acetate seems promising, since acetate is often produced during pre-fermentation or anaerobic sludge digestion. This may be boosted by addition of low-cost carbon sources such as fermentable industrial or food waste.

For on-site integration and final P recovery, the re-dissolution process needs to be followed by a phase separation step (e.g. sedimentation). The P-depleted AS may be returned to the aerobic stage of the WWTP and the P-enriched aqueous phase may be used to precipitate a P-fertilizer. Ongoing work is related to the implementation of a pilot-scale fluidized bed reactor where milk of lime is used to increase the pH and recover a calcium phosphate as proposed in Anders et al. (2021).

CRediT authorship contribution statement

Annika Anders: Conceptualization, Investigation, Formal analysis, Visualization, Writing – Original Draft, Review & Editing | **Harald Weigand:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition | **Harald Platen:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition

Conflicts of interest

There are no conflicts to declare.

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ESI - Supplementary material

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

Annika Anders, Harald Weigand, Harald Platen

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

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Table S4-3| Best-fit parameters (I[0 min; 220 min]) of the modified Gompertz model used to describe the kinetics of P re-dissolution after supplementation of activated sludge with formate, acetate, propionate and butyrate.

	Formate	**Acetate**	**Propionate**	**Butyrate**
A_{max} [mmol P/L]	0.25	1.45	0.56	0.46
μ [mmol P/ (L×min)]	1.41×10^{-3}	17.20×10^{-3}	4.29×10^{-3}	2.58×10^{-3}
λ [min]	28.57	8.49	14.93	29.71
R^2	0.990	0.991	0.991	0.977

Calculation of indicators of the P re-dissolution stoichiometry

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

$P_{release}/C_{uptake}$ ratios were calculated for the time t_μ of the maximum P release rate μ as predicted from the modified Gompertz model (equation 4-1). For each of the different VFA supplementations t_μ was located by equating the second derivative of the modified Gompertz model (equation 4-2) to zero. Employing the best-fit parameters summarized in Table S4-3 t_μ values were 40 min, 63 min, 94 min and 96 min for acetate, propionate, formate and butyrate, respectively. Equation 4-1 was solved for $P_{i,rel}$ (t_μ) to obtain the numerator of the $P_{release}/C_{uptake}$ ratio. Its denominator was found from the corresponding VFA time profile (Figure 4-1B). Therefore, linear regression lines fit to an appropriate time interval were evaluated for the t_μ values. C_{uptake} was calculated from obtained aqueous phase VFA concentration converted in C-mol.

Modified Gompertz Model:

$$P_{i,rel}(t) = A_{max} \cdot \exp\left\{-\exp\left[\frac{\mu \cdot \exp(1)}{A_{max}}(\lambda - t) + 1\right]\right\} \tag{4-1}$$

Second derivative:

$$P_{i,rel}''(t) = \frac{\mu}{A_{max}} \cdot \left\{\exp\left[\frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}}\right] - 1\right\} \cdot \exp\left\{\frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}} + 3 - \exp\left[\frac{\mu \cdot \exp(1) \cdot (\lambda - t)}{A_{max}} + 1\right]\right\} \tag{4-2}$$

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

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

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

$$\frac{P_{Yield}}{VFA_{spike}} = \frac{P_{i,rel,\,t=220\,min}}{VFA_{spike,\,t=0\,min}} \tag{4-3}$$

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

Table S4-4| Key enzymes and transporter involved in polyP hydrolysis, glycogen degradation and PHB synthesis.

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

[a] obtained using the MetaCyc database[S1]

Additional information on Figure 4-3: PolyP composition

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

For simplification, in Figure 4-3, we assumed that polyP has Mg^{2+} as a counter ion, only. The stoichiometric ratio of P to Mg^{2+} of 2:1 was derived from the molecular formula $(NaPO_3)_n$ (Graham's salt). Therefore, during anaerobic P re-dissolution and with hydrolysis of polyP 1 mol P will be released together with 0.5 mol Mg^{2+}.

References of supplementary material

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

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

Chapter 5

Anders et al. (2024) *Process Saf. Environ. Prot.*

Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions

This chapter[8] has been published in the Journal *Process Safety and Environmental Protection* in December 2024 as a research article entitled:

"Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions"

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[8] For consistency, the formatting and orthography, including citation style, of the following article have been adapted to the dissertation style.

Full research article:

Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions[9]

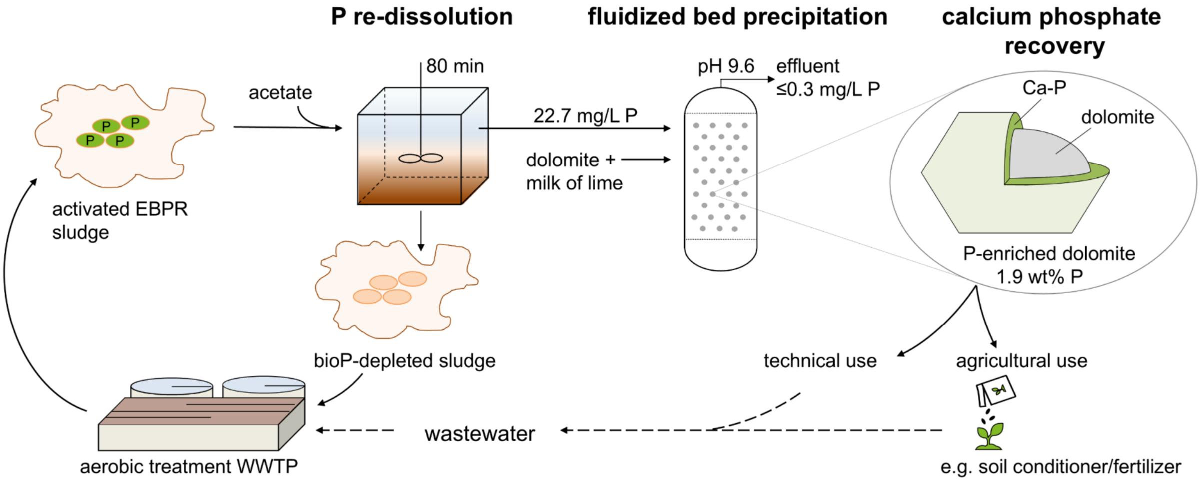
Annika Anders[a], Harun Cakir[b], Frank Ohnemüller[b], Harald Platen[a], Ulrich Kornhaas[c], Harald Weigand[a]

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

[b] *Forschungsgemeinschaft Kalk & Mörtel e.V., Annastr. 67-71, D-50968 Cologne, Germany*

[c] *Ingenieurbüro Alwin Eppler GmbH & Co. KG, Gartenstr. 9, D-72280 Dornstetten, Germany*

Graphical Abstract



Keywords

Activated sludge, Enhanced biological phosphorus removal (EBPR), Dolomite, Phosphorus recovery**,** Pilot plant, Volatile fatty acids (VFA)

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[9] Supplementary material availabe.

Abbreviations

AS, activated sludge; CPR, chemical P removal; Dol-P, P enriched dolomite; EBPR, enhanced biological P removal; $\eta_{P\ recovery}$, efficiency of P recovery; $\eta_{precipitation}$, efficiency of fluidized bed precipitation; $\eta_{re\text{-}dissolution}$, efficiency of re-dissolution; P, phosphorus; PAO, polyphosphate accumulating organism; PHA, polyhydroxyalkanoate; PolyP, polyphosphate; $P_{yield}/Ac_{consumed}$, molar ratio of cumulative P yield to total acetate consumption; P_{yield}/Ac_{spike}, molar ratio of cumulative P yield to initial acetate spike level; RAS, return activated sludge; SEM, scanning electron microscopy; TSP, triple super phosphate; TSS, total suspendedsolids; VFA, volatile fatty acid; WWTP, wastewater treatment plant; Y_P, yield of re-dissolved P

Abstract

The recovery of phosphorus (P) from wastewater is crucial for circularity of plant nutrients. While wastewater treatment plants (WWTPs) employing enhanced biological P removal offer potential for P recovery by biological P re-dissolution, its scalability and performance under real-world conditions remain underexplored. Here we report on a pilot-scale test (15 m^3 return sludge per day) for rapid P recovery from return sludge at a full-scale municipal WWTP induced by acetate supplementation and subsequent P precipitation as a soil amendment and fertilizer. In a total of 54 re-dissolution batches (treatment time approx. 80 min), supernatant P concentrations varied greatly. Batches were combined and used as the feed at 19.2–22.7 mg P/L for fluidized bed precipitation with dolomite seed grains. This step was highly efficient since upon milk of lime addition (pH >9.6), 99% of the P-input load precipitated onto the dolomite, forming a calcium phosphate layer with 0.9–1.9 wt% P. Trace element levels in the product complied with the German Fertilizer Ordinance. Residual ortho-P effluent levels were ≤0.3 mg P/L. The precipitate is valuable in terms of soil pH regulation, while providing Ca, Mg, and P as plant nutrients. However, the poor overall recovery of 1.9% of total sludge P clearly highlights the challenges of re-dissolution and phase separation at the pilot-scale. These arise from the combined biological and chemical P elimination strategy used at the particular WWTP, variations in acetate-induced re-dissolution kinetics, and inefficient sedimentation for solid/liquid separation. Perspectives for process optimization and improved overall recovery are discussed.

5.1 Introduction

Phosphorus (P) is an essential nutrient for all life and is classified a critical raw material by the European Commission since 2014 (European Commission, 2014). Its high market demand is primarily driven by the utilization of P fertilizers in the agricultural sector. Typically, these P fertilizers are obtained by mining and processing phosphate rock. Although there are no imminent shortages of phosphate rock (Jasinski, 2024), the reliance on reserves concentrated in geopolitically vulnerable regions has raised concerns about future accessibility, affordability, and import security (Baker et al., 2024; Elser et al., 2024). At the same time, agricultural P run-offs and P discharges into waterbodies, reduce water quality and contribute to environmental pollution (Chen et al., 2006). Therefore, recovery of P from secondary resources such as wastewater is a key step towards a more sustainable and circular P management.

In wastewater treatment plants (WWTPs), P is removed from the aqueous phase to prevent eutrophication of the effluent-receiving waterbodies using enhanced biological P removal (EBPR) or chemical P removal (CPR). In EBPR, polyphosphate accumulating organisms (PAOs) abundant in the activated sludge (AS) accumulate excess P from the wastewater. Under aerobic conditions, P is intracellularly stored as polyphosphate (polyP), thereby creating a P-rich AS and providing net P removal from the water phase (Fuhs and Chen, 1975; Mino et al., 1998). CPR typically relies on the addition of iron or aluminum salts for P precipitation as sparingly soluble metal-phosphate complexes retained in the solid phase (Prot et al., 2020; Wilfert et al., 2016). Large WWTPs frequently employ a combination of EBPR and CPR to ensure compliance with effluent standards. Ultimately, both EBPR and CPR eliminate P from the system via the sludge disposal.

With 1.3 Mt P/year globally removed in WWTPs (Li and Li, 2017), sewage sludge provides a valuable P resource. It is estimated that P recovered from wastewater treatment can substitute 15–20% of the worldwide P rock demand (Yuan et al., 2012) and up to 40–50% of the mineral fertilizer annually applied to farmland in Central Europe (Egle et al., 2016).

Although the most straightforward P reuse option is the direct agricultural utilization of the sewage sludge, this approach has raised concerns regarding the accumulation of toxic trace metals, pharmaceuticals, microplastics and other wastewater-borne substances (Ott and Rechberger, 2012; Tahiri et al., 2023; van den Berg et al., 2020). In Germany, this was acknowledged in the 2017 amendment to the German Sewage Sludge Ordinance, which prohibits agricultural sewage sludge utilization for WWTPs with more than 50,000 population equivalents by 2032 at the latest (AbfKlärV, 2020). Instead, German WWTPs with a P content above 20 mg/g sludge (dry weight) need to implement a P recovery technology by 2029 (AbfKlärV, 2020), affecting more than half of countries WWTPs (Destatis, 2018; Sichler et al., 2022).

In this context, several technologies have been developed to recover P from wastewater, sewage sludge, and sewage sludge ash. To meet regulations, the P content of the sewage sludge has to be reduced below 20 mg/g. Alternatively 50% and 80% of the total P has to be recovered from the sludge and mono-incineration ash, respectively (AbfKlärV, 2020).

Downstream recovery of ash-borne P can be realized by wet-chemical or thermochemical treatment. These processes achieve high recovery rates, but on the downside also require a large infrastructure and have a relatively high energy demand. In addition, the products may require additional processing to meet regulatory standards with respect to trace elements. WWTP-integrated recovery focuses on the aqueous phase or aims to re-dissolve P from the sludge matrix using biological, chemical or thermal pretreatment methods (Kabbe and Rinck-Pfeiffer, 2019; Li and Li, 2017; Takiguchi et al., 2003). Wet-chemical sludge re-dissolution processes with strong acids are fast but often involve a high consumption of chemicals and co-solubilization of metals considered unfavorable.

Thus, anaerobic digestion is a widely established target point for more environmentally friendly P recovery, as the biological hydrolysis of the sludge can produce a P-rich stream suitable for struvite precipitation, especially in EBPR plants. Larger scale implementations that rely on P re-dissolution by hydrolysis and acidification include the DHV Crystalactor® (Royal Haskoning DHV), AirPrex® (EasyMining Sweden AB), and Ostara Pearl® (Ostara Nutrient Recovery Technologies Inc.). However, sludge disintegration during anaerobic digestion solubilizes trace elements and organic matter. This can affect the precipitation and reduce the quality of the recovery product (Selling et al., 2008; Vasenko and Qu, 2019; Yee et al., 2019). Few technologies specifically target the main wastewater treatment line prior to anaerobic digestion. Targeting these earlier stages such as the end of the aerobic EBPR process, offers an opportunity to recover high levels of biomass P and prevent unwanted struvite scaling in the anaerobic digester and downstream equipment (Melia et al., 2017). Instead of relying on sludge disintegration and concomitant cell lysis, P can be biologically released from AS by adapting the EBPR mechanism. This considers that under anaerobic conditions, PAOs, such as the putative genus *Candidatus* Accumulibacter, take up volatile fatty acids (VFAs), e.g., acetate, which are converted to polyhydroxyalkanoates (PHAs) and stored inside the cell (Comeau et al., 1986; Mino et al., 1998). The energy required for uptake is provided by the hydrolysis of intracellularly stored polyP, upon which ortho-phosphate (ortho-P) is released into the aqueous phase (Smolders et al., 1994).

Despite its potential, the biological recovery of P from AS by VFA-induced re-dissolution remains underexplored, especially at larger scales (Cheng et al., 2024; Di Capua et al., 2022; Gonzalez-Silva et al., 2022; Vučić et al., 2024). One of the few full-scale technologies, the Phostrip process, relies on the internal VFA production from organic matter (and subsequent P release) through anaerobic side stream storage (up to 20 h) of the sludge (Kaschka and

Weyrer, 1999; Kim et al., 2000; Levin and Della Sala, 1987). However, prolonged anaerobic storage can alter the AS structure, biotic community and likely affects PAO activity (Chen et al., 2003; Wilén et al., 2000), which could be detrimental to EBPR process itself. A suitable engineering of this approach for a fast and targeted P re-dissolution could allow for a simple P recovery process design as a main-stream integration (Zhang et al., 2022). Previous studies have shown a fast P re-dissolution by acetate supplementation (Acevedo et al., 2015; Anders et al., 2021; Gonzalez-Silva et al., 2022; Xia et al., 2014). Since the PAOs in EBPR systems proved resistant to short-term starvation periods and released P from their polyP pool without loss of activity (Acevedo et al., 2015), acetate supplementation could enable an environmentally friendly P release while maintaining the microbiome. Nevertheless, so far, most researchers have focused on laboratory-scale experiments with enriched PAO cultures (Acevedo et al., 2015; Welles et al., 2017; Xia et al., 2014) or non-acclimated AS (Anders et al., 2021; Fernando et al., 2019; Petriglieri et al., 2022). Since the conduction of large-scale experiments has been strongly recommended (Zhang et al., 2022), this study aims to test the viability of P recovery processes under real-world conditions. Under these conditions, specific challenges that are typically not addressed in laboratory studies arise. These include the temporal variability of the WWTP influent, changing ambient conditions, and alterations in WWTP operation. All of these may enhance microbial interactions and competition within the AS and may feedback on P re-dissolution. Coping with these challenges is a prerequisite for the successful and cost-efficient implementation of a bio-based P recovery route for municipal sewage sludge.

Beyond the biological P re-dissolution, the choice of the value-added recovery product plays an important role for its potential use. To date, once P is dissolved, it is mainly recovered by crystallization of struvite or calcium phosphate (Kabbe and Rinck-Pfeiffer, 2019). Also, P recovery as vivianite has been reported (Li and Li, 2017; Prot et al., 2020, 2019; Wilfert et al., 2018, 2016). For example, chemicals used for struvite recovery, such as magnesium salts conflict with the economics of the recovery process (Deng and Dhar, 2023). Among various materials that have been tested for recovery of dissolved P, dolomite ($CaMg(CO_3)_2$), is a low cost and naturally abundant mineral with great efficiency and selectivity for P (Boeykens et al., 2017; Piol et al., 2019; Roques et al., 1991; Xu et al., 2014). Only recently, dolomite has been used as a seed material in a laboratory-scale fluidized bed crystallization process to enhance P removal efficiency (Ha et al., 2022).

In agriculture dolomite is a well-established liming material to amend acidic soils, improving soil structure and crop growth (Lamar, 1961; Shaaban et al., 2015; Shreckhise et al., 2020). This is of particular relevance as the prevalence of acidic soils is an increasing challenge for agriculture (Dai et al., 2017). Therefore, the recovery of wastewater P as P-enriched dolomite may offer an option for a dual purpose product, combining pH regulation with the provision of

Mg, Ca and P as a slow release fertilizer, particularly beneficial for acidic soils. Unlike conventional water-soluble fertilizers, such as triple superphosphate, that are prone to P run-offs due to low plant use-efficiency and risk of overapplication, dolomite phosphate rock fertilizers have been proven suitable to reduce these P leaching losses (Chen et al., 2006; Yang et al., 2012). As a result, combining wastewater-derived P with dolomite may not only address the need for a sustainable P recovery but also promote agricultural sustainability.

This study pursued three objectives by (i) integrating a bio-based P re-dissolution from AS with the precipitation of a Ca/P fertilizer from the supernatant, (ii) upscaling the process and (iii) testing it under real-word conditions. Therefore, we designed a pilot-scale plant for P recovery from AS by fast biological P re-dissolution with acetate and subsequent fluidized bed precipitation using dolomite as a seed material and milk of lime as a precipitant (Re-BioP-Cycle). The process was evaluated under real-world conditions by feeding the pilot plant with return activated sludge (RAS) from a full-scale municipal WWTP. The product was characterized in terms of P solubility in different extractants to assess its bioavailability value. By addressing challenges under real-world conditions, this work aims to advance the field towards the operational implementation of bio-based P recovery in municipal WWTPs, contributing to a more sustainable P management and circular economy.

5.2 Materials and methods

The Re-BioP-Cycle pilot plant was installed at the Giessen municipal WWTP (Mittelhessische Wasserbetriebe, Germany) and was operated during the months of August and September. The WWTP has a capacity of 300,000 population equivalents. During standard operation, P is removed from the wastewater in the aerobic treatment stage by combined EBPR/CPR, where CPR relies on the addition of ferrous chloride.

For logistical reasons, the AS feed of the Re-BioP-Cycle pilot plant system was provided from the return sludge line as RAS. The sludge composition is listed in Table 5-1.

Table 5-1| Characteristics of the influent RAS used in the Re-BioP-Cycle pilot plant (return activated sludge from Giessen WWTP). Data are presented as mean ± standard deviation. Samples were collected during a three-week campaign.

Parameter	Concentration	Unit	Number of samples [b]
RAS			
TSS	9.2 ± 1.8	g/L	12
P_{total}[a]	30.6	mg/g_{TSS}	-
aqueous phase			
ortho-P	4.5 ± 3.5	mg P /L	12
sludge solids			
P	30.1 ± 2.6	mg/g_{TSS}	3
Mg	6.5 ± 3.7	mg/g_{TSS}	3
Ca	13.5 ± 0.8	mg/g_{TSS}	3
Fe	36.3 ± 4.3	mg/g_{TSS}	3

[a] P_{total} was calculated as the sum of ortho-P and sludge solid P concentrations, [b] sampled on individual dates.

The RAS comprised the aqueous phase and the sludge solids. During plant operation, the average total suspended solids (TSS) concentration of the RAS was (9.2±1.8) g_{TSS}/L. The dissolved ortho-P concentration in the aqueous phase varied between 0.88 mg P/L and 13.56 mg P/L. Compared to AS samples collected directly after the aerobic treatment (dissolved ortho-P < 0.1 mg P/L), ortho-P was higher in the RAS. This may indicate partial re-dissolution of P within the clarifier and/or during passage of the return sludge line. The initial ortho-P concentration in the RAS fluctuated over a wide range providing a highly variable feed to the pilot plant system. The P content of the sludge solids was (30.1±2.6) mg P/g_{TSS}. Thus, P recovery is mandatory according to the German Sewage Sludge Ordinance. Sludge-borne Fe, Ca and Mg concentrations were 36 mg Fe/g_{TSS}, 13 mg Ca/g_{TSS} and 6 mg Mg/g_{TSS}, respectively. The high iron levels in the sludge mirrors the use of ferrous chloride for CPR in the aerobic treatment.

5.2.1 Design and operation of the Re-BioP-Cycle pilot plant

The pilot plant consisted of three process units (Figure 5-1). In unit A, P was biologically re-dissolved from EBPR/CPR-RAS upon supplementation with acetate where induced anaerobiosis produced a P-rich supernatant. This was facilitated by the ability of PAOs to selectively hydrolyze stored polyP and release ortho-P through uptake of a carbon source as part of their anaerobic maintenance. In unit B (storage tank), the supernatant was stored until an appropriate volume had accumulated and was delivered to unit C for precipitation of the ortho-P as a P-enriched dolomite product via milk of lime and dolomite seed addition in a fluidized bed. In the units the following tentative reactions occur:

Unit A: $(\text{polyP})_n + CH_3COO^- \rightarrow (\text{polyP})_{n-1} + \text{PHA} + HPO_4^{2-}$

Unit C: $Ca^{2+} + 2\ OH^- + HPO_4^{2-} + \text{dolomite} \rightarrow CaHPO_4 - \text{dolomite} \downarrow + H_2O$

Please see Figure S5-5 of the supplement for photographic documentation of the pilot-scale plant.

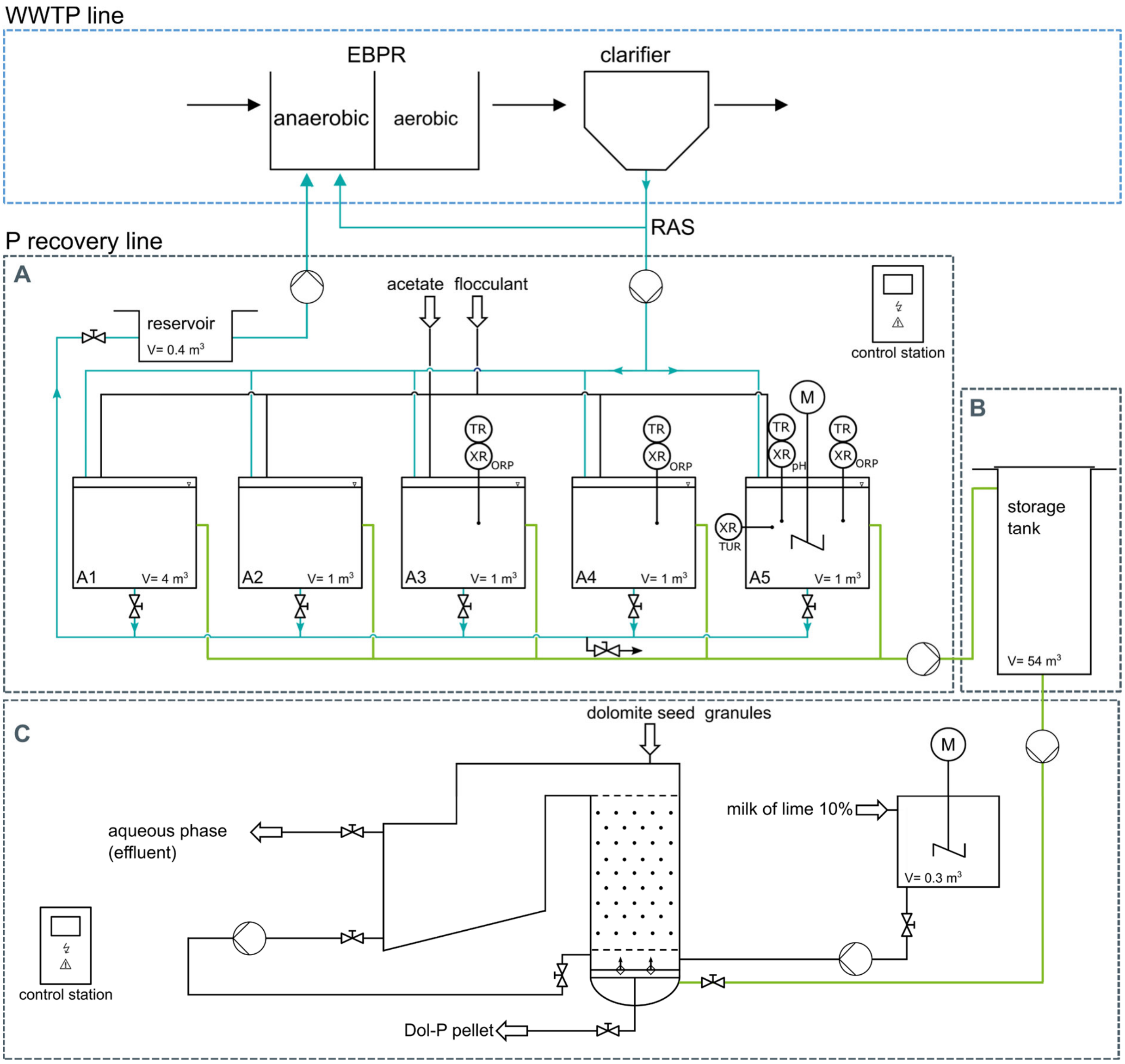

Figure 5-1| Configuration of the Re-BioP-Cycle pilot plant operated at the Giessen WWTP. Unit A: Five P re-dissolution reactors, A1–A5. Unit B: Collection of the P-rich supernatant. Unit C: Fluidized bed reactor for precipitation of P-enriched dolomite pellet. Colors indicate the flow paths of the return activated sludge (cyan) and the P-rich supernatant (green). TR: temperature sensor/logger, XR: oxidation reduction potential (ORP), pH, turbidity (TUR) sensors/loggers, Dol-P: P-enriched dolomite product.

5.2.1.1 P re-dissolution – Process description and reactor operation

Process unit A consisted of five re-dissolution reactors (three 1 m^3 IBC tanks and two cylindrical pools). The volume of reactor A1 was 4 m^{3,} and the volumes of reactors A2 through A5 were 1 m^3, each. Reactors A3, A4, and A5 were equipped with 1200-S sc digital pH/ORP sensors and a temperature probe (Pt100; Hach Lange, Düsseldorf, Germany; immersion depth 0.4 m). The oxidation reduction potential (ORP) was measured against an Ag/AgCl reference electrode (3 M KCl). The ORP values were converted and reported as E_H (potential against standard hydrogen electrode). Reactor A5 was additionally equipped with a 1200-

S sc SOLITAX t-line sc turbidity-suspended solids sensor (Hach Lange, Düsseldorf, Germany) at an immersion depth of 0.5 m. All sensors were connected to a sc-1500 controller (Hach Lange, Düsseldorf, Germany). Mobile Sensor Management Claros^{TM} software (Hach Lange, Düsseldorf, Germany) was used for on-line data monitoring and acquisition. A peristaltic pump (ALH 40RSF Albin Pump SAS, max. flow rate 8.19 m^3/h, Montélimar, France), supplied the RAS and delivered the supernatant of reactors A1 through A5 to the storage tank (unit B). The pump was operated by a control station equipped with a frequency converter (VF-S15 2004 PL, Toshiba, Tokyo, Japan) and a control panel. The treated sludge was recirculated to the WWTP using a submersible pump (7000/D, max. flow rate 7 m^3/h, Gardena Deutschland GmbH, Ulm, Germany).

Reactors A1 through A5 were operated in batch mode. Reactor A5 served as a reference reactor for on-line temperature, pH, ORP and turbidity measurement and was operated in parallel with the other reactors at all times.

Each P re-dissolution cycle consisted of five phases (Table 5-2). First, the reactors were fed with RAS at a rate of 2.3 m^3/h (phase I). The pH and temperature of the RAS ranged from 6.8–7.0 and 18–20 °C and was not adjusted. Sodium acetate trihydrate (Ph. Eur., USP, E262, WHC GmbH, Schweitenkirchen, Germany) was manually dosed as a concentrated solution (15% (w/w) in H_2O) to yield a final in-reactor acetate concentration of 200 mg/L. This was based on previous laboratory studies performed with freshly sampled AS from the same WWTP (Anders et al., 2023, 2021). In accordance with these laboratory findings, the batches were incubated for 1–2 h for acetate-induced P re-dissolution (phase II). During incubation, reactor A5 was continuously agitated at 38 rpm using a worm gear container mixer (SRT-IBC-0235, Schwarzer Rührtechnik GmbH, Delmenhorst, Germany). A conical-shaped foldable cup impeller was used for gentle homogenization of the sludge with low shear stress and minimized air intake. Reactors A1 to A4 were homogenized by manual mixing in 10–15 min intervals. Phase III was initiated by stopping the mixer and allowing the sludge flocs to settle. Settling times varied among batches, as judged by visual inspection of the settling progress and the turbidity signal of reactor A5. If necessary, a flocculant (GWT3278; GWT Gesellschaft für Wassertechnik mbH, Wiesbaden, Germany) was added as a concentrated solution. In phase IV, the supernatant was withdrawn at a flow rate of 1.3–1.5 m^3/h and stored in a light-proof storage tank (unit B, Figure 5-1) until it was transferred to the precipitation reactor (unit C, Figure 5-1). Ultimately, the settled and P-depleted RAS was recirculated to the EBPR system of the WWTP (phase V).

Table 5-2| Operating phases of P re-dissolution.

Phase	Process
I	RAS feeding to reactors
II	supplementation of acetate and P re-dissolution
III	settling
IV	withdrawal of supernatant to storage tank
V	drainage and recirculation of settled RAS

5.2.1.2 P precipitation – Process description and reactor operation

Two pooled batches of P-rich supernatant comprising 7 m^3 and 30 m^3 were collected in unit B (Figure 5-1) and used for P precipitation.

The automated precipitation unit C (Figure 5-1) was based on the fluidized bed reactor concept employed in commercial drinking water softening (ALWIN Eppler GmbH & Co. KG, Dornstetten, Germany). Therein, an increase in pH induces the growth of limestone pellets around a sand grain core, resulting in softened effluent water. This principle was adopted to precipitate re-dissolved P as calcium phosphate using ground dolomite (grain size 0.1–0.5 mm) as seed granules.

As shown in Figure 5-1, the reactor consisted of a column (diameter 250 mm), the upper section of which was connected to a settling zone. Re-dissolved P was upstream fed at a speed of 30–120 m/h to maintain a fluidized bed of seed granules. Particles that escaped the reaction zone were retained in the settling zone and returned to the column reactor. For the start-up, the precipitation reactor was run with water from the WWTP clarifier for 2 h at a high flow rate (>2.5 m^3/h) to wash the seed granules. Subsequently, the feeding of re-dissolved P was started (flow rate 1.8–2.4 m^3/h). For precipitation of the P, finely dispersed, highly reactive milk of lime (10 wt%) was added at 2.1–3.4 L/h maintaining a pH of around 9.3. Product pellets exceeding a flow-rate-dependent critical diameter deposited on the ground of the fluidized bed reactor and were harvested manually at the pellet discharge. The reactor was operated under semi-continuous to continuous conditions and monitored via a control station.

5.2.2 Sampling and analytical methods

To characterize the input to the re-dissolution unit, the RAS was analyzed for P, magnesium (Mg), calcium (Ca) and iron (Fe). Ortho-P in the aqueous and solid phase was determined directly and after aqua regia digestion, respectively (see supplementary material). Therewith sludge total P (P_{total}) was calculated in units of mg/g_{TSS} accounting for the TSS (APHA, 1999). All P concentrations reported in this study are expressed as phosphorus-P. The kinetics of P re-dissolution in unit A were determined as the evolution of the free ortho-P concentration over time. The feed of the precipitation reactor (P-rich supernatant) was characterized in terms of pH, conductivity, acid-neutralizing capacity, and the concentrations of ortho-P and acetate. Effluent samples from unit C were analyzed for ortho-P, pH, and turbidity. Harvested pellets were air-dried. The pellets were analyzed by light microscopy and scanning electron microscopy (SEM). Dry pellet characterization accounted for bulk contents of macro nutrients and trace elements as well as for operationally defined P fractions using four different extractants. Commercially available triple superphosphate (TSP) was used for comparison. Further details of the analytical methods are provided in the supplementary material.

5.2.3 Data evaluation

The yield of re-dissolved P, Y_P [%], was calculated according to equation 5-1. It represents the fraction of RAS-borne P solubilized and corresponds to the P depletion in the RAS matter.

$$Y_P = \frac{P_{t,\text{re-dissolved}} - P_{t0}}{TSS \cdot P_{RAS}} \cdot 100\ \% \tag{5-1}$$

with $P_{t,\text{re-dissolved}}$ [mg/L]: concentration of ortho-P at t_{end} in unit A, mean of all daily batches
P_{t0} [mg/L]: initial concentration of ortho-P in unit A, cf. Table 5-1
TSS [g_{TSS}/L]: initial total suspended solids of the RAS, cf. Table 5-1
P_{RAS} [mg/g_{TSS}]: initial P content in the RAS solids, cf. Table 5-1

To evaluate the efficiency of P recovery in the partial processes of re-dissolution and precipitation as well as in the overall process of P recovery, we first determined the individual P loads that entered these processes (see Figure S5-6). For the calculation of P loads, please refer to equations S5-1–S5-4 presented in the supplementary material.

Based on the P loads entering and exiting the respective units, efficiencies for the re-dissolution ($\eta_{\text{re-dissolution}}$), fluidized bed precipitation ($\eta_{\text{precipitation}}$), and overall P recovery ($\eta_{\text{P recovery}}$) were determined according to equations (5-2)–(5-4), where $P_{\text{load,in}}$ and $P_{\text{load,out}}$ [g P] are the input and output mass of P in the re-dissolution and precipitation units, respectively. $P_{\text{load,supernatant}}$ [g P] is the mass of P in the collected supernatant. $P_{\text{load,pellets}}$ [g P] is the mass of P recovered in the pellets taken from the precipitation unit.

$$\eta_{\text{re-dissolution}}\ [\%] = \frac{P_{\text{load,superatant}}}{P_{\text{load,in}}} \cdot 100\% \tag{5-2}$$

$$\eta_{\text{precipitation}}\ [\%] = \frac{P_{\text{load,pellets}}}{P_{\text{load,supernatant}}} \cdot 100\% \tag{5-3}$$

$$\eta_{\text{P recovery}}\ [\%] = \frac{P_{\text{load,pellets}}}{P_{\text{load,in}}} \cdot 100\% \tag{5-4}$$

To further evaluate the re-dissolution process in terms of substrate consumption and substrate spike, the molar $P_{\text{yield}}/Ac_{\text{consumed}}$ and $P_{\text{yield}}/Ac_{\text{spike}}$ ratios were calculated with equations 5-5 and 5-6, as described by Anders et al. (2023).

$$\frac{P_{\text{yield}}}{Ac_{\text{consumed}}} = \frac{P_{\text{re-dissolved, feed}}}{Ac_{\text{spike}} - Ac_{\text{end}}} \tag{5-5}$$

$$\frac{P_{\text{yield}}}{Ac_{\text{spike}}} = \frac{P_{\text{re-dissolved, feed}}}{Ac_{\text{spike}}} \tag{5-6}$$

with $P_{\text{re-dissolved, feed}}$ [mmol/L P]: concentration of ortho-P in the collected supernatant, corresponds to the feed of the precipitation reactor

Ac_{spike} [mmol/L acetate]: initial concentration of the supplemented acetate

Ac_{end} [mmol/L acetate]: aqueous acetate concentration at the end of the re-dissolution

5.3 Results and discussion

5.3.1 P re-dissolution from activated sludge

Previous laboratory results suggested that an acetate supplementation of 200 mg/L was suitable for P re-dissolution (Anders et al., 2023). This was confirmed by preliminary pilot-scale tests, where 200 mg/L of acetate induced a 9-fold increase in dissolved ortho-P within 120 min (supplementary material Figure S5-7). In the same timeframe, the acetate level in the aqueous phase decreased to around 2 mg/L (Figure S5-7). Overall, the kinetics of P release and acetate uptake were similar to those identified in the laboratory experiments (Anders et al., 2023).

Figure 5-2 shows the time course of process parameters monitored in the P-re-dissolution unit (unit A) in an exemplary cycle. Operation happened under roughly isothermal conditions at 20 °C in the medium. The pH was stable at approximately 7. The E_H values of the RAS indicated an immediate prevalence of anoxic conditions in the RAS. Upon addition of acetate, the E_H value decreased further, indicating anaerobic conditions (no free molecular and no bound oxygen), as classified by Tanwar et al. (2008) and Wareham et al. (1993). By the time acetate was supplemented, the P re-dissolution process was initiated (phase II, Figure 5-2) and continued during the settling process (phase III), where the E_H of the supernatant gradually decreased further, reaching approximately 50 mV, after which the maximum ortho-P concentration was observed. Process phases I through IV (cf. Table 5-2) also went along with a characteristic turbidity evolution. During the RAS feeding, turbidity increased to 2000–3000 FNU. The signal was unsteady, possibly caused by turbulence. Equalization of turbidity signals around 1200 FNU occurred when the sludge was stirred, apparently resulting in a rather homogeneous suspension (phase II). Sedimentation (phase III) was again characterized by a scattering turbidity. This may be related to the vertical passage of sludge flocs at the immersion depth of the turbidity sensor (0.5 m). After a settling time of 100 min approximately, a constant minimal turbidity of 9 FNU was observed. A maximum concentration of 42.1 mg P/L was recorded in the supernatant, of which 38.9 mg P/L had been released from the sludge. Surprisingly, the maximum concentration was not observed at the end of phase II (re-dissolution) but shortly after phase III (settling). One possible explanation is the ongoing re-dissolution after acetate consumption, which has previously been observed in laboratory experiments (Anders et al., 2023).

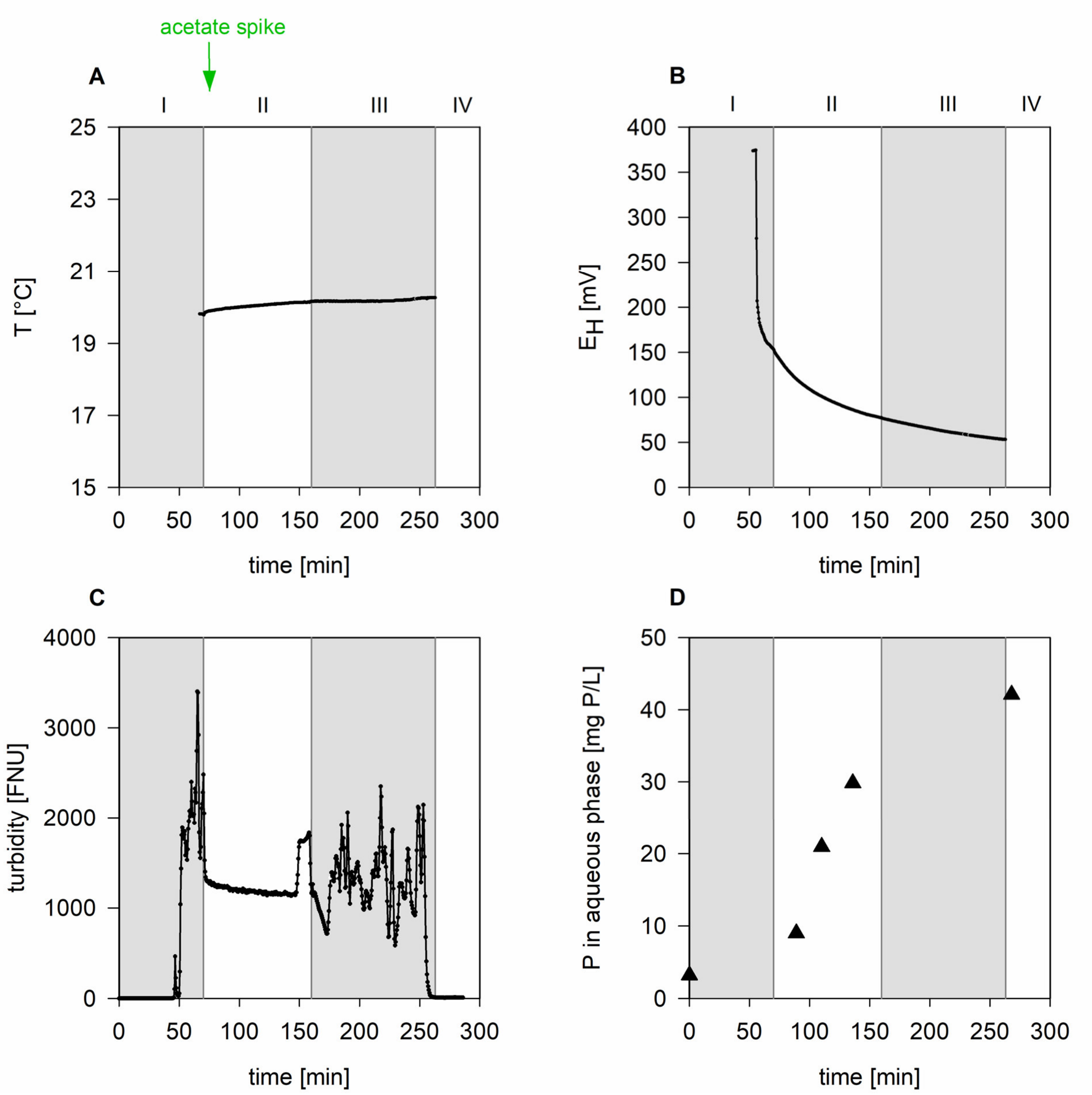

Figure 5-2| Process parameters monitored during an exemplary P re-dissolution cycle (1 of 86 re-dissolution experiments); (A) in-reactor temperature (T), (B) redox potential (E_H), (C) turbidity immersion depth of 0.5 m, and (D) ortho-P concentration in the aqueous phase. The green arrow indicates the timing of acetate spiking. Roman numerals depict the operating phases of RAS feeding (I), P re-dissolution (II), settling (III), and withdrawal of the supernatant (IV), as listed in Table 5-2. Parameter acquisition in phase I was offset from t_0 due to the filling process.

Figure 5-3 summarizes the re-dissolved P concentrations obtained in multiple batches on different days of pilot plant operation. The mean daily ortho-P concentrations were in the range of 8.99–31.38 mg P/L. On days 5 and 6 of operation, the average levels of re-dissolved P were broadly comparable to laboratory findings (Anders et al., 2023). Otherwise, the values were lower.

The data suggest lower mean P re-dissolution in the August batches (18.2 mg/L, n = 13) compared to September (25.7 mg/L, n = 41) which may have resulted from differences in average sludge pH (7 vs. 7.2) and temperature (20 °C vs. ≤ 19 °C). Yet, differences in mean

P re-dissolution proved non-significant based on a one-sided two-sample t-test ($p = 0.95$). On average, approximately 6% of the total P was re-dissolved from the sludge as calculated from the mean P re-dissolved values of all batches and sludge-borne P content according to equation 5-1. This is much lower than that in our laboratory studies with sludge from the same WWTP where around 21–24% P could be re-dissolved (Anders et al., 2023, 2021).

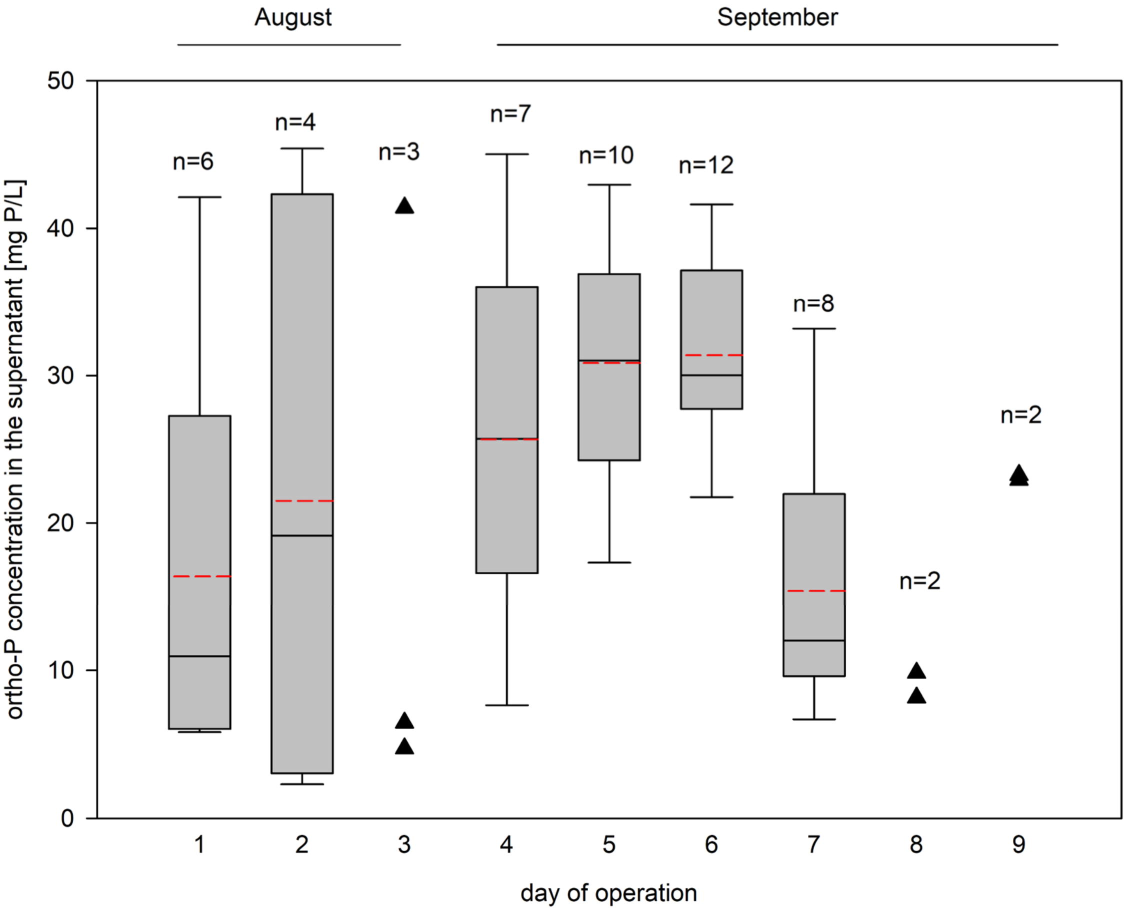

Figure 5-3| P re-dissolution from RAS on individual days of pilot plant operation with n = number of daily batches. The upper and lower frame of the boxes denote the 25th and 75th percentiles, respectively, the black line represents the median, and the red dashed line is the arithmetic mean. The 0th and 100th percentiles are represented by whiskers. Percentiles were calculated median exclusive with Microsoft Excel 2016. Triangles represent individual data points when n was ≤3.

All individual August supernatants of the re-dissolution batches were pooled to provide a feed solution (7 m^3) for the precipitation unit (Aug-feed). Similarly, all September batches were pooled to a volume of 30 m^3 (Sept-feed). This was done to provide two distinct feed solutions with a large volume for the continuous operation of the precipitation unit. The ortho-P concentration of the pooled supernatant was 19.2 mg P/L and 22.7 mg P/L, respectively (Table 5-3). The residual acetate concentration was below 1.2 mg/L, indicating almost complete uptake by the RAS microorganisms. This is supported by the finding that the $P_{yield}/Ac_{consumed}$

ratio of 0.22 mol P/mol Ac (Table 5-3) is similar to the P_{yield}/Ac_{spike} ratio. The P re-dissolution efficiency $\eta_{re\text{-}dissolution}$ was calculated based on the parameters of the collected and pooled supernatants (equation 5-2), resulting in a recovery of only 2% of the available influent RAS P load. The low value is, in part, attributed to the small amount of supernatant obtained through sedimentation in comparison to the volume of RAS treated and the significant loss of re-dissolved P with the discharge of the settled RAS at the end of phase V.

5.3.2 Precipitation of re-dissolved P as a P-enriched dolomite product

Table 5-3 summarizes important parameters of the Aug-feed and Sept-feed entering the precipitation unit. The low total volume of the Aug-feed was topped up to 18 m^3 with water from the clarifier and the ortho-P level was roughly compensated for by the addition of phosphoric acid. The Sept-feed was used without alteration.

Table 5-3| Characterization of feed to the precipitation unit.

Parameter	August feed	September feed
volume [m^3]	7 (18)*	30
pH	7.40 (7.25)*	7.10
ortho-P [mg P/L]	19.2 (32.3)*	22.7
acetate [mg/L]	n.d.	<1.2
conductivity [µS/cm]	1080 (780)*	960
$ANC_{4.3}$ [mmol/L]	5.4 (3.1)*	4.8
$P_{yield}/Ac_{consumed}$ [mol /mol]	0.18	0.22
P_{yield}/Ac_{spike} [mol /mol]	0.18	0.22

n.d.: not detected, ANC: acid neutralizing capacity, *values in brackets depict the composition of the August feed after topping it up to 18 m^3 with water from the WWTP-clarifier and roughly compensating the P level by phosphoric acid addition.

Our precipitation experiments were performed at a pH between 9.3 and 9.9 adjusted by adding milk of lime to unit C. Since the supernatant was virtually free of acetate (Table 5-3) its carryover into the precipitate is deemed negligible. Rather, acetate will be stored in the RAS microorganisms during the re-dissolution step and therewith returned to the biological stage of the WWTP. Operating conditions of the precipitation step were determined in preliminary tests with water from the WWTP clarifier synthetically spiked with phosphoric acid to an ortho-P level in the range of 40–100 mg P/L. The P concentration of the P-rich stream is a critical parameter for process efficiency (Ha et al., 2022) and for P precipitation with the DHV Crystalactor® a benchmark value >20 mg P/L in the aqueous phase is recommended for 50% removal efficiency (Reinout Vreugdenhil, product manager Crystalactor, Royal HaskoningDHV, personal communication, September 26, 2023). For an economically viable process Jupp et al. (2021) suggested levels of at least 50–60 mg P/L. Thus, our feed concentration was in the lower range of the recommendations (Deng and Dhar, 2023).

In spite of the latter, we observed a very satisfactory and reproducible precipitation efficiency, ($\eta_{precipitation}$, of 99% P; cf. equation 5-3) with the Aug-feed and Sept-feed. In both cases, the dissolved ortho-P levels in the effluent of the fluidized bed were as low as 0.3 mg/L. Using the Sept-feed, total effluent P (i.e., dissolved and particulate) amounted to 5.6 mg P/L suggesting breakthrough of a small share of particulate P (e.g., calcium phosphate precipitates), which was also supported by a relatively high turbidity of 135 FNU. Nevertheless, in both cases, $\eta_{precipitation}$ was substantially above those reported in previous studies on fluidized bed precipitation with $Ca(OH)_2$ (75–95%) (Deng and Dhar, 2023).

Despite the high precipitation efficiency, the strict regional ortho-P discharge standards for WWTPs in Hesse, Germany (<0.2 mg P/L; HMUKLV, 2021) and the total P levels set by the EU Urban Wastewater Treatment Directive (Council of the European Union, 1991) were not met. This calls for a modification of the treatment scheme, e.g., by recycling the effluent of the precipitation unit to the WWTP together with the sediment from the re-dissolution unit. Additionally, the high pH of the final effluent may necessitate further treatment or adjustment to comply with effluent pH regulations before discharge.

Table 5-4 summarizes important characteristics of the pellets produced in the fluidized bed reactor. The pellets had a high loss on ignition (LoI) pointing to substantial contents of hydrates and carbonates. The elemental composition was dominated by Ca (24.6%), followed by Mg (10.1%). Low amounts of Fe, Al, Si, Na, and Mn were also present. The P content of the pellets was between 0.9–1.9%. All other elements had levels <0.5%. The high calcium and magnesium content reflects the use of dolomite seeds and the use of milk of lime resulting in a Ca/P molar ratio between 11 and 20. The P level was therefore significantly lower than in designated fertilizers such as TSP (19–23% P) or struvite (21–27% P). Thus, the product may be regarded a compound fertilizer rather than a designated P fertilizer.

Table 5-4| Characteristics and chemical composition of three P-enriched dolomite pellet samples (Dol-P1, Dol-P2, Dol-P3) produced from re-dissolved P from RAS using dolomite as a seed material and milk of lime. Limit values of the German Fertilizer Ordinance (DüMV, 2012).

Element	Mean	Min	Max	DüMV 2012
LoI [wt%]	43.8	43.0	45.2	
P [wt%]	1.41	0.90	1.93	
Ca [wt%]	24.64	23.14	25.57	
Mg [wt%]	10.15	9.53	11.34	
C [wt%]	11.53	11.15	12.05	
As [mg/kg$_{\text{dry weight}}$]	0.07	0.03	0.09	40
Cd [mg/kg$_{\text{dry weight}}$]	0.14	0.13	0.16	1.5*
Cr [mg/kg$_{\text{dry weight}}$]	2.66	2.60	2.72	-
Hg [mg/kg$_{\text{dry weight}}$]	<0.01	<0.01	<0.01	1
Ni [mg/kg$_{\text{dry weight}}$]	1.33	1.08	1.59	80
Pb [mg/kg$_{\text{dry weight}}$]	<0.2	<0.2	<0.2	150
Cu [mg/kg$_{\text{dry weight}}$]	0.99	0.84	1.18	900
Zn [mg/kg$_{\text{dry weight}}$]	8.60	8.13	8.86	5000

LoI: loss on ignition, *only applicable for P fertilizers containing 50 mg/kg P_2O_5 or more

Regarding their fertilizer value, the P solubility of the pellets was determined in extracts with water, formic acid and neutral ammonium citrate. In contrast to commercial TSP, the pellets showed a low P water solubility of 1.4 wt% (Table 5-5), which could be beneficial to avoid runoff and seepage water losses at high precipitation intensities. In turn, full solubility of P in formic acid and neutral ammonium citrate indicated that pellets may provide a slow release P fertilizer in acidic soils or for crops excreting week organic acids.

Typically, concerns are raised about trace metals in sewage sludge-derived fertilizer products. The data presented in Table 5-4 show that levels of As, Cd, Cr, Hg, Ni and Pb in the pellets complied with the limit values of the German Fertilizer Ordinance (DüMV, 2012) and not even a labelling would be required. Inorganic contaminant carryover into the product is therefore irrelevant for this particular re-dissolution and precipitation technology. Organic contaminants regulated by the Fertilizer Ordinance were out of the scope of this investigation but will be included in future work. In terms of pathogens inactivation, the precipitation at pH >9 is regarded as favorable.

Table 5-5| Total mineral acid solubility of the P-enriched dolomite (Dol-P) produced in this study compared to commercial triple superphosphate (TSP) and corresponding relative P solubility in different extractants.

		Relative P solubility in *		
Sample	MinA-P [g P/kg$_{\text{dry weight}}$]	Water	Formic acid	Neutral ammonium citrate
Dol-P	14.4±2.9	1.4 wt%	137.3 wt%	104.7 wt%
TSP	193.1±3.4	106.4 wt%	105.6 wt%	89.5 wt%

MinA-P: mineral acid soluble P, *relative P solubility based on total MinA-P

Microscopic analysis of the pellets (Figure 5-4) revealed that fluidized bed precipitation formed a shell around the dolomite seed granules. The precipitated layer likely consisted of new Ca phases formed by the reaction of aqueous HPO_4^{2-} with milk of lime.

The composition of the surface layer that formed on the dolomite was calculated as presented in the supplementary material S5-3 using the measured Mg as a marker for the pellets' dolomite content. Notably, the presence of carbon in the surface layer suggested the inclusion of calcium carbonates ($CaCO_3$), while P pointed to the formation of calcium phosphate phases. The estimated formula for the surface layer was approximately $Ca_{4.32}(PO_4)_1(CO_3)_{2.75}$ (cf. supplement S5-3 and Table S5-8). The molar Ca/P ratio ranged from 3.9 to 4.9. When excluding the Ca attributed to $CaCO_3$, the Ca/P ratio was 1.4–1.8. A minor charge imbalance derived from the Ca^{2+} and PO_4^{3-} ions suggested the formation of calcium hydrogen phosphate ($CaHPO_4$) and calcium hydroxide ($Ca(OH)_2$) to maintain charge neutrality (see supplement S5-3). Consequently, we hypothesize that in addition to $CaCO_3$, a mixture of Ca phases including calcium phosphate ($Ca_3(PO_4)_2$), $CaHPO_4$ and $Ca(OH)_2$ may have formed. Thus, surface layer may be possibly described as $[Ca_3(PO_4)_2]_1 \cdot [CaHPO_4]_{0.01} \cdot [CaCO_3]_{1.14}$, $[Ca_3(PO_4)_2]_1 \cdot [CaCO_3]_{3.79} \cdot [Ca(OH)_2]_{0.95}$ and $[Ca_3(PO_4)_2]_1 \cdot [CaHPO_4]_{0.04} \cdot [CaCO_3]_{0.85}$ for Dol-P1, Dol-P2 and Dol-P3, respectively (Table S5-9). The formation of mixed calcium phosphate minerals and precursor phases has been previously observed (Wang and Nancollas, 2008). Previous studies reported that at pH levels below 10, brushite ($CaHPO_4 \cdot 2H_2O$) is the predominant phase, while hydroxyapatite ($Ca_5(PO_4)_3OH$) forms above pH 10 (Cichy et al., 2019; Wang and Nancollas, 2008). Our theoretical estimation, however, excluded the formation of hydroxyapatite for simplification, but we acknowledge that hydroxyapatite may form from precursor phases during the aging process (Meesschaert et al., 2020; Wang and Nancollas, 2008). The observed variations in pellet composition are consistent with literature, indicating that minor changes in precipitation conditions such as the residence time in the fluidized bed reactor may have influenced the chemical characteristics of the precipitates (Han and Louhi-Kultanen, 2018; Hoeher et al., 2021; Nikolenko et al., 2020). Further studies should focus on confirming the specific phases present in the surface layer through X-ray diffraction analysis of the surface layer.

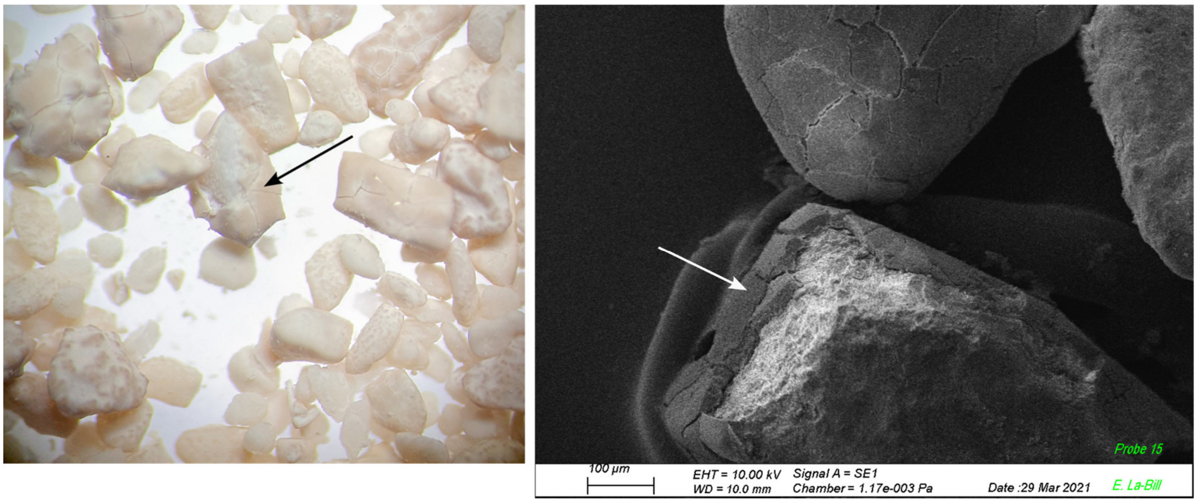

Figure 5-4| Light microscopy image (left) and SEM image at 300x magnification (right) of the recovered pellets produced from the P-rich supernatant. Arrows mark the newly formed shell around the dolomite seed granules during the fluidized bed precipitation.

The shell-like structure suggests a sequential solubilization process when the product is applied as a soil amendment. Initially, the outer surface, which is enriched with P and Ca, may dissolve under acidic conditions. Subsequent dissolution of the inner dolomite core goes along with the known benefits of dolomite as a soil conditioner, i.e., provision of Mg, Ca, and soil pH adjustment. Based on previous studies that demostrated the effectiveness of dolomite phosphate rock fertilizers for raising the soil pH, improving soil structure, and increased nutrient availability (Chen et al., 2006; Yang et al., 2012), we suggest that the P-enriched dolomite may offer similar agronomic benefits.

5.3.3 Critical evaluation and economic assessment

To evaluate the efficiency of P recovery in the partial processes of re-dissolution and precipitation as well to highlight the overall efficiency of the P recovery process, we calculated the corresponding P loads (see simplified process scheme in supplementary Figure S5-6).

As mentioned in section 5.3.1, the $\eta_{\text{re-dissolution}}$ values were quite low and a large share of available P could not be transferred to the feed of the precipitation unit. Thus, in spite of excellent $\eta_{\text{precipitation}}$, $\eta_{\text{P recovery}}$ of the complete process was as low as 1.9% (equation 5-4). Drawbacks in the re-dissolution unit were related to (i) the low and scattering biological P release and (ii) the employed solid/liquid separation via sedimentation. The latter most likely resulted in the loss of re-dissolved P together with the discharge of treated sludge (as a slightly thickened RAS) in operational phase V. The volume ratio of recovered supernatant to treated RAS was between 1:3 and 1:4. Thus, a considerable part of the liquid volume was lost. This limited $\eta_{\text{re-dissolution}}$, (and hence $\eta_{\text{P recovery}}$). In comparison, the total fraction of re-dissolved P (Y_P) averaged 6% in the individual batches (cf. Figure 5-3). Hypothetically, $\eta_{\text{re-dissolution}}$ could increase to 7% using a belt thickener for solid/liquid separation at an 85% thickening ratio. Similarly, the long sludge settling time was one of the main operational drawbacks. Settling times of typically ~ 80 min clearly exceeded the re-dissolution timeframe. As previously demonstrated on the laboratory-scale (Anders et al., 2023) and confirmed here, the re-dissolution process itself cannot be accelerated. In a re-engineered setting, this may be circumvented by the use of a lamella clarifier or a belt thickener.

A further drawback rested with the mismatch of the dimensions of the re-dissolution and the precipitation unit. Since the latter was laid out for a volumetric flux of > 2 m^3/h, the volume of the re-dissolution unit should have been larger such as to avoid interim storage of feed. Obviously, for a full-scale application, both units should be dimensioned according to the rate of RAS production at the corresponding WWTP. Additionally, increasing the retention time of the pellets in unit C could potentially raise the pellet P content. Probably the most critical aspect in terms of a full-scale application is the large scattering in re-dissolved P concentrations since it may feed-back into the product quality. In the applied fluidized bed precipitation, the addition of milk of lime is independent from the P-concentration in the feed. Thus, low P-concentrations would result in low P/Ca-ratios while high P-concentrations would increase the P/Ca-ratio. This is likely to bring about changes in the solubility of the product and affect the plant-availability of P in the recycled fertilizer. Besides a possible seasonality of sludge pH and temperature, this may also have been due to unknown operation specifics of the CPR-unit. In the pilot-scale tests $\eta_{\text{re-dissolution}}$ was lower than in the previous laboratory-scale experiments conducted with the same AS (Anders et al., 2021). Additionally, laboratory studies of pure EBPR sludge (i.e., without additional CPR) have shown a significantly higher $\eta_{\text{re-dissolution}}$ (Anders et al., 2021). Thus, in terms of P re-dissolution efficiency, a full-scale application of the process seems more advisable for pure EBPR systems rather than for the mixed EBPR/CPR considered here.

A preliminary cost estimate for a full-scale scenario for the WWTP investigated in this study with 280,000 active population equivalents showed that treating the daily surplus AS volume of 500 m^3 would incur operating costs of 0.86–1.47 €/(a×population equivalent). This estimate takes into account current prices for technical grade sodium acetate trihydrate (1.56 €/kg), milk of lime (10%, 50 €/m^3), as well as the dolomite seed granules (88 €/t) and manpower (24,750 €/a). Energy costs were excluded as they cannot be reliably estimated at this point. The cost to treat 1 m^3 of surplus AS would thus be about 1.31–2.78 €. In addition, there would be construction and maintenance costs for a re-dissolution basin (60 m^3) and an up-scaled fluidized bed reactor, as well as dosing peripheries (acetate, milk of lime, seed granules).

Depending on the WWTP configuration, the existing periphery of pumps for sludge transport, solid/liquid separation etc. of the WWTP may be used.

Obviously, under current conditions the low overall P recovery efficiency renders the process uneconomical. It is clear that acetate accounts for a large proportion of the costs incurred. Prospectively, however, acetate obtained as a by-product from fermentation or anaerobic digestion, could be used to reduce the chemical costs associated with need for acetate supplementation. This would also contribute to the idea of a circular economy. Interestingly, Kamiyama et al. (2024) have recently shown that the addition of primary sludge is suitable as an alternative carbon source for P re-dissolution. The authors hypothesized that dissolved organic matter provides substrate for the anaerobic release of P from surplus AS, but present organic acids were not analyzed (Kamiyama et al., 2024). Compared to struvite recovery, the total chemical cost of calcium phosphate recovery with milk of lime is likely lower due to the high costs of magnesium salts (Deng and Dhar, 2023). In addition to the chemical cost considerations, the approach investigated here can hold potential benefits for WWTPs. Similar to the well-known struvite recovery from the digester liquid, such as with Ostara Pearl®, implementation of this bioP recovery scheme could provide for lower P backloads to the WWTP. A reduction in struvite scaling can be expected, as the supernatant with re-dissolved P and possibly Mg is separated from the sludge before entering the digester. This can be particularly beneficial in WWTPs employing EBPR and anaerobic digestion to reduce main maintenance costs. Compared to methods that effectively target the anaerobic digester liquid, the targeted acetate-induced P re-dissolution after the aerobic stage enables a faster processing that preserves sludge integrity. This can prevent solubilization of unwanted contaminants while maintaining microbial activity. On the one hand, this allows for reuse of the sludge for renewed P uptake in the wastewater treatment. On the other hand, the P-depleted but PHA enriched biomass offers potential to be recovered as a biopolymer or utilized for biogas generation (carbon source).

5.4 Conclusion and outlook

In this study we evaluated the feasibility of a bio-based P recovery approach that combined the acetate-induced P re-dissolution from municipal RAS with precipitation of a Ca/P fertilizer. Under pilot-scale (capacity 15 m^3/d RAS) real-world conditions, the process achieved a rapid P release from the RAS within approx. 80 min and a downstream precipitation efficiency of 99%. The re-dissolution process was considered particularly beneficial for treating large sludge volumes and maintaining microbial activity when returning the sludge to the wastewater treatment. The P-enriched dolomite product containing 0.9–1.9wt% P complied with the trace metal limits of the German Fertilizer Ordinance and holds promise as a slow-release fertilizer and soil conditioner for acidic soils.

The product could serve both P recovery and soil health, bridging the gap between wastewater treatment and agricultural sustainability. Therewith the results contribute to the development of a broader range of value-added products that use low-cost, abundant material like dolomite and milk of lime aligning with the principles of circular economy. In addition, the study is a step forward to the bio-based P recovery under real-world conditions.

Despite these merits, the low overall P recovery efficiency (1.9%) highlights critical bottlenecks of the up-scaled process, including the slow sedimentation of P-depleted RAS resulting in a low recovered supernatant volume and the lower than expected re-dissolved P concentrations. To overcome these bottlenecks technical improvements in the liquid/solid separation (e.g., employing a belt clarifier) are needed. Also, the process stability and temporal variation in sludge characteristics require further investigation. Moreover, the variability of EBPR/CPR sludge and its suitability for the acetate-induced P re-dissolution under unknown plant operating conditions remains to clarified. Therefore, further research should first focus on applying identical field-test conditions at a WWTP with a pure EBPR, optimizing the process for higher P yields and evaluating its cost-effectiveness, bearing in mind the P recovery requirements set out by the German Sewage Sludge Ordinance. Additionally, the agronomic effectiveness of the P-enriched dolomite product should be assessed including solubility under various soil conditions, fertilizer value and its long-term safety. This is crucial to validate its potential as an alternative to conventional fertilizers.

By integrating P recovery in a real- world setting the study demonstrates a bio-based pathway towards a circular P management. Most importantly, further development will require collaboration between researchers, WWTP operators and policy makers.

CRediT authorship contribution statement

Annika Anders: Conceptualization, Investigation, Formal analysis, Visualization, Writing – Original Draft, Review & Editing | **Harald Platen:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition | **Harun Cakir:** Project administration, Investigation | **Frank Ohnemüller:** Project administration, Investigation, Funding acquisition | **Ulrich Kornhaas:** Methodology, Resources | **Harald Weigand:** Writing - Review & Editing, Supervision, Funding acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions

Annika Anders[a], Harun Cakir[b], Frank Ohnemüller[b], Harald Platen[a], Ulrich Kornhaas[c], Harald Weigand[a]

Affiliations:

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

[b] Forschungsgemeinschaft Kalk & Mörtel e.V., Annastr. 67-71, D- 50968 Cologne, Germany.

[c] Ingenieurbüro Alwin Eppler GmbH & Co. KG, Gartenstr. 9, D-72280 Dornstetten, Germany.

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S5-1: Analytical methods and instrumental details

The dissolved ortho-P concentration in all aqueous samples (sludge aqueous phase, digestates, pellet extracts) was determined photometrically at 880 nm based on the molybdenum blue method[S1] after 0.45 µm membrane filtration. Analysis of total P in effluent samples was performed using LCK350 (2.0–20.0 mg P/L) cuvette tests from Hach Lange (Düsseldorf, Germany) according to manufacturer's instructions. Acid neutralization capacity down to pH 4.3 ($ANC_{4.3}$) and water hardness were determined photometrically using cuvette tests LCK362 (0.5–8.0 mmol/L) and LCK327 (1–20 °dH) from Hach Lange (Düsseldorf, Germany), respectively. For acetate quantification, the samples were filtered through a 0.2 µm membrane filter. Acetate was determined in technical triplicates by ion chromatography (Metrohm 861 Advanced Compact IC system equipped with Metrosep A Supp 5 column, 150 mm × 4.0mm, particle size 5 µm, Metrohm, Herisau, Switzerland) with suppressed conductivity detection. Isocratic elution of 20 µL of the sample was performed within 20 min with 1.0 mmol/L $NaHCO_3$/3.2 mmol/L Na_2CO_3 at a flow of 0.7 mL/min. Data was processed using MagIC Net 3.1 software.

Filtered sludge biomass (white ribbon filter, ash-free, pore size of 8–12 µm) was dried at 105 °C for 24 h and ground in a ball mill prior to triplicate aqua regia digestion of aliquots according to the European standard procedure DIN EN 16174:2012-11[S2]. The digestates were analyzed for the P, Ca, Mg, and Fe contents in the sludge. Ca, Mg, and Fe were determined by atomic absorption spectroscopy (acetylene/air flame, AAnalyst 100, Perkin Elmer, Waltham, MA, USA equipped with N3050208 Lumina™ and 3036037 Intensitron® Hollow Cathode Lamp) at 422.7 nm, 285.2 nm, and 248.3 nm, respectively. Samples were 0.2 µm filtered and diluted in milliQ water, if necessary. For Ca and Mg quantification, the samples were diluted in a 10% cesium chloride-lanthanum chloride buffer solution (10 g/L CsCl and 100 g/L La, Merck KGaA, Darmstadt, Germany). External standards of calcium nitrate tetrahydrate (pro analysi, Merck KGaA, Darmstadt, Germany), magnesium sulfate heptahydrate (p.a. ≥99%, Honeywell Fluka, Thermo Fisher Scientific Inc., Waltham, MA, USA) and iron(II)sulfate heptahydrate (analytical grade, Merck KGaA, Darmstadt, Germany) prepared in 1% HNO_3 were used for instrument calibration.

Dried and partially crushed fertilizer pellets were analyzed by scanning electron microscopy using Zeiss EVO LS10 (Carl Zeiss Microscopy Deutschland GmbH, Oberkochen, Germany) with secondary electron detection. Chemical analyses analysis was performed at the Institute for Lime and Mortar Research e.V., Cologne, Germany using the combustion method at 1200 °C (elemental analyzer Eltra CS 2000), complexometric titration (Metrohm Titrando 888) and inductively coupled plasma – optical emission spectrometry (Spectro ICP-OES Spectroblue) according to DIN EN 459-2[S3] and DIN EN 12485[S4]. Trace elements (As, Cd, Cr, Hg, Ni, Pb, Cu and Zn) were analyzed after microwave digestion according to DIN EN 12485

(Perkin Elmer AAS Analyst 600 with a THGA Graphite Furnace) using atomic absorption spectrometry. Loss on ignition was determined gravimetrically according to DIN EN 495-2[S3]. The chemical P solubility of the pellets was analyzed at the Federal Research Centre for Cultivated Plants, Braunschweig, Germany using the following extraction methods. Water extraction was performed according to DIN EN 15958[S5], formic acid extraction according to DIN EN 15919[S6], neutral ammonium citrate extraction according to DIN EN 15957[S7], and mineral acid digestion according to DIN EN 15956[S8]. P content in the extracts was determined photometrically using the molybdenum blue method.

Figure S5-5| Re-BioP-Cycle pilot plant for P recovery built at the municipal WWTP Giessen. a) Overview; front: precipitation unit; middle: storage tank unit B; back: 20 ft container with the P re-dissolution reactors. b) P re-dissolution unit A. c) precipitation unit C designed by ALWIN Eppler GmbH & Co. KG.

S5-2: Calculation of the P loads

To evaluate the efficiency of P recovery in the partial processes of re-dissolution and precipitation, as well as in the overall process of P recovery, we first determined the individual P loads that enter these processes (see Figure S5-6).

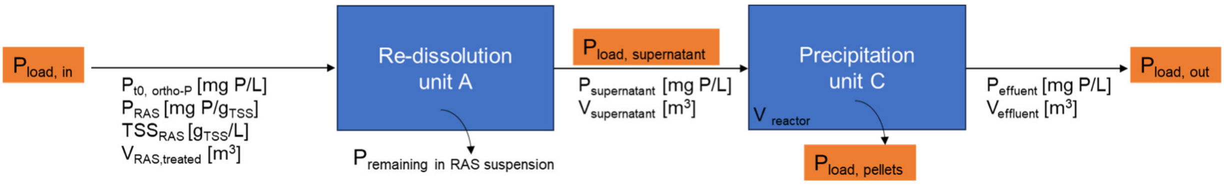

Figure S5-6| Visualization of the P load along the treatment steps.

$P_{\text{load,in}}$ and $P_{\text{load,out}}$ are the input and output mass of P in the re-dissolution unit and precipitation unit, respectively. $P_{\text{load,supernatant}}$ is the mass of P recovered in the collected supernatant. $P_{\text{load,pellets}}$ is the mass of P recovered with the pellets at the outlet of the precipitation unit. The calculation of the respective P loads [g P] is as follows:

$$P_{\text{load,in}} = V_{\text{RAS}} \cdot P_{\text{RAS}} \cdot \text{TSS}_{\text{RAS}} + V_{\text{RAS}} \cdot P_{\text{t0,ortho}-P} \tag{S5-1}$$

$$P_{\text{load,supernatant}} = V_{\text{supernatant}} \cdot \mathrm{P}_{\text{supernatant}} \tag{S5-2}$$

$$P_{\text{load,out}} = V_{\text{effluent}} \cdot \mathrm{P}_{\text{effluent}} = \left(V_{\text{supernatant}} - V_{\text{reactor}}\right) \cdot P_{\text{effluent}} \tag{S5-3}$$

$$P_{\text{load,pellets}} = P_{\text{load,supernatant}} - \mathrm{P}_{\text{load,out}} \tag{S5-4}$$

with P_{RAS} [mg P/g_{TSS}] : initial P content in the RAS solids
V_{RAS} [m^3]: volume of RAS treated
TSS_{RAS} [g_{TSS}/L]: total suspended solids concentration of the RAS
$P_{\text{t0,ortho-P}}$ [mg P/L]: initial concentration of ortho-P in unit A
$V_{\text{supernatant}}$ [m^3]: volume of the produced supernatant (unit B) after re-dissolution
$P_{\text{supernatant}}$ [mg P/L]: concentration of ortho-P in the produced supernatant (unit B)
V_{effluent} [m^3]: volume of effluent leaving unit C
P_{effluent} [mg P/L]: concentration of ortho-P in effluent leaving unit C
V_{reactor} [m^3]: reactor volume of the precipitation unit C left with liquid (dead volume)

For the operation of the plant in August V_{RAS} and P_{effluent} were 22 m^3 and 0.31 mg P/L, respectively. For the operation in September V_{RAS} and P_{effluent} were 123 m^3 and 0.27 mg P/L, respectively. V_{reactor} was 0.28 m^3.

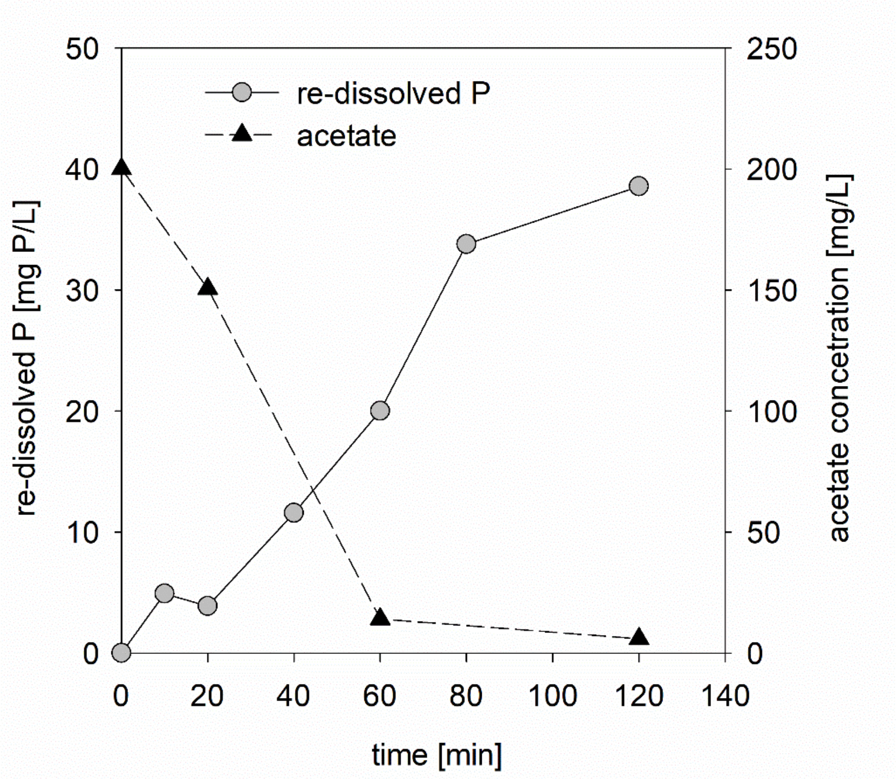

Figure S5-7| Kinetics of P re-dissolution and acetate uptake from RAS supplemented with 200 mg/L acetate at pilot-scale (one batch, 1 m^3). The measured P concentration was normalized to the P values of the control batch (RAS without acetate).

S5-3: Calculation of the pellet surface layer and stoichiometric ratios

Given the lack of access to X-ray diffraction analysis to identify the specific Ca phases in the surface layer of the pellet, we employed a theoretical approach:

1. Elemental composition of the pellets

The measured elemental composition of the P-enriched dolomite pellets Dol-P1, Dol-P2, Dol-P3 (Table S5-6) served as the basis for further calculations.

Table S5-6| Elemental composition of fertilizer pellets.

Sample	Element	wt%	g / g pellet
Dol-P1	P	1.52	0.0152
	Ca	25.21	0.2521
	Mg	9.53	0.0953
	C	11.4	0.114
Dol-P2	P	1.81	0.0181
	Ca	25.57	0.2557
	Mg	9.59	0.0959
	C	11.15	0.1115
Dol-P3	P	0.89	0.0089
	Ca	23.14	0.2314
	Mg	11.34	0.1134
	C	12.05	0.1205

2. Theoretical dolomite composition

Table S5-7 shows the elemental composition of dolomite, $CaMg(CO_3)_2$

Table S5-7| Elemental composition of dolomite.

Element	wt%	g / g dolomite	g/ mol dolomite
Ca	21.7	0.217	40
Mg	13.19	0.1319	24.3
C	13.02	0.1302	24

3. Dolomite content of pellets based on the Mg content

We assumed that the Mg content in the pellets originates exclusively from the dolomite seed material. Therefore, the Mg content was used to determine the moles of dolomite per gram of pellet, using equation S5-5.

$$\text{mol dolomite}/\text{g}_{\text{pellet}} = \text{Mg content pellet } [\text{g}_{Mg}/\text{g}_{\text{pellet}}]/24.3\ \text{g}_{\text{Mg}}/\text{mol}_{\text{dolomite}} \tag{S5-5}$$

Dol-P1: $0.0953\ \text{g}/\text{g}_{\text{pellet}}/24.3\ \text{g}_{\text{Mg}}/\text{mol}_{\text{dolomite}} = 3.92 * 10^{-3}\ \text{mol}_{\text{dolomite}}/\text{g}_{\text{pellet}}$

Dol-P2: $0.0959\ \text{g}/\text{g}_{\text{pellet}}/24.3\ \text{g}_{\text{Mg}}/\text{mol}_{\text{dolomite}} = 3.95 * 10^{-3}\ \text{mol}_{\text{dolomite}}/\text{g}_{\text{pellet}}$

Dol-P3: $0.1134\ \text{g}/\text{g}_{\text{pellet}}/24.3\ \text{g}_{\text{Mg}}/\text{mol}_{\text{dolomite}} = 4.67 * 10^{-3}\ \text{mol}_{\text{dolomite}}/\text{g}_{\text{pellet}}$

These values allowed to calculate the amount of Ca, Mg and C derived from the dolomite by multiplication of the moles of dolomite per gram of pellet with the amount of each dolomite-derived element (Table S5-7).

<u>Example for Ca derived from dolomite in Dol-P1:</u>

$$3.92 \cdot 10^{-3}\text{mol dolomite}/\text{g pellet } \cdot 40\ \text{g}_{\text{Ca}}/\text{mol}_{\text{dolomite}} = 0.1569\ \text{g Ca}/\text{g pellet}$$

4. Elemental composition of the surface layer

The elemental composition of the surface layer is determined by subtracting the contributions of the dolomite core from the total pellet composition (cf. Table S5-8). The molar ratio of Ca/P in the surface layer is 3.9–4.9. To reflect the likely co-existence of calcium phosphate and calcium carbonate species under the experimental conditions[S9] the general formula for the surface layer can be represented as $Ca_x(PO_4)_y(CO_3)_z$, where x, y, z corresponds to the mmol/ g pellet values for Ca, P and C in the surface layer from Table S5-8, respectively. This results in:

Sample	x	y	z	$Ca_x(PO_4)_y(CO_3)_z$*
Dol-P1	2.38	0.49	1.66	$Ca_{4.86}(PO_4)_1(CO_3)_{3.38}$
Dol-P2	2.54	0.58	1.40	$Ca_{4.19}(PO_4)_1(CO_3)_{2.40}$
Dol-P3	1.12	2.89	7.08	$Ca_{3.90}(PO_4)_1(CO_3)_{2.47}$
mean	-	-	-	$Ca_{4.32}(PO_4)_1(CO_3)_{2.75}$

*values normalized to 1 mol PO_4

Balancing the charges of the components suggests a small deficit in positive charge for Dol-P1 and Dol-P3 and a deficit in negative charge for Dol-P2. The former imbalance may indicate the formation of calcium hydrogen phosphates, while the latter suggests the formation of calcium hydroxide to maintain charge neutrality. The theoretical composition was calculated as $[Ca_3(PO_4)_2]_a \cdot [CaHPO_4]_b \cdot [CaCO_3]_c \cdot [Ca(OH)_2]_d$ employing the stoichiometric coefficients summarized in Table S5-9.

The composition of the fertilizer pellets is summarized in Table S5-8.

Table S5-8| Elemental composition of the total pellet (measured) as well as dolomite core, surface layer and the surface layer without $CaCO_3$ contribution (calculated) for the P-enriched dolomite (Dol-P1, Dol-P2 and Dol-P3).

Sample	**Element**	**Total pellet**	**From dolomite**	**Surface layer**			**Surface layer - $CaCO_3$ subtracted**	
		g / g pellet	g / g pellet	g / g pellet	mmol/ g pellet	molar Ca/P ratio	mmol/ g pellet	molar Ca/P ratio*
Dol-P1	P	0.0152	0	0.0152	0.49	4.86	0.49	1.48
	Ca	0.2521	0.1569	0.0952	2.38		0.72	
	Mg	0.0953	0.0953	0	0			
	C	0.114	0.0941	0.0199	1.66			
Dol-P2	P	0.0181	0	0.0181	0.58	4.19	0.58	1.79
	Ca	0.2557	0.1579	0.0978	2.54		1.05	
	Mg	0.0959	0.0959	0	0			
	C	0.1115	0.0947	0.0168	1.40			
Dol-P3	P	0.0089	0	0.0089	0.29	3.90	0.29	1.43
	Ca	0.2314	0.1867	0.0447	1.12		0.41	
	Mg	0.1134	0.1134	0	0			
	C	0.1205	0.1120	0.0085	0.71			

*According to pure $Ca_3(PO_4)_2$ with 3 mol Ca and 2 mol P (molar ratio of 1.5) this suggests that (i) at values <1.5: Ca^{2+} ions are replaced by H^+ and (ii) at values > 1.5 PO_4^{3-} are replaced by OH^-.

Table S5-9| Stoichiometric coefficients for the composition of the surface layer for $[Ca_3(PO_4)_2]_a \cdot [CaHPO_4]_b \cdot [CaCO_3]_c \cdot [Ca(OH)_2]_d$.

Sample	a	b	c	d
Dol-P1	1	0.01	1.14	0
Dol-P2	1	0	3.79	0.95
Dol-P3	1	0.04	0.85	0

Supplementary references

S1 APHA. Standard methods for the examination of water and wastewater. 20th ed.. American Public Health Association. American Water Works Association. Water Environment Federation. Washington DC. 1999.

S2 CEN. DIN EN 16174:2012-11 Sludge. treated biowaste and soil-Digestion of aqua regia soluble fractions of elements. European committee for standardization. Brussels. 2012.

S3 CEN. DIN EN 459-2:2021-09 Building lime - Part 2: Test methods. European committee for standardization. Brussels. 2021.

S4 CEN. DIN EN 12485:2017-10 Chemicals used for treatment of water intended for human consumption - Calcium carbonate. high-calcium lime. half-burnt dolomite. magnesium oxide. calcium magnesium carbonate and dolomitic lime - Test methods. European committee for standardization. Brussels. 2017.

S5 CEN. DIN EN 15958:2012-02 Fertilizers – Extraction of water soluble phosphorus. European committee for standardization. Brussels. 2012.

S6 CEN. DIN EN 15919:2011-08 Fertilizers – Extraction of phosphorus soluble in 2 % formic acid. European committee for standardization. Brussels. 2011.

S7 CEN. DIN EN 15957:2011-12 Fertilizers – Extraction of phosphorus which is soluble in neutral ammonium citrate. European committee for standardization. Brussels. 2011.

S8 CEN. DIN EN 15956:2011-12 Fertilizers – Extraction of phosphorus soluble in mineral acids. European committee for standardization. Brussels. 2011.

S9 Seckin, I. Y., Altiner, M., Kayranli, B., & Yilmaz, T.. Acidification and alkali precipitation for phosphorus recovery from municipal digested sludge. *Water and Environment Journal* 38, 259-268. 2024.

Chapter 6

General conclusions and outlook

6.1 A bio-based approach to P recovery

The sustainable use of resources is a major challenge for today’s society. As an essential nutrient for plant growth, P plays a fundamental role for agricultural productivity and global food security. Despite its widespread abundance in nature, P used in fertilizers is largely derived by mining finite PR deposits. As the global demand for P fertilizers continues to rise, and easily accessible PR deposits decrease, a sustainable P management is becoming more critical. But the relevance of sustainable P management extends beyond agricultural productivity to wastewater management and ecosystem health. In this context WWTPs have traditionally focused on the removal of P through an effective wastewater treatment. Moving forward, WWTPs are now aiming to transition into biorefineries for the recovery of resources, including P (Solon et al., 2019).

In this thesis, a bio-based approach to P re-dissolution from AS and the subsequent recovery of P as a multi nutrient product and soil amendment was investigated across laboratory- and pilot-scale systems. Wastewater is commonly treated using microbial communities to remove P, but up today there is no clear path for a biological P recovery ahead of anaerobic digestion, where sludge P concentration is the highest (Vučić and Müller, 2021). In order to bridge the gap between existing laboratory studies performed with highly PAO-enriched AS and future large-scale applications, non-acclimated AS from full-scale municipal WWTPs was investigated to mirror the real-world microbiology of the respective WWTP. The thesis is structured around three consecutive studies, focusing on a rapid VFA-mediated P re-dissolution that targets the polyP pool of PAOs. Together the results provide insights into the variations across WWTPs, optimization of the carbon supplementation, as well as challenges and limitations of scaling the process to pilot-scale.

In the following the major findings from the studies, presented in chapters 3–5, are summarized. Insights and practical implications for the feasibility of a bio-based P recovery strategy and a more sustainable P management in both EBPR and mixed EBPR/CPR systems are presented. The results of this research highlight practical challenges and limitations of bio-based P recovery in real-world systems and hold implications for the operational practices in wastewater treatment

In chapter 3, the potential for a biological P re-dissolution from AS from three German full-scale WWTPs, that employ (i) simultaneous Fe-based CPR/EBPR, (ii) Al-based CPR post-EBPR and (iii) pure EBPR, was evaluated by examining kinetics of P release and acetate uptake. The kinetics of P release and acetate uptake followed the general trend of rapid P re-dissolution previously observed in enriched laboratory cultures (Acevedo et al., 2015), suggesting substantial PAO activity. FISH analysis of the microbial community confirmed that *Accumulibacter* dominated over its competitor, *Candidatus* Competibacter spp., across all of the WWTPs studied (Figure 3-2), although its abundance was less in the mixed EBPR/CPR systems and significantly lower than in laboratory SBRs. Thus, the conditions for re-dissolution by inversion of EBPR are generally met, even in WWTPs with a long history of co-dosing iron salts within the biological treatment (Anders et al., 2023, 2021). Comparison of the re-dissolution yields demonstrated that among the three WWTPs, AS from pure EBPR performed best, while the P yield was reduced in mixed EBPR/CPR systems (Figure 3-3). In detail, 56% of total P in the EBPR-AS was re-dissolved within 4 h of anaerobic treatment at neutral pH facilitated by complete uptake of the supplemented carbon source.

The substantial biomass P-depletion achieved under these conditions aligns with the requirements of the German Sewage Sludge Ordinance allowing for co-incineration of the resulting sludge or use in cement kilns. In contrast high iron dosage interfered with the P re-dissolution (Anders et al., 2024, 2023, 2021) by formation of dense flocs, likely limiting substrate transport and reducing bioavailability of P (Liu et al., 2011; Oikonomidis et al., 2010). The high inorganic fraction of the sludge (35% non-VSS) is likely at the expense of PAO activity and the intracellular polyP pool, as evident from the low abundance of *Accumulibacter* in chapter 3 (Anders et al., 2021). Despite these limitations, a maximum P re-dissolution yield of 19–22% was observed. These findings highlight the importance of bioavailable P and minimizing chemical interference in optimizing PAO activity for effective P recovery and suggest that AS from pure EBPR systems offer a superior recovery potential.

However, CPR is often used in addition to EBPR to comply with effluent limits (Korving et al., 2019). Thus, the focus in chapter 4 was on optimizing the re-dissolution from mixed EBPR/CPR AS with the aim of facilitating a high biological P re-dissolution for recovery. Since the microbial sludge community, especially PAOs are typically adapted to their habitat and shaped by the individual plant influent (Dottorini et al., 2023), suitability of carbon sources for P re-dissolution was further investigated. Comparison of the VFA formate, acetate, propionate and butyrate confirmed acetate as the most suitable carbon source, that allowed for a consistent and rapid P release upon supplementation, including a high acetate uptake and P release rate. However, the lower $P_{release}/C_{uptake}$ ratios compared to previous studies (López-Vázquez et al., 2007; Qiu et al., 2019) highlight the influence of the individual microbial community on the carbon uptake, with a portion being utilized for processes beyond P release.

It has to be acknowledged that the supplementation of carbon is necessary to drive a rapid P re-dissolution. Therefore, for a real-world implementation significant substrate costs may be expected, even if acetate is a promising and readily available substrate (Kiefer et al., 2021). Consequently, the optimal substrate dosage for maximal P re-dissolution was investigated. In short, acetate at a one-time dosage level of 200 mg/L was the most cost-beneficial for the P re-dissolution, while higher dosage levels exhausted the P re-dissolution capacity. Compared to chapter 3 this allowed for a reduction in substrate supplementation of 50% without compromising the P re-dissolution yield. Surprisingly, additional P release was observed when acetate uptake ceased. Theoretical stoichiometric metabolic modeling of the P re-dissolution process in *Accumulibacter* suggested that the prolonged P release after acetate cessation was possibly linked to glycogen degradation coupled with the membrane potential. Clearly, further investigations are needed to reveal underlying processes, possibly by incorporation of meta-transcriptomics to study active metabolic pathways. While the presented studies focused on *Accumulibacter* for P re-dissolution, recently Liu et al. (2019) highlighted the abundance of *Tetrasphaera* spp. in Danish and Polish WWTPs operating at low temperatures. Given the similar climatic conditions, extending the FISH analysis to include *Tetrasphaera* spp. for the German WWTPs could provide additional insights and help to explore alternative carbon sources tailored to their metabolic potential.

It was demonstrated that short-term anaerobic treatment with acetate at neutral pH facilitates a rapid P re-dissolution from non-acclimated AS within 1–3 h, possibly offering an advantage over conventional biological recovery methods that require prolonged anaerobic conditions. Although the sludge treatment through anaerobic digestion usually offers an opportunity for P recovery, provided that WWTPs have the appropriate facilities, it often goes hand in hand with uncontrolled struvite scaling and release of organic and inorganic contaminants solubilized by sludge degradation and pH reduction (Marti et al., 2008; Selling et al., 2008). Minimizing the anaerobic exposure can be beneficial to maintain the sludge structure as well as PAO activity, keeping potential contaminants bound within the biomass (Chen et al., 2003; Wilén et al., 2000). Additionally, the rapid P release is beneficial for the treatment of larger volumes as well as for recycling of the sludge to the aerobic treatment stage, potentially selectively enriching PAOs and optimizing the microbial community for efficient P removal and recovery. Thus, by specifically targeting the intracellular polyP pool through carbon source supplementation, the process allows for a selective P re-dissolution. However, it is important to note that achieving complete sludge-P recovery with this strategy is not feasible. Even in pure EBPR systems a substantial portion of P can be attributed to the inorganic P fraction (>38%) and microbial cell components such as DNA and cell membranes (3–4%), that will remain unavailable (Petriglieri et al., 2022).

To transfer the process from laboratory to full-scale implementation and to identify possible limitations, chapter 5 focused on testing the scalability and practical feasibility of the optimized carbon dosing strategy (developed in chapter 4) for P re-dissolution and recovery. This was evaluated in a pilot-scale setup with a total re-dissolution unit volume of 8 m³, treating real return AS (15 m^3/d). The goal was to achieve high P release in < 2 h for the subsequent precipitation in a fluidized bed reactor, producing a P-enriched recovery product. The optimized dosing strategy demonstrated potential at larger scale, allowing for efficient recovery of P from the supernatant (99%) by precipitation using low cost materials dolomite and milk of lime. However, the individual P re-dissolution yields were less consistent compared to the laboratory experiments, likely due to process variabilities in the RAS feed and WWTP operation. The overall recovery rate of 1.9% highlighted the need for process optimization to minimize P losses and enhance P recovery efficiency.

The recovered P-enriched dolomite pellet contained 0.9–1.9 wt% P (Chapter 5, Anders et al., 2024). While the P content in the pellet was lower than anticipated due to a limited volume of the P-rich feed (30 m^3) and low P concentration (22.7 mg/L), the final product showed promising characteristics for the use as dual-purpose soil amendment. The pellets' non-water solubility and full solubility in formic acid and neutral ammonium citrate (Table 5-5) highlights the potential to mitigate P run-offs, a major challenge of conventional water-soluble fertilizer applications such as with triple superphosphate (TSP). The trace metal concentrations were found to fall within the limits set by the German Fertilizer Ordinance. Only with these limits in mind would it be possible to use the product in agriculture without requiring a declaration. Hence, the P-enriched dolomite pellet may act as a slow-release fertilizer and soil conditioner to provide nutrients P, Ca, and Mg as well as a pH adjustment, particularly for acidic soils. Soil acidification is a widespread problem that affects a large part of arable soils worldwide and decreases nutrient availability and crop growth (Dai et al., 2017; von Uexküll and Mutert, 1995). Traditionally this is addressed by liming (Shaaban et al., 2015; Wu et al., 2021). The in chapter 5 proposed sequential solubilization of the P-enriched pellet may allow the pellet's surface to first release Ca and P, followed by the dolomite's effect for pH adjustment and improving soil structure as well as nutrient availability (Chen et al., 2006; Shreckhise et al., 2020; Yang et al., 2012). Products with similar functionalities such as Dolophos® (DüKa Düngekalkgesellschaft) - a mixture of SSA and dolomite - are already marketed as fertilizers for grassland and farming. Given the established use and consumer familiarity these may serve as a reference when considering the market potential of P-enriched dolomite pellets. Given the likely decline of direct land application of SSA due to its low P bioavailability (Clemens and Teloo, 2020; Kabbe and Rinck-Pfeiffer, 2019) the P-enriched dolomite pellets could offer an alternative with a lower trace metal contamination risk. Efficiency of the pellet however may be limited in neutral and alkaline soils, or depend on presence of P solubilizing microorganisms, mycorrhizal

symbioses, or plant root exudates to mobilize the P (Deng and Dhar, 2023; Piol et al., 2019). Plant growth tests are needed to evaluate the pellets' performance in diverse soil types, which was out of scope in the presented study (Anders et al. 2024).
Beyond agriculture, the P-enriched dolomite pellet could serve as a raw material for established industrial processes, precursors for the fertilizer production or as a livestock feed, promoting a diverse P recycling way (Clemens and Teloo, 2020; Deng and Dhar, 2023).

Despite potential, a further process optimization is required to increase the P content in the precipitated surface layer of the pellet. Enhancing re-dissolution of P from the AS and optimizing the scaled-up process is critical here to produce an aqueous phase with consistently high P concentrations. Additionally, characterizing the specific Ca-P phases and their crystallinity will be essential, as different phases (e.g., HAP or amorphous Ca-P) exhibit varying solubility that would affect the nutrient availability (Deng and Dhar, 2023). Although the product could create a revenue stream for the WWTP, the current marketing of P recovery products faces limitations by the small size of the individual recovery stream, which cannot compete with the production of large quantities of comparably low-cost PR-derived fertilizers. Here policy frameworks are essential for the adoption of the recovered P products by emphasizing their environmental benefits and promoting market acceptance (Cheng et al., 2023).

Overall, the findings demonstrate that non acclimated AS from pure EBPR systems is most suited for a bio-based P recovery, when acetate is employed as a carbon source. It allowed for a high re-dissolution of >50% of total sludge P, thus achieving a depletion of the sludge P concentration below the “P recovery threshold” set out by the German Sewage Sludge Ordinance. However, as shown for WWTPs with simultaneous CPR usage during EBPR, the P recovery yield will be significantly reduced (21–24%). Therefore, prioritizing EBPR as the main P removal strategy during wastewater treatment is recommended to enhance the activity of PAOs, the size of the polyP pool and thus facilitate a more efficient P recovery from the AS directly on-site as part of the treatment process.

Adopting EBPR may offer a viable way to enhance sustainability and cost-effectiveness of wastewater treatment (Bertanza et al., 2020), while meeting the demands for P recovery. By reducing the chemical precipitant usage, WWTPs could not only lower chemical costs but also the sludge volume and disposal costs. Decreasing the reliance on precipitant usage is especially relevant given rising costs and supply chain vulnerabilities, which have been highlighted during recent crises such as the Covid-19 pandemic and the Russia-Ukraine conflict (Könemann, 2022). In this case, the dependence of precipitant production on industry by-products and pre-cursors has causes product shortages in Germany and undermined the compliance with the P discharge limits (DWA, 2022). Thus, focusing on EBPR as the primary strategy for P removal can provide a greater resilience to external supply disruptions while offering operational benefits and making P recovery more viable.

Yet, it has to be acknowledged that when effluent standards are especially stringent, combining EBPR with occasional CPR may still be necessary to ensure compliance. In such cases, moving the chemical precipitation step post-biological treatment (cf. WWTP2, chapter 3) or even post-clarifier could be beneficial. This adjustment would increase the P availability for PAOs and minimize chemical interference. Alternatively, implementing a P-dependent dosing strategy may be also effective. While such a dual approach leads to the formation of some non-bioavailable iron- or aluminum-bound P fractions (Oikonomidis et al., 2010), it is likely to result in fewer of such fractions compared to simultaneous dosing. By depleting the sludge of P, early on in the treatment process, the risk of pre- and post-digestion struvite scaling as well as the P backflow to the plant is minimized, both providing operational benefits for the WWTP.

The diverse influent and operating parameters of each plant as well as the available infrastructure will likely require a case-by-case evaluation of the optimal approach to P recovery (Cheng et al., 2023; Wang et al., 2024; Zhang et al., 2022). Additionally, WWTPs operate under different requirements for effluent limit, which so far had impacted the employed P removal strategies. Since regulatory stringency varies regionally across Germany, regional context in the development of P recovery efforts may be necessary. However, a simple experimental setup for a short-term re-dissolution, as described in chapter 3, could help to assess the general potential for a bio-based P recovery and evaluate it based on the performance indicators $P_{yield}/VFA_{consumed}$ and P_{yield}/VFA_{spike} introduced in chapter 4.

6.2 Towards a circular economy

Biological technologies for the P recovery are still at an early stage of development. Based on the results presented, the following areas are proposed for future work.

It is highly recommended to conduct continuous pilot-scale field tests, especially in pure EBPR WWTPs, to evaluate the short-term P re-dissolution and optimize the process units to reduce P losses and improve recovery efficiencies. If the P re-dissolution yields consistently mirror the high yields observed in the laboratory-scale study (Anders et al., 2021), a more detailed techno-economic and cost analysis can be carried out. Furthermore, transitioning from the batch/semi-continuous to continuous pilot plant operation is likely essential to ensure practical feasibility in a full-scale setting (Zhang et al., 2022).

Moreover, the long-term effects of repeated P re-dissolution and P uptake cycles on PAO activity, microbial diversity and WWTP performance need to be assessed. While some laboratory-studies suggest that PAOs might adapt to such conditions with an increased P removal activity (Guisasola et al., 2019; Wong et al., 2018), a biomass P extraction threshold of 60% of influent P has been proposed as the biological limit of PAOs to ensure long-term operation (Zhang et al., 2022).

In order to reduce substrate costs and the carbon footprint, future research should explore alternatives to fossil-fuel derived VFAs. The research may be directed towards the use of non-targeted fermentation of primary sludge or the use of specialized microorganisms for autotrophic/heterotrophic production of acetate or tailored VFA-mixtures. Here, organic waste or CO/CO_2 may be used as raw materials for biotechnological VFA production (Xie et al., 2015; Zhang et al., 2020). As many WWTPs have access to biomass streams, this could allow a more economically viable provision of substrates (Kamiyama et al., 2024). However, the integration must be carefully evaluated, to ensure the purity of the VFAs produced and to adjust the concentration to the specific requirements of P re-dissolution. Combining P recovery with the recovery of other materials such as PHA or biogas could provide additional value streams to enhance the overall economic viability and sustainability of WWTPs as future resource recovery facilities (Solon et al., 2019).

Beyond this, further research is required to assess the agricultural value of the P-enriched dolomite pellet as a soil amendment. Studies on the influence on soil health, provision of nutrients (P, Mg and Ca) and crop yield, as well as their long-term environmental safety, are necessary. Additionally, the use of the recovery product as a raw material for industrial processes or as an animal feed should be explored in order to expand its applicability or tailor the seed material of fluidized bed precipitation accordingly.

In conclusion, this work provides a comprehensive view on a bio-based approach to P recovery from AS, detailing underlying mechanisms and conditions for P re-dissolution and P precipitation. The feasibility of a biological and rapid P re-dissolution was demonstrated and highlighted the potential of an acetate-mediated strategy for a targeted P depletion from the sludge prior to anaerobic digestion, especially in pure EBPR systems. The results emphasize the influence of WWTP operation and sludge characteristics on the P recovery yield, identifying acetate as the most efficient carbon source for maximum P re-dissolution from mixed EBPR/CPR systems. Result insights support the adoption of pure EBPR systems as a sustainable and potentially more environmentally friendly P management strategy, aligning with the growing recognition of EBPR’s potential in wastewater treatment. While scale-up challenges and reduced P recovery yields in mixed EBPR/CPR systems remain, the findings highlight the potential for integrating the process into existing WWTPs. This may facilitate the development of on-site nutrient recovery technologies, supporting the broader vision of a circular economy.

Chapter 7

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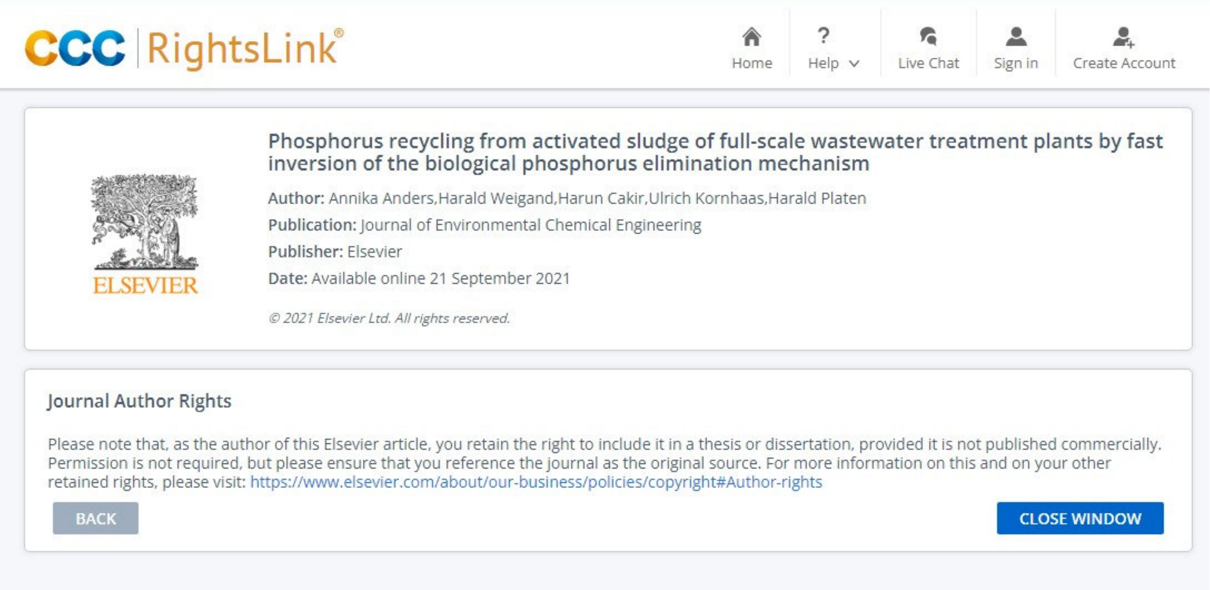

Phosphorus recycling from activated sludge of full-scale wastewater treatment plants by fast inversion of the biological phosphorus elimination mechanism

Author: Annika Anders,Harald Weigand,Harun Cakir,Ulrich Kornhaas,Harald Platen

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For Chapter 4: "Phosphorus recovery by re-dissolution from activated sludge – Effects of carbon source and supplementation level revisited" https://doi.org/10.1039/D2EW00356B:

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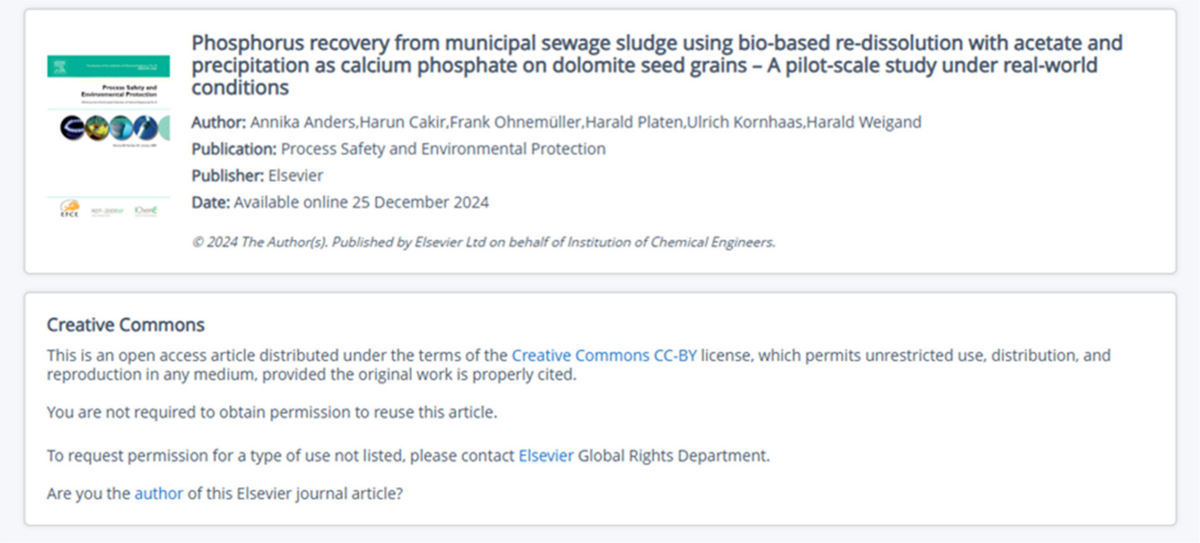

Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions

Author: Annika Anders,Harun Cakir,Frank Ohnemüller,Harald Platen,Ulrich Kornhaas,Harald Weigand

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Chapter 8

List of publications

8.1 Peer-reviewed publications included in this thesis

I. Phosphorus recycling from activated sludge of full-scale wastewater treatment plants by fast inversion of the biological phosphorus elimination mechanism
Annika Anders, Harald Weigand, Harun Cakir, Ulrich Kornhaas, Harald Platen
J. Environ. Chem. Eng, Volume 9, Issue 6 https://doi.org/10.1016/j.jece.2021.106403.

II. Phosphorus recovery by re-dissolution from activated sludge – Effects of carbon supplementation and carbon source revisited
Annika Anders, Harald Weigand, Harald Platen
Environ. Sci.: Water Res. Technol., 2023, Volume 9, Issue 1
https://doi.org/10.1039/D2EW00356B

III. Phosphorus recovery from municipal sewage sludge using bio-based re-dissolution with acetate and precipitation as calcium phosphate on dolomite seed grains – A pilot-scale study under real-world conditions
Annika Anders, Harun Cakir, Frank Ohnemüller, Harald Platen, Ulrich Kornhaas, Harald Weigand
Process Saf. Environ. Prot., online December 2024, in press
https://doi.org/10.1016/j.psep.2024.12.088

8.2 Research report – Re-BioP-Cycle Project

Recycling von Phosphor aus kommunalen Kläranlagen durch gekoppelte Bio-P-Rücklösung und Kristallisation/ Pelletierung im Wirbelbettreaktor (Re-BioP-Cycle), Annika Anders, Harald Weigand, Harald Platen, Harun Cakir, Frank Ohnemüller, Forschungsbericht Nr. 1/2021, Abschlussbericht zum Forschungsvorhaben AiF-Nr. 19746N, Forschungsgemeinschaft Kalk und Mörtel e.V.

8.3 Poster and Presentations

I. Conference Poster:

Phosphorus Release from Activated Sludge by Inversion of Biological P-Elimination for Fertilizer Production in a Ca-Fluidized Bed, Microbiotec Congress 2019, Coimbra, Portuguese Microbiology Society (SPM) and the Portuguese Biotechnology Society (SPBT), University of Coimbra, Portugal, December 5–7, 2019

II. Conference Abstract:

Perspectives and Limitations of the Inverted Biological P-Elimination for P-Fertilizer Production from Sewage Sludge, ESPC4-4th European Sustainable Phosphorus Conference, Vienna, June 2020 (postponed)

III. Presentations:

Ausloten der Grenzen: Biobasiertes P recylcing aus kommunalen Klärschlamm und herstellung von Mehrnährstoffdüngern im Wirbelbettkristallisationsreaktor, DPP-Forum 2024, Deutsche-Phosphor-Platform e.V., Frankfurt am Main, Germany, October 23, 2024

Bio-Based Phosphorus Recycling from Municipal Sewage Sludge: A Pilot-Scale Study - Evaluating Efficiency, Product Characteristics, and Future Directions, ISESIE 2024 - 4th International Symposium in Environmental Science and Industrial Ecology, Bangkok, Thailand, August 23–24, 2024

Half-yearly reports on the project status (2019–2021) – Re-Bio-P-Cycle, Arbeitskreis Kalk im Umweltschutz, Bundesverband der Deutschen Kalkindustrie e.V. Cologne, Germany

8.4 Student theses created during the research project

I. Lukas Künkel (2020) „Phosphorrückgewinnung aus Belebtschlamm der Kläranlage Gießen mit biologischer Phosphorelimination - Untersuchung von Eisenphosphat-Verbindungen nach Phosphorrücklösung mit Hilfe einer Laborkläranlage, Bachelor thesis

II. Nikolai Bannwitz (2019) Untersuchungen zur Freisetzung biologisch gebundenen Phosphats („BioP“) aus Belebtschlamm durch Acetat, Bachelor thesis

Chapter 9

List of Figures and Tables

9.1 List of Figures

9.2 List of Tables

Chapter 10

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Eidesstattliche Erklärung – Declaration of Authorship

Ich erkläre: Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Ich stimme einer evtl. Überprüfung meiner Dissertation durch eine Antiplagiat-Software zu. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der entsprechenden Satzung der federführenden Hochschule niedergelegt sind und die mir ausgehändigt wurde, eingehalten.

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the regulations of the leading University which were delivered to me in carrying out the investigations described in the dissertation.

Giessen, 31.12.2024