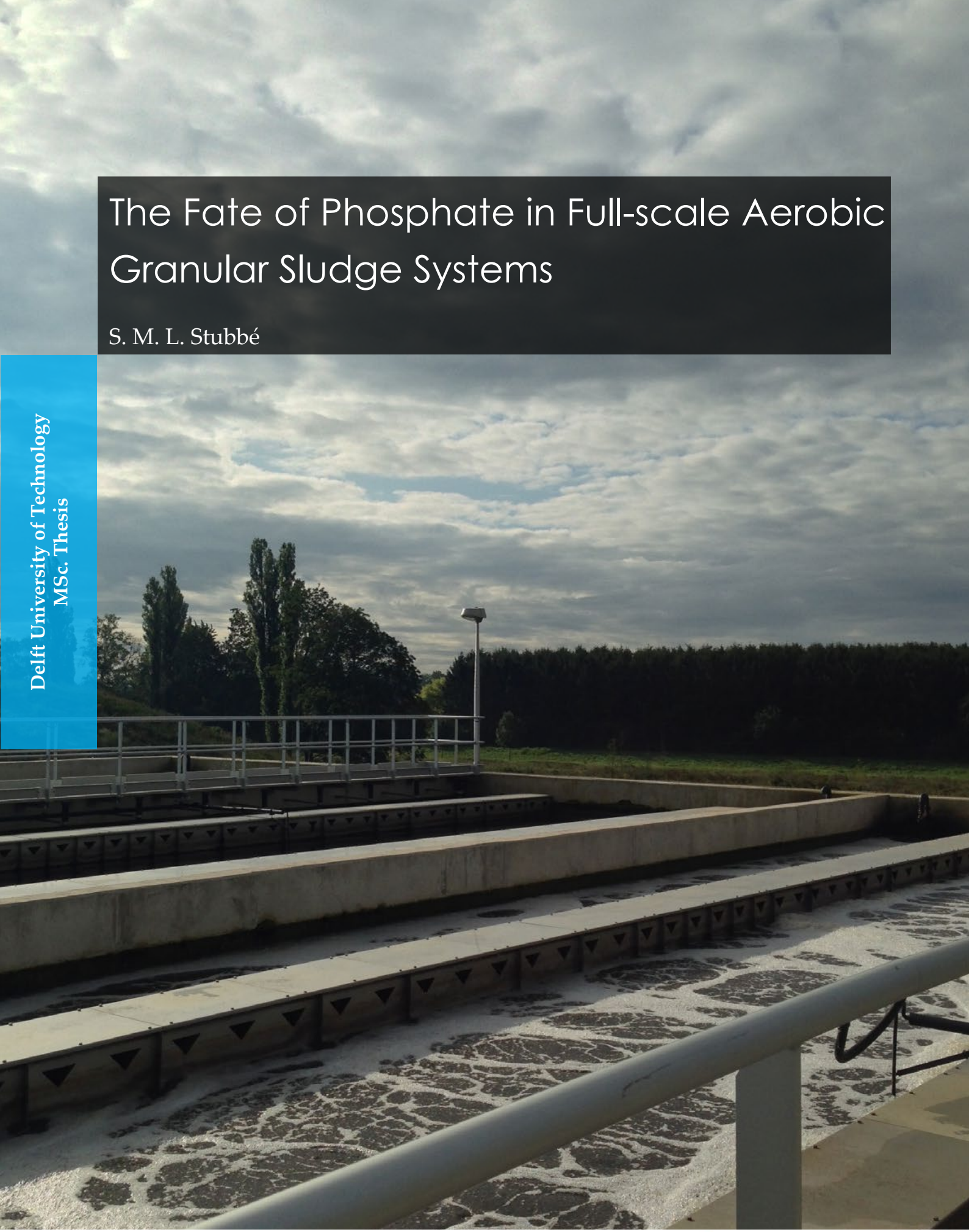
The Fate of Phosphate in Full-scale Aerobic Granular Sludge Systems

S. M. L. Stubbé

Delft University of Technology
MSc. Thesis



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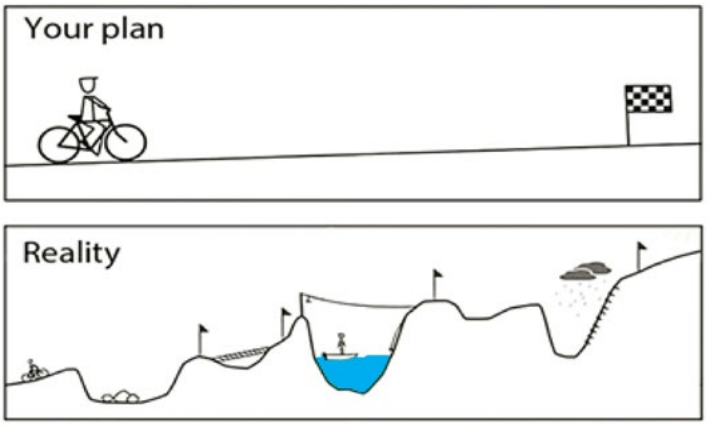
It is the journey that makes you happy, not the end goal
- Dan Millman



Title	The Fate of Phosphate in Full-scale Aerobic Granule Sludge Systems
Author	Stefanie Maria Lousie Stubbé
Studentnumber	4212789
Daily Supervisor	Dr. Mario Pronk, Applied Sciene
Chair Assessment Committee	Dr. Ir. Merle K. de Kreuk, Civil Engineering
Assessment Committee	Prof. Dr. Ir. Mark C. M. van Loosdrecht, Applied Science
	Dr. Ir. Robbert Kleerebezem, Applied Science
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The Fate of Phosphate in Full-scale Aerobic Granule Sludge Systems

By

Stefanie Maria Louise Stubbé

The Fate of Phosphate in Full-scale Aerobic Granular Sludge Systems

phosphate as a potential additional process control parameter for aerobic granular sludge systems

by S.M.L. Stubbe[1,2,3] supervised by M. Pronk[3,4], R. Kleerebezem[4,] M.C.M. van Loosdrecht[4] and M.K. de Kreuk[1],

[1]Delft University of Technology, Civil Engineering, Sanitary Engineering, Stevinweg 1, 2628 CN, Delft, The Netherlands
[2]Wetsus European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, 8911 MA, Leeuwarden
[3]Royal HaskoningDHV, P.O Box 1132, 3800 BC Amersfoort, The Netherlands
[4]Delft University of Technology, Kluyvewr Institute for Biotechnology, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

Abstract

In previous works, biologically induced phosphate precipitation was observed in lab-scale aerobic granular sludge (AGS) reactors. In this study, the contribution of biologically induced phosphate precipitation to the total removal of phosphate was investigated in full-scale AGS installations in Utrecht and Garmerwolde, the Netherlands. A longitudinal phosphate balance showed that under current operating conditions, phosphate does not accumulate noticeably in mature full-scale AGS systems. The total phosphate content of granule fractions, the scanning electron microscope (SEM) images coupled with energy dispersive X-rays (EDX) and the X-ray fluorescence (XRF) analyses on reactor granules showed no substantial phosphate crystals in the granules. The X-ray diffractions (XRD) revealed the presence of quartz crystals (SiO_4) and, at some moments in time, different types of other minerals (e.g., potassium struvite, dolomite, magnesium calcite, brushite). Other XRD measurements however, showed no crystals at all besides quartz. It is suggested that a dynamic precipitation - dissolution process occurs in full-scale AGS systems within each process cycles or over multiple cycles. Overall, no indications were found phosphate precipitation contributed significantly to the total phosphate removal. In addition, the potential of phosphate to contribute to the process control of full-scale AGS installations is discussed.

1. Introduction

A promising innovation in wastewater treatment was implemented with the first full-scale Aerobic Granular Sludge (AGS) installation in Epe, the Netherlands in 2011 (van der Roest et al. 2011, Giesen et al. 2013). The first full-scale installation was the result of successful research on discontinuous fed sequencing batch reactors with aerobic granules conducted in the nineties (Morgenroth et al. 1997, Beun et al. 1999). AGS technology enables final clarification and all necessary conversions (organics and nutrients removal) in one tank due to the advantageous settling properties and unique structure of the aerobic granules. AGS technology can reduce the space requirements of the installation by ± 80% and is cost-effective compared to activated sludge technology (De Bruin et al. 2004, De Kreuk et al. 2004). The granules consist of a layered structure: slow autotrophic (e.g., nitrification) and a little heterotrophic growth takes place on the outside while only heterotrophic growth (e.g., denitrification) takes place on the outside (Beun et al. 2001, De Kreuk et al. 2005, De Kreuk 2006).

Phosphate removal is an important step towards protecting the environment against eutrophication (Balcerzak 2006). In AGS systems, phosphate removal may occur biologically or chemically (Yeoman et al. 1988). Biological phosphate removal by phosphate accumulating organisms (PAOs) has been researched extensively and the effectiveness has been proven in practice (Bonting et al. 1993, Schönborn et al. 2001, Seviour et al. 2012, Acevedo et al. 2015, Welles 2015). Chemical removal of phosphate by dosing metal salts is widely applied, although the method has certain disadvantages as outlined by Röske et al. (1994) (e.g., higher costs, additional metal hydroxide sludge). The chemical removal of phosphate can also take place through biologically induced phosphate precipitation, when biologically conditions are created to initiate phosphate precipitation. This mechanism was already discovered in the eighties (Arvin 1983, Appeldoorn et al. 1992), but was recently revived as a feasible option, as the granule's gradients combined with the phosphate release of PAOs enhance the conditions for precipitation. Phosphate precipitation products such as struvite and hydroxyapatite are especially interesting since they are reusable products in agriculture (De-Bashan et al. 2004). Additionally, precipitation was proposed to be beneficial for the settling properties of both granules and flocs (Peeters et al. 2011, Winkler 2012). Phosphate precipitates in the form of amorphous calcium phosphate ($Ca_3(PO_4)_2{:}6H_2O$, *ACP*), hydroxyapatite ($Ca_5(PO_4)_3OH{:}6H_2O$, *HAP*), whitlockite ($MgFe(PO_4)_6PO_3OH$) and

Nomenclature

ACP	Amorphous calcium phosphate ($Ca_3(PO_4)_2.6H_2O$)
ASM2d	Activated Sludge Model No 2d
AGS	Aerobic granular sludge
Bio-P	Biological phosphate removal
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EBPR	Enhanced biological phosphorus removal
EDX	Energy Dispersive X-ray
EPS	Extracellular polymeric substances
Fe	Iron
FISH	Fluorscence in situ hybridization
HAP	Hydroxyapatite ($Ca_5(PO_4)_3OH.6H_2O$)
K_p	Solubility product of mineral phase *p*
N	Nitrogen
NH_4	Ammonium
NH_4 : TP	Ammonium : Total Phosphatewwv influent ratio
Nkj	Kjeldahl-Nitrogen (organic-N, NH_3, NH_4)
P	Phosphate
PAO	Phosphate Accumulating Organism
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PO_4	Ortho-phosphate
Poly-P	Poly-Phosphate
PRT	Phosphate retention time
SEM	Scanning Electron Microscopy
SI	Supersaturation Index
SRT	Sludge Retention Time
STR-NH_4	Ammonium magnesium phosphate ($MgNH_4PO_4.6H_2O$)
STR-K	Potassium magnesium phosphate ($MgKPO_4.6H_2O$)
STR-Na	Sodium magnesium phosphate ($MgNaPO_4.6H_2O$)
SS	Suspended solids
TN	Total Nitrogen
TP	Total Phosphate
TSS	Total Suspended Solids
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
WW	Wet Weight
XRD	X-ray diffractogram
XRF	X-ray fluorescence

potassium-struvite ($MgKPO_4{:}6H_2O$ STR-K, iso-structural and iso-morphous of ammonium struvite, $MgNH_4PO_4{:}6H_2O$ STR-NH_4) are found in aerobic granules harvested from lab reactors (Barat et al. 2008, Mañas et al. 2011, Lin et al. 2012, Li et al. 2014, Wan et al. 2015). The occurrence and potential of phosphate precipitates in full-scale granules remains unknown. Although Li et al. (2014) reported iron, silicate, calcium and phosphate precipitation in granules from a full-scale SBR system, their non-distinguishable evidence was solemnly based on XRF analyses of the influent and sludge. These elements (Fe, Si, Ca and PO_4), however, could accumulate as part of extracellular polymeric substances (EPS), polyphosphates (poly-P) or entrapped sand and clay particles instead of biologically induced precipitation.

Beside the phosphate removal mechanisms, this study investigated the potential contribution of phosphate measurements to the process control of full-scale AGS systems. It is investigated whether a total phosphate (TP) balance can provide an additional method to estimate the sludge retention time (SRT) and sludge production of AGS systems. As phosphate is inert and no volatile gases are involved in any conversion products, this parameter is advantageous over other parameters such as nitrogen or chemical oxygen demand (COD) to establish a mass balance. The dry weight measurements, currently used for determining the SRT and sludge production, are known to have complications with measuring additional, non-biomass related waste (Tchobanoglous et al. 1991). In addition, handling the samples is considerably more complicated in AGS systems than in activated sludge systems, considering the granules' fast settling behaviour, the dynamics of the cycle phases and the reactor design. The AGS technology provides the opportunity to study the operating parameters used in activated sludge systems and determine whether they are reasonable applicable to AGS systems.

This study aimed to determine the fate of phosphate in full-scale AGS installations. This is done by identifying both the biologically induced phosphate precipitation and the potential contribution of the phosphate balance to the process control of AGS systems. Furthermore, a detailed protocol for establishing an accurate TP-balance was created during this study and provided here.

2. Materials & Methods

Description of full-scale installations

Samples were taken from the full-scale AGS installations in Utrecht and Garmerwolde, the Netherlands. Each installation was operated with a process cycles of ± 6 hours; one hour of anaerobic feeding in plug-flow from the bottom with simultaneous effluent withdrawal at the top. This was followed by 15 min of excess sludge discharge, four hours of aeration, depending on the applied set-points, and one hour for settling. During rain weather conditions, the cycle was shortened to minimal 3 hours. At both treatment sites, a conventional activated sludge treatment line was operated in parallel next to the AGS system.

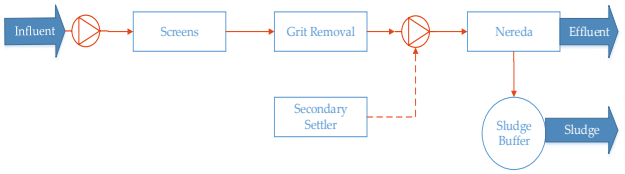
At the full-scale AGS installation in Utrecht (hereafter 'Utrecht'), only biological phosphate removal was applied to obtain a low phosphate effluent concentration. The full-scale prototype AGS installation had a maximum hydraulic capacity of 125 m^3 h^{-1}, a volume of 1,000 m^3 and was 6 m height. Wastewater influent entered the installation through the screens (6 mm) via the grit removal in the intermediate pumping station (1,050 m^3 h^{-1}) (Fig. 1). Clean water was added from time to time to the influent of the AGS installation to obtain a higher upward velocity during feeding (3 - 5 m h^{-1}). This water originated from the secondary settler of the existing AB-plant that is operated in parallel next to the AGS system. The excess sludge of this AGS installation was transported to the on-site sewer system. The effluent of the AGS reactor was not directly discharged in the nearby river and therefore there were no effluent requirements for this installation.

The full-scale AGS installation in Garmerwolde (hereafter 'Garmerwolde') operated since June 2013 with a hydraulic capacity of 4,200 m^3 h^{-1} (Pronk et al. 2015). Wastewater entered the installation through the screens (6 mm), via the grit removal, in the influent buffer (4,000 m^3). Two AGS reactors (height 7.5 m and 9,600 m^3 volume each) were operated simultaneously with half a cycle time-difference (Pronk et al. 2015). The excess sludge was stored in the sludge buffer tank (400 m^3) before being transported to the on-site sludge treatment facilities. Additional iron dosing for phosphate removal was frequently used until May 2015, after which only occasional dosing was applied, when the effluent phosphate levels exceeded the requirements.

Total phosphate balance per day

The total phosphate (TP) balance of the installations was made by subtracting the effluent and excess sludge phosphate loading rate (kgTP d^{-1}) from the influent phosphate loading rate (see Eq. 1). The resulting term (Acc.) represents the accumulation or loss of phosphate out the reactor. No steady state was assumed. The TP concentrations of the influent and effluent were provided by the water authorities (Stichtse Rijnlanden en Noorderzijlvest). These analyses were done every 3 to 5 days as part of the water authority's

Utrecht



Garmerwolde

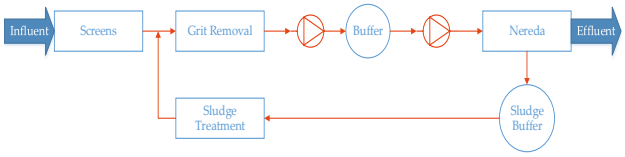


Fig. 1 Photographic and schematic overviews of the full-scale aerobic granule sludge installations in Utrecht (top) and Garmerwolde (below), the Netherlands (modified from STOWA (2015)).

required water quality analyses. The influent and effluent samples were taken by an automated sampler that acquired a discharge-dependent sample volume. In such, the average concentrations of that day were obtained. To obtain the excess sludge loading rate, excess sludge samples were analysed from December 2015 until June 2016. The samples were taken at least every two weeks by taking periodic samples (1L every minute) of the sludge decant flow. During this research, the representativeness of the excess sludge sampling method needed to be improved as the sludge content decreased exponentially during the excess sludge decant flow. The same periodic samples (1L every minute) were taken during the whole excess sludge decant flow, thus the large changes at the beginning in sludge content were not well represented. From February 2015 onwards, the sampling method was improved in Utrecht by (1) increasing the sample frequency to twice a week and (2) by continuously tapping a fraction of the sludge decant flow. The measured excess sludge TP concentration was assumed to be representative for that day.

Besides these offline measurements, online measurements of phosphate, ammonium, nitrate and dissolved oxygen were made by sensors 0.5 m submerged at the top of the reactor. Since the sludge was settled during the feeding phase, the sensors measured the effluent concentrations during the first hour. The measurements were done semi-continuous (5 - 10 min interval) during the cycle by an automatic sampling, filtration and analysis device (Hach Lange; PHOSPHAX type LXV421.99, AMTAX type LXV294, Germany). The pH sensor was hanging at the sludge bed level to record the pH changes during the feeding phase.

The daily influent discharge of the full-scale installations was provided by the water authorities of the full-scale installations (Stichtse Rijnlanden en Noorderzijlvest). When the daily influent exceeded the designed dry weather influent (50,000 m^3 d^{-1} and 26-33,000 m^3 d^{-1} for Utrecht and Garmerwolde), the day was considered as rain weather conditions. Excess sludge discharges were calculated by multiplying the reactor surface (A) by the water level difference during sludge decant summed over one day (see Eq. 2). The effluent discharge was calculated by subtracting the excess sludge discharge from the influent discharge. The TP loading rates were calculated by multiplying the (calculated) discharge with the measured TP concentrations (see Eq. 2)

$$0 = \mathrm{IN} - \mathrm{OUT}_{\text{excess sludge}} - \mathrm{OUT}_{\text{effluent}} + \mathrm{Acc.} \quad (\mathrm{kg\ d^{-1}}) \tag{1}$$

$$0 = (Q_{in} * TP_{in}) - (\sum_{0}^{1d} \Delta h * A * TP_{exc}) - ((Q_{in} - Q_{exc}) * TP_{eff}) + Acc. \quad (\mathrm{kg\ d^{-1}}) \tag{2}$$

where, Q_{in} and Q_{exc}, are the influent and excess sludge discharge (m^3 d^{-1}), TP_{in}, TP_{eff}, TP_{exc} are the influent, effluent and excess sludge total phosphate concentration (kg m^{-1}), Δh is the level difference during sludge decant (m d^{-1}), A is the area of the reactor (m^2). The average sludge retention time (SRT_{av}) was calculated over 10 consecutive days in order to illustrate the global trend of this parameter. The SRT was calculated according to Eq. 3 and the phosphate retention time (PRT) according to Eq. 4. The PRT is similar to the SRT, but based on TP instead of TSS measurements.

$$\mathrm{SRT} = \frac{V_r * X_r}{Q_{ex} * X_{ex} + (Q_{in} - Q_{exc}) * X_{eff}} \ (\mathrm{d}) \tag{3}$$

$$\mathrm{PRT} = \frac{V_r * TP_r}{Q_{exc} * TP_{exc} + (Q_{in} - Q_{exc}) * TP_{eff}} \ (\mathrm{d}) \tag{4}$$

where, V_r is the reactor volume (m^3), X_r, X_{ex}, X_{eff} the reactor, excess sludge and effluent total suspended solids (TSS) concentration (g m^{-3}), Q_{in}, Q_{exc}, Q_{eff} the influent, excess sludge and effluent discharge (m^3 d^{-1}) and TP_r, TP_{ex}, TP_{in}, TP_{eff} the total phosphate concentration of the reactor, excess sludge, influent and effluent (g m^{-3}). When either the reactor TP_r or excess sludge TP_{ex} content was missing, the average was used since both were needed to calculate the PRT.

Total phosphate balance per cycle

Since the daily average phosphate concentration can be substantially different than the real experienced influent phosphate concentration ($TP_{in,exp}$) in each cycle, a method was created to estimate the $TP_{in,exp}$ from the maximum ammonium concentration measured online (see Eq. 5). It was expected that the influent phosphate and ammonium concentration were closely related. The influent concentrations of ammonium and phosphate concentration were obtained from the databases of the water authorities (Stichtse Rijnlanden en Noorderzijlvest). The exchange ratio (EX) was calculated by dividing the influent volume by the reactor volume, to account for dilution after mixing. As indicated by Bassin et al. (2011), ammonium adsorption (AD) occurs in AGS systems during feeding The data of the online ammonium sensor in the full-scale AGS installations was used to investigate the ammonium adsorption in full-scale AGS systems. The influent ammonium concentration was compared to the experienced ammonium concentration in the reactor after mixing. Since ammonium was not converted during feeding, the difference between the influent and experienced ammonium concentration gave an indication for the AD. The exchange ratio and residual effluent ammonium concentration were taken into account when calculating the experienced ammonium concentration

after mixing. To verify the equations, manual samples were taken from the raw wastewater and compared with the obtained calculated results. The manual samples were analysed with an ion chromatograph as described in Stubbé et al. (2016).

$$TP_{in,exp} = \frac{NH_{4,max}}{1 - AD} * \frac{R_{N:P}}{EX} - \frac{(1 - EX) * TP_{eff}}{EX} (mg\ L^{-1}) \quad (5)$$

where, EX is the exchange ratio (-), V_{in} and V_r the cycle influent and reactor volume (m^3), $TP_{in,exp}$ and TP_{eff} the experienced influent and effluent phosphate concentration, $NH_{4,max}$ the maximum online measured ammonium concentration (g m^{-3}), AD the ammonium adsorption ratio (-) and $R_{N:P}$ the influent ammonium : phosphorus ratio.

In Utrecht, excess sludge samples were taken by tapping a fraction of the sludge decant flow instead of the more conventional periodic samples (see total phosphate balance per day). These excess sludge samples were taken by tapping the sludge decant flow of multiple, consecutive cycles overnight. In this case, the excess sludge phosphate concentration was averaged over multiple cycles within ± 12 h. To obtain the total phosphate balance per cycle, these excess sludge results were inserted at the first cycle of the second day.

Analytical characterization

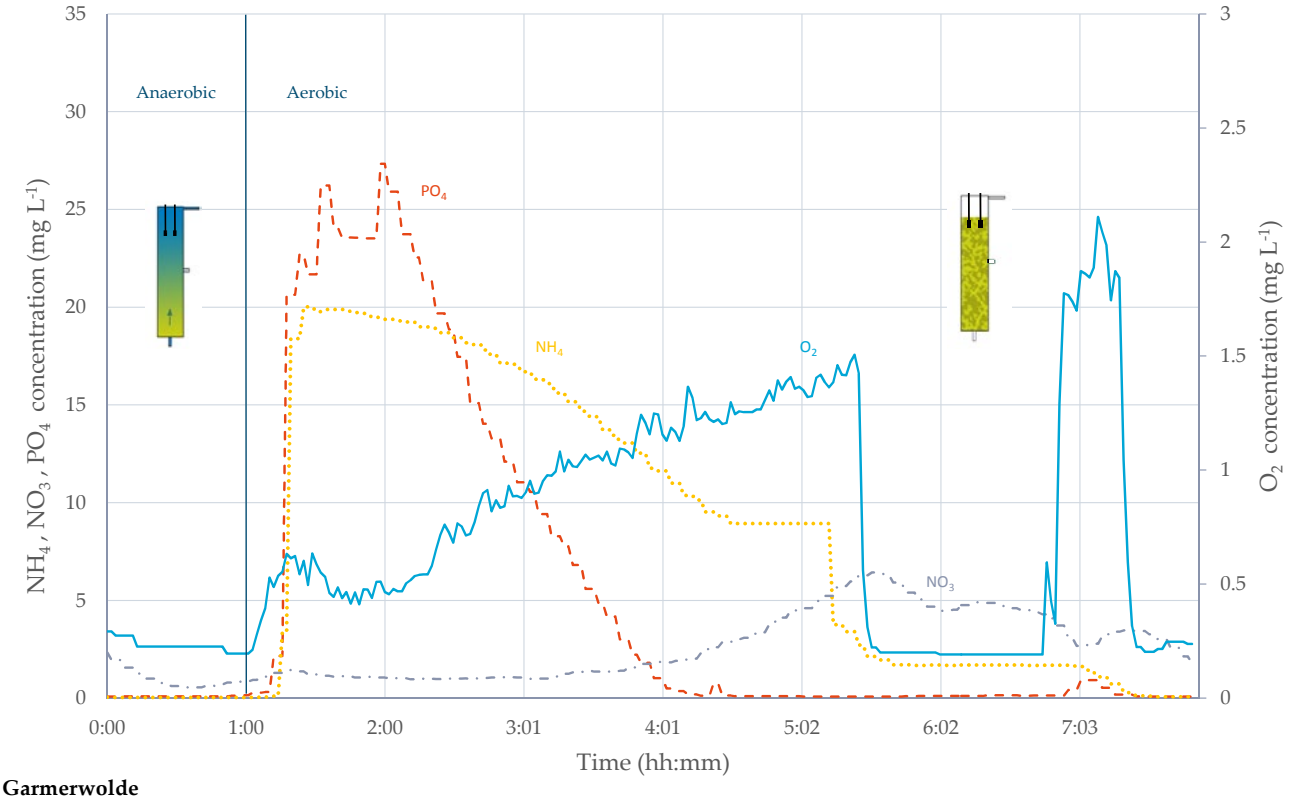
Microscopic pictures were made with the Image Analyser Leica 80 in combination with the LAS Image Analysis software. X-ray diffraction (XRD) analyses were performed on reactor granules (> 0.2 mm) sampled in the aerobic phase and air-dried for > 48 h. Removing the organic fractions by calcification (2hours at 550°C) was not an option, as studies have identified decomposition and amorfication of minerals at elevated temperatures (Bhuiyan et al. 2008, Čermáková et al. 2015, Ramlogan et al. 2016). The XRD measurements were done with Bruker D8 Advance diffractometer Bragg-Brentano geometry, lynxeye position sensitive detector and divergence slit V12 with data evaluation by Bruker software Diffrac. EVA vs 4.1. A cobalt tube scattered from 5° to 90° while sample was spinning, using step size 0.020° and counting time 1s per step. The same granular sample was used to perform X-ray fluorescence (XRF) analysis. The XRF measurement was performed with a Panalytical Axios Max WD-XRF spectrometer and data evaluation done with SuperQ5.0i/Omnian software. With X-ray Fluorensce (XRF), the metal : phosphorus (mol $molP^{-1}$) ratios can be calculated by dividing the weight percentages by the molecular weight. This ratio indicated the maximal amount of phosphate that could be bound to the respective cation. The granules analysed with scanning electron microscopy (SEM) were sampled during the aerobic phase and freeze dried for >24 h at 0 bar and -80 °C with a Labconco Freezone 4.5 (Beun de Ronde, Abcoude, the Netherlands) and cut in half. The SEM analyses were performed with Jeol JSM-6480LV in combination with an energy dispersive X-ray spectroscopy probe (EDX) using 20 keV.

Analytical methods

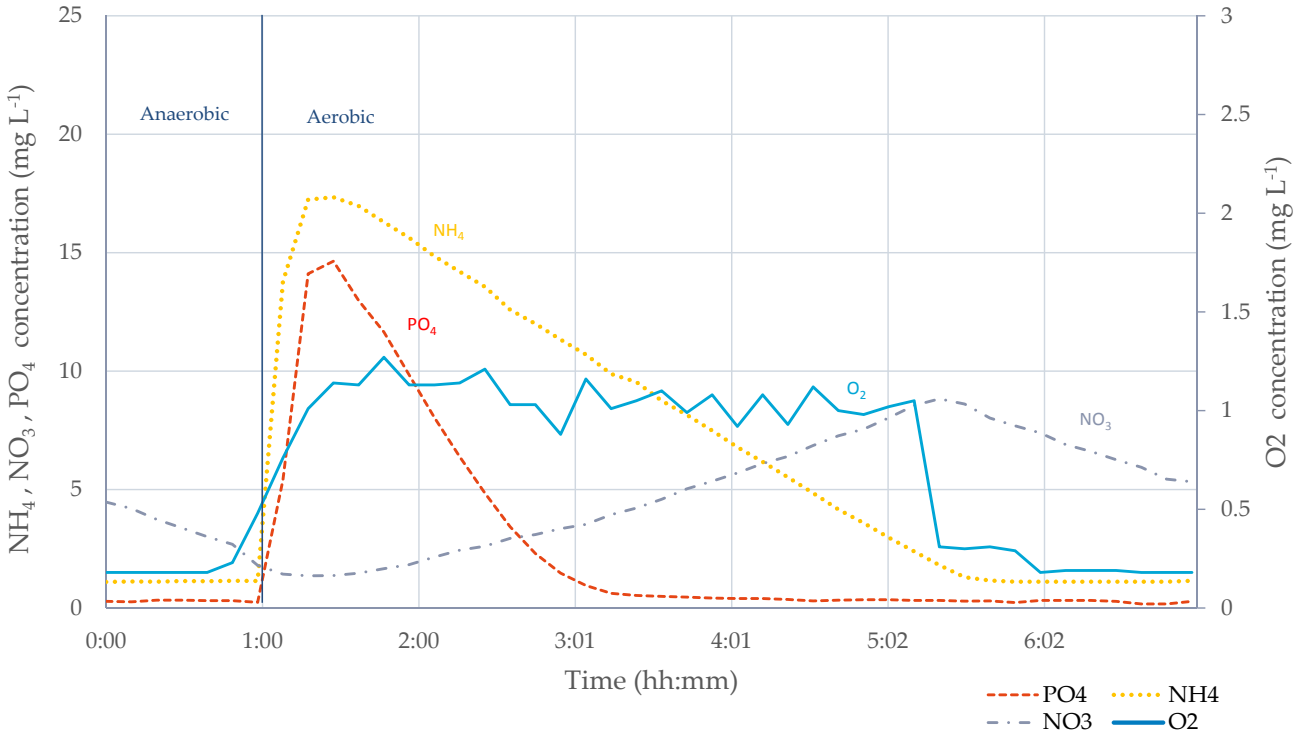
For the granular sludge, a crusher was used for the analytical methods to ensure homogeneous samples (Janke & Kunkel type Ultra-Turrax T25, manufacturer IKA-labortechnik, Germany). Chemical analyses (e.g., TP, PO_4-P and iron) were performed spectrophotometrically by using standard test kits (Dr. Lange type LCK350 and 302; manufacturer: Hach Lange, Dusseldorf, Germany). The measurements of TP were treated with an oxidation reagent describe in Valderrama (1981) prior to chemical analysis. The total and volatile suspended solids (TSS and VSS) analyses were done according to standard method (APHA 1915) with the use of coffee filters instead of standard filters, as much larger volume samples needed to be analysed in order to obtain representative samples (≥ 1 L). Triplicate samples were performed to investigate the standard deviation.

Five granule fractions (> 2 mm, > 1 mm, > 0.6 mm, > 0.355 and > 0.2 mm) were created by sieving the samples within 24 h after sampling from the aerobic phase. On the sieves, the granules were washed gently and excess water was removed with paper tissues from underneath. To measure the TP content of each fraction (gTP $gTSS^{-1}$), wet granules of the mentioned fractions were crushed and suspended in tap water. The measured TP concentration was divided by the resultant wet granule concentration and multiplied by the wet weight, dry $weight^{-1}$ (WW DW^{-1}) ratio. The WW DW^{-1} ratios were obtained by drying separately wet granules according to standard methods (APHA 1915).

Utrecht



Garmerwolde



3. Results

Full-scale aerobic granular sludge installations

The waste water characteristics of the full-scale AGS installations in Utrecht and Garmerwolde are shown in Tables 1 - 2. In Utrecht, there were no effluent requirements dictated as the effluent was not yet directly discharged to surface water (see Table 1). The effluent requirements in Garmerwolde are on average fulfilled (see Table 2). The concentration profiles of a typical cycle are illustrated for each installation in Fig. 2. During feeding no concentration changes were measured, because the sensors were located at the top of the reactor and feeding happened from the bottom of the reactor. The concentration peaks after feeding were caused by aeration which mixed the bulk of the reactor. The phosphate maximum concentration is a mixture of release poly-phosphate and influent phosphate. The phosphate concentration profile during each cycle turned out to give valuable information about the performance of the biological phosphate removal (Bio-P). When the decrease of phosphate was a straight linear line towards effluent quality, the bio-P was functioning well. However, when the phosphate concentration profile had a decreasing slope towards effluent quality, it became evident during this research that the biopolymer content of the PAOs was not balanced. This is further elaborated in the *Discussion*.

Total phosphate content of granule fractions

The measured ash contents of the granule fractions were between 10 and 14 ± 0.74 % in Utrecht and between 17 and 22 % in Garmerwolde (Fig. 3). No clear pattern in the ash contents was found between the granular fractions, as also demonstrated by Robertson (2014). The absolute ash content was lower in Utrecht compared to Garmerwolde, which can be explained by iron dosing in Garmerwolde (until May 2015 frequently, with occasional dosing until December 2015). As the measurement was done in January, long after frequent dosing, it is put forward that iron remained longer than expected in the AGS reactor. For activated sludge systems, ash contents between ± 25 - 55 % were reported (Tchobanoglous et al. 1991).

The TP contents of the granule fractions were between 0.7 and 1.9 ± 0.56 % in Utrecht and between 1.2 and 2.2 % (gTP $gTSS^{-1}$) in Garmerwolde with a slightly upward trend for bigger granule fractions. This trend can be explained by either more phosphate precipitates or more poly-P inside the larger granules. The absolute values for ash and TP contents however, were rather low to indicate substantial phosphate precipitates. An ash and TP content of > 30 % and ± 5.5 % are believed to be necessary to contain phosphate crystals, as these values appeared in the studies with phosphate precipitates inside aerobic granules from lab reactors (Mañas et al. 2011, Lin et al. 2012). It should be taken into consideration that the high standard deviation

Table 1 Average waste water characteristics in Utrecht from August 2015 to April 2016 (Royal HaskoningDHV 2016)

Parameter	Influent mg L^{-1}	Effluent mg L^{-1}	Effluent Requirement mg L^{-1}
COD	706	45.3	-*
BOD	-	10.06*	-
SS	-	10.6	-
Nkj	41.67	6.8	-
TP	9.2	0.7	-
PO_4-P	5.14	0.3	-
TN	-	6.4	-
NO_3-N	-	4.08	-

*No effluent requirements were active as the effluent was not directly discharged to surface water

Table 2 Average waste water characteristics in Garmerwolde from April 2014 to March 2015 (STOWA 2015)

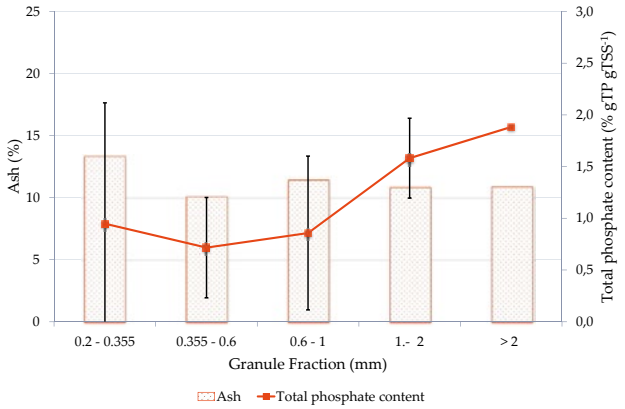
Parameter	Influent mg L^{-1}	Effluent mg L^{-1}	Effluent Requirement mg L^{-1}
COD	495	60	125
BOD	218	8.9	20
SS	228	17.9	30
Nkj	33.15*	2.74*	
TP	6.6	0.83	1
PO_4	-	0.40*	
TN	49.4	7.8**	7
NO_3-N	0.27*		

*Database water authority Garmerwolde from December 2015 to March 2016
** TN effluent was higher than the effluent requirement as the installation is operated with a higher than design load since November 2014

of the TP content made the overall trend debateable. The TSS measurement of the smallest fraction (0.2 – 0.355 mm) was sensitive for measurement errors. For the other granule fractions, both the TP and TSS concentrations varied in time, depending on influent concentrations and operation conditions.

The granule fraction distribution of the AGS reactors in Utrecht and Garmerwolde is depicted in Fig. 4. The fractions < 0.2 mm, 1 – 2 mm and > 2 mm had in both installations the biggest contribution to the total reactor sludge. Illustrations of the sieved and washed granule fractions are shown in Fig. 5. The excess sludge fractions displayed a different distribution; the largest contributions were the smallest fractions < 0.2 mm and 0.2 – 0.4 mm. The sludge in the reactor (or 'reactor sludge') and the excess sludge samples were analysed on TP content (% gTP TSS^{-1}) in order to investigate whether preferred phosphate selection took place. Preferred phosphate selection occurred when the excess sludge TP content was substantially lower than the reactor sludge. This would lead to TP accumulation in the reactor, either biologically or chemically-bound. The results in Fig. 6 show that, although the average values of the excess sludge differ from the reactor, the standard deviations overlapped. Therefore, preferred phosphate selection cannot be confirmed with these experimental data. The number of measurements was 7 and 16 for the reactor samples, and 46 and 38 excess sludge samples, in Utrecht and Garmerwolde respectively. Since the excess sludge pictures (see *Retention time based on solids and phosphate*) show considerable amounts of cellulose, it cannot be excluded that cellulose caused a discrepancy in the TP content between the reactor and the excess sludge. Cellulose fibres are commonly found in wastewater treatment plants and they are hard to degrade, especially at lower temperatures (Verachtert et al. 1982, Ruiken et al. 2013). A higher cellulose concentration in the excess sludge would increase the TSS concentration, therefore increasing the non-phosphate related TSS compounds (e.g. leaves, sand) which lead to a lower TP content. In Utrecht, it was found that the reactor TP content was, on average, lower than the excess sludge TP content. Also, the absolute reactor TP content in Utrecht was lower than in Garmerwolde. Both these results can be caused by the relatively young granules in Utrecht, as this reactor is operated only since 2015 and the granules have had less time to accumulate poly-P. Besides, large extractions of reactor sludge were taken from the reactor in Utrecht during the research period, which lowered the TP content as well. More about the extractions can be found in *TP balance per day*.

Utrecht



Garmerwolde

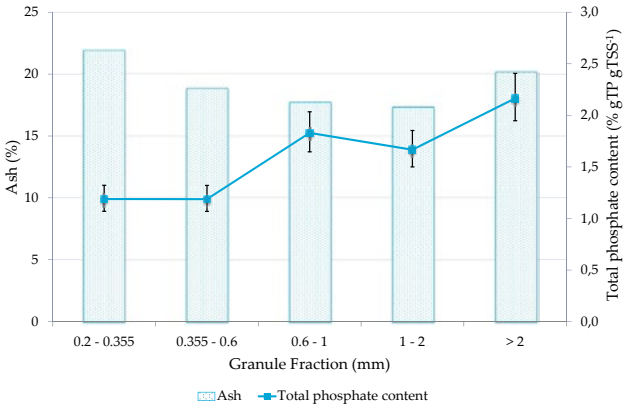


Fig. 3 Total phosphate and ash content of different granule fractions in Utrecht (top) and Garmerwolde (below) with standard deviations in black. The standard deviation of the ash content was on average ± 0.74.

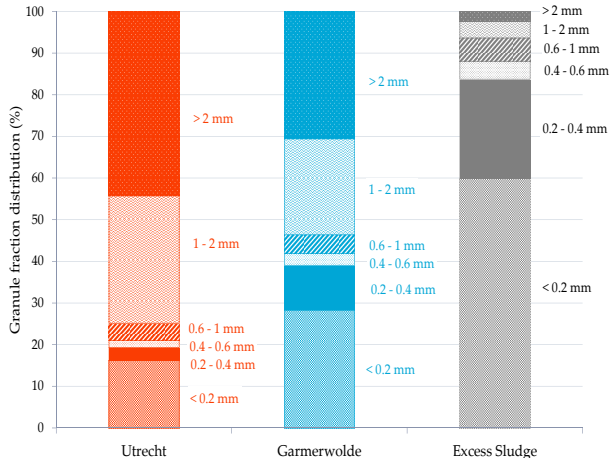


Fig. 4 Granule fraction distribution of full-scale AGS reactors in Utrecht and Garmerwolde averaged over > 7 months (Royal Haskoning DHV 2016). Excess sludge measurements were from Garmerwolde.

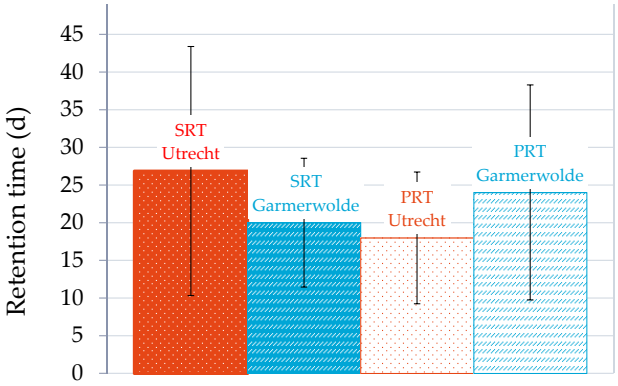


Fig. 6 TP content of reactor sludge and excess sludge in Utrecht (N = 7 for reactor and N = 46 for excess sludge) and Garmerwolde (N = 16 for reactor, N = 38 for excess sludge)

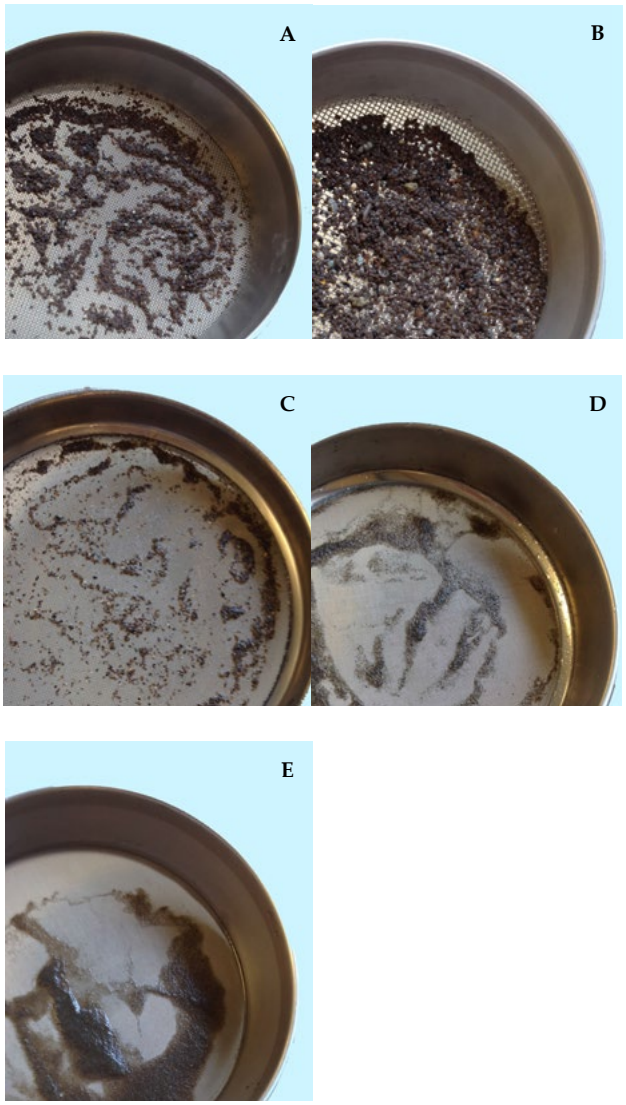


Fig. 5 Sieved and washed granule fractions from Utrecht with A: > 2 mm, B: 1 – 2 mm, C: 0.6 – 1 mm, D: 0.4 – 0.6 mm and E: 0.2 – 0.4 mm.

Dynamic XRD results

X-ray diffractograms (XRDs) were performed on aerobic granules in order to detect whether and which type of phosphate crystals were present inside the granules from full-scale AGS systems. In January, the XRD peaks in the reactor sludge from Utrecht coincided with the reference spectra of ammonium phosphate (NH_4PO_3), dolomite ($CaMg(CO_3)_2$) and magnesium calcite ($Mg_{0.06}Ca_{0.94}CO_3$) (see Fig. 7). In December, some indications of STR-K ($MgKPO_4.6H_2O$) peaks were found in the XRD of reactor sludge from Garmerwolde, although it should be taken into account that the spectra of STR-K, STR-NH_4 ($MgNH_4PO_4.6H_2O$) and STR-Na ($MgNaPO_4.6H_2O$) are difficult to distinguish. In both XRDs, the highest peaks coincide with the reference spectra of quartz (SiO_4), demonstrating the presence of sand grains in the granules. These sand grains can easily originate from the influent. The mountain-shaped line between 15 - 50° and 15 - 30° for Utrecht and Garmerwolde indicated a substantial amount of amorphous or organic phases in the samples. The smaller crystal peaks were disturbed by the noise of the amorphous or organic fraction. Some peaks therefore, remained unidentified. The XRD technique is limited in detecting micro minerals in a multi-phase system (Li et al. 2014).

Repeated XRD measurements over time presented different results. In March, peaks similar to brushite ($CaHPO_4.2H_2O$) were found in reactor sludge from Utrecht (Stubbé et al. 2016). In January, XRD measurements of reactor sludge from Garmerwolde did not show anything besides quartz. These results pointed out that the precipitation process in full-scale AGS systems could be dynamic; precipitates are formed and dissolved within cycles, or in consecutive cycles, possibly influenced by operation conditions (e.g., pH, aeration regime, cation concentration). The samples from Garmerwolde in January could be taken after a time of dissolution, whereas the sample in March could have been taken after a period of different influent concentrations, inducing a different precipitate.

Multiple XRDs of the excess sludge from a conventional activated sludge waste water treatment plant (WWTP) in Leeuwarden, the Netherlands showed indications of struvite and vivianite in all measurements (Wilfert et al. submitted). This WWTP consisted of biological phosphate removal in aerobic and anoxic carrousels. As sludge treatment, this WWTP had a digestion on-site. This comparison could indicate that the precipitation processes in AGS systems is more divers than in activated sludge systems, as a variety of minerals were found in aerobic granules in multiple measurements.

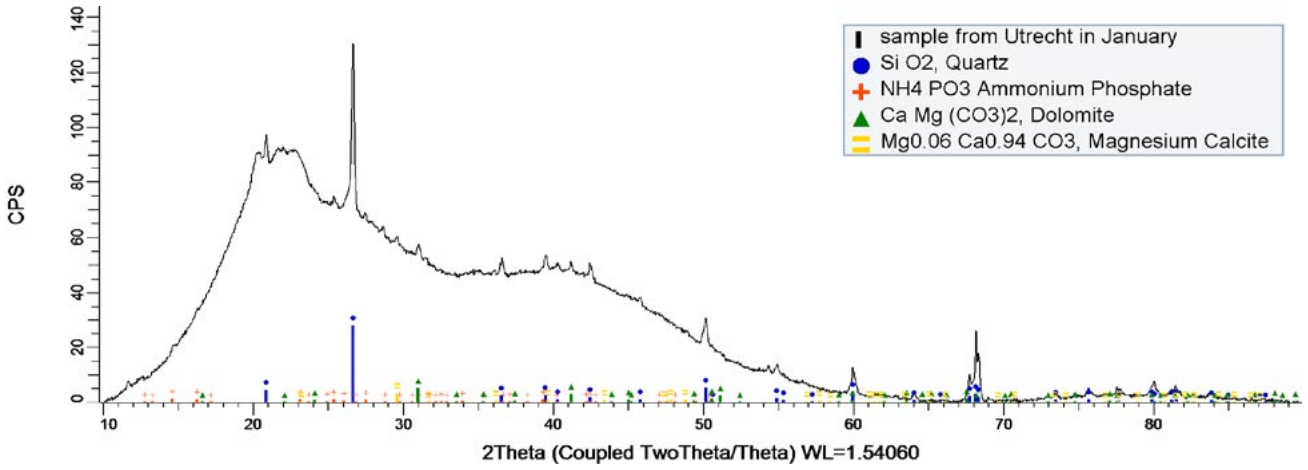
Metal : Phosphate ratios

The ratio of metal to phosphate indicates the maximal amount of phosphate bound to that specific metal ion. This ratio only gives the upper limit of these bindings (Me-P), as the metal ions can simple be bound to other anions (e.g., CO_3^{2-}, OH^-) or be in solution. When this ratio is higher than or close to the stoichiometric ratio of the phosphate precipitate including that metal ion, it could indicate significant presence of that respective precipitate.

The metal to phosphate ratios of reactor granules from Utrecht were as follows: 0.09 Fe:P, 0.74 Ca:P, 0.22 Mg:P, 0.22 Al:P and 0.28 K:P (mol $molP^{-1}$) (see table 3). The metal to phosphate ratios of reactor granules from Garmerwolde, were: 0.72 Fe:P, 0.60 Ca:P, 0.20 Mg:P, 0.11 Al:P and 0.27 K:P (see table 3). These results suggested that none of the precipitates reach substantially the stoichiometric ratios of the phosphate precipitate (see table 3). The stoichiometric ratio of possible phosphate precipitates with the mentioned metals are 1 - 2.5 Fe:P, 1 - 1.67 Ca:P, 0.5 - 1.5 Mg:P, 1 K:P and 1 Al:P for iron, calcium, magnesium, potassium and aluminium (van Kemenade et al. 1987, Luedecke et al. 1989, Minteq database 2006, Madsen et al. 2014). Besides, the stoichiometric ratio of Poly-P ($Mg_{0.36}K_{0.28}H_2PO_4$) should be taken into account, which would lower the phosphate ions available for precipitates even more (Barat et al. 2005).

A higher Fe:P ratio was found in Garmerwolde compared to Utrecht, as a logical consequence of the iron dosing in Garmerwolde. Although this was only done frequently until May 2015, a sporadically dose was applied in December 2015 to maintain the required effluent phosphate concentration. After iron, the Ca:P ratio was the highest in both installations, followed by magnesium, potassium and aluminium. As the solubility product (pK) of iron phosphate bonds are highest, followed by calcium, magnesium, potassium and aluminium, the available phosphate is most likely to bind with the metals in this order. The likelihood of phosphate to bind with a certain metal ion can however, be influenced by factors other than the pK, such as solution speciation, temperature, pH, etc (Song et al. 2002). Therefore these results are not a proof, but an indication.

Utrecht



Garmerwolde

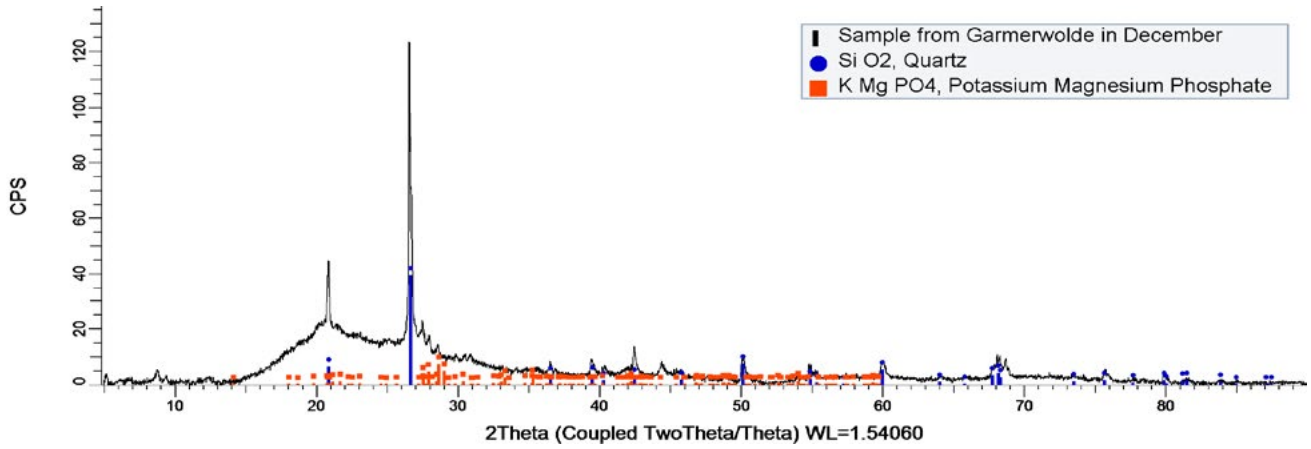


Fig. 7 X-ray diffractograms of granule samples (> 0.2 mm) from Utrecht (above) and Garmerwolde (below) with the matching reference spectra of crystal peaks in color. On the x-axis, the 2Theta indicates the angle of the detector with respect to the incident beam and on the y-axis, the counts of pulses are plotted.

Table 3 Molar ratios of cation : phosphate in reactor sludge from Utrecht and Garmerwolde calculated from the weight percentages of EDX-SEM and XRF measurements

Compound	Utrecht mol mol-P^{-1}	Garmerwolde mol mol-P^{-1}	Max.ratio mol mol-P^{-1}	Mineral* unhydrated	Reference	pK -	Reference
Fe	0.09	0.72	2.5	$Fe_{2.5}PO_4(OH)_{4.5}$	Luedecke et al. (1989)	96.7	Luedecke et al.
Ca	0.74	0.60	1.67	$Ca_5(PO_4)_3OH$	van Kemenade et al.	48.6	Murray et al. (1996)
Mg	0.22	0.20	1.5	$Mg_3(PO4)_2$	Minteq database (2006)	23.28	Minteq database
K	0.28	0.27	1	$MgKPO_4$	Taylor et al. (1963)	11.68	Luff et al. (1980)

*Only most thermodynamically stable mineral displayed, other minerals exist with different ratios

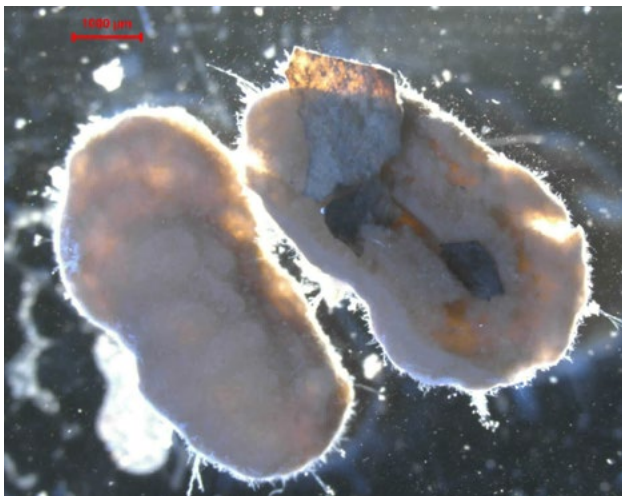
Inside the granule

Scanning electron microscope (SEM) images coupled with energy dispersive X-rays (EDX) facilitated a close-up look inside of aerobic granules in search for inorganic material. The EDX analysed the electromagnetic emission spectrum of the granule for the unique set of peaks corresponding to a specific element. Together these techniques enabled to study the inside of the granules for precipitates.

The SEM-EDX images of cut granules showed no inorganic core or clear phosphate crystals, supporting the results of the XRD and XRF analyses (see Fig. 8). The results suggested that no phosphate crystals were prominently present inside the granules The element maps showed presence of carbon over the complete granule from both installations with some shadows created by the cutting. The EDX spectra of both installations showed that carbon and oxygen are the most present elements in the samples (see Fig. 9). These results indicate that organic material covered the whole inside of the granule. The PO_4^{3-}, Mg^{2+}, K^+, Ca^{2+} and $Fe^{2+/3+}$ maps showed presence over the whole granule, with some locations showing an increase in intensity. The EDX spectra however, showed that these elements are only faintly present (see Fig. 9). Slightly higher PO_4^{3-} concentrations were measured at the outer layer on the left side of a granule from Utrecht (see A1 in Fig. 8). It is unclear why only the left side showed increased phosphate concentrations. The PO_4^{3-} location coincided with higher concentrations of Mg^{2+} and K^+ in the same granule from Utrecht, which is the characteristic composition of poly-P (Barat et al. 2005, Günther 2011). This location was indicated as the preferred location of PAOs by Lemaire et al. (2008), who studied a mixed culture aerobic SBR in the lab. Although De Kreuk et al. (2005) and Kagawa et al. (2015) showed microbial images and modelled the location of PAOs also in the core of the granule, their granules were smaller (± 0.6 mm) than the ones measured here (> 2 mm). In Garmerwolde, the location of more intense phosphate concentration coincided with higher calcium and iron concentrations, which could point to (amorphous) precipitates (e.g., ACP, ferrihydrite). The difference between Utrecht and Garmerwolde can be explained by the iron dosing in Garmerwolde until May 2015.

The microscopic pictures also did not show inorganic cores (see Fig. 10). The inside of wet granules was often filled with small natural material (e.g., little leaves, sand grains, twigs, seeds), especially in the non-spherical granules. This material could have functioned as seeding material for the biomass to grow on. The light brown colour showed the biomass, which is clear of black spots in these pictures.

Utrecht



Garmerwolde

Fig. 10 Microscopic pictures of sieved granules (> 2 mm) from Utrecht (top) and Garmerwolde (below). Organic material (e.g., little leaves, sand grains, twigs, seeds) was found inside that could have functioned as seeding material. Bars indicate 1000 µm.

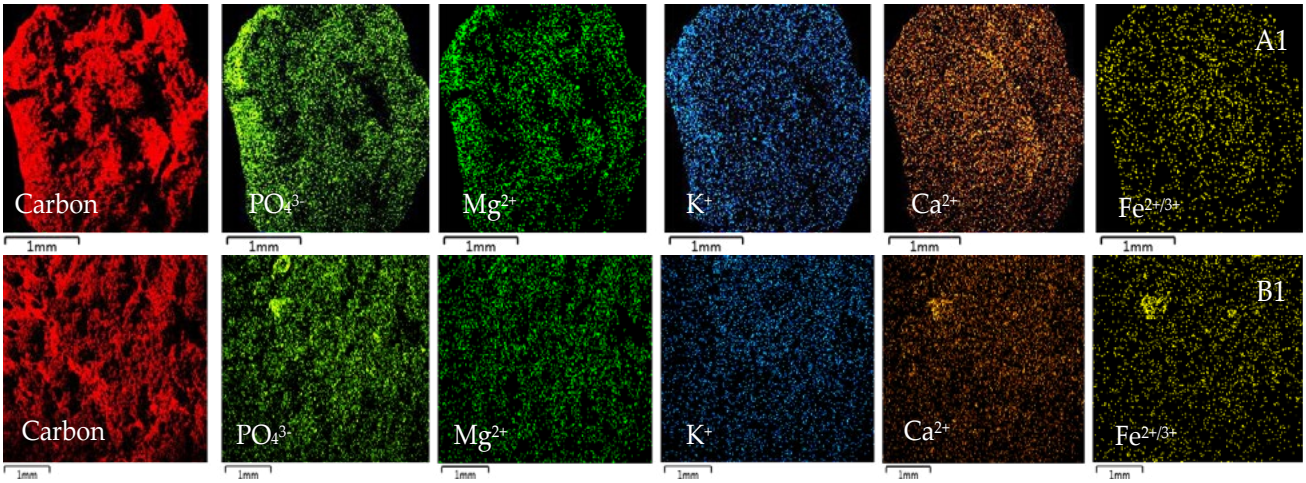


Fig. 8 EDX illustrations of SEM pictures taken of granules from Utrecht (A1) and Garmerwolde (B1). From left to right, the images show carbon (red), phosphate (green), magnesium (dark green), potassium (blue), calcium (orange) and iron (yellow).

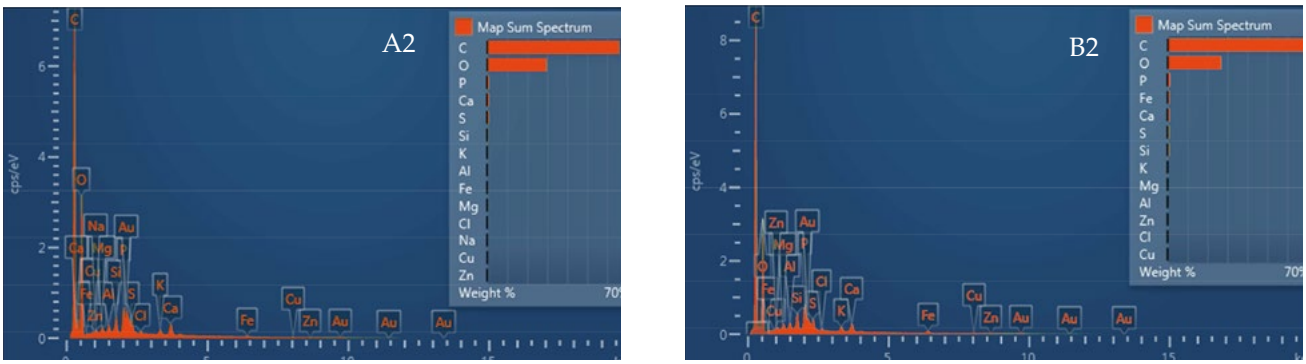


Fig. 9 The EDX spectra from Utrecht (A2) and Garmerwolde (B2) indicates the electromagnetic emission spectra and weight percentages of each element of the SEM samples of Fig. 8.

The total phosphate balance per day

Total phosphate (TP) balances per day were made for both full-scale AGS systems to investigate whether phosphate was systematically accumulated or lost out of the reactor. The total phosphate load in, the total phosphate load out (effluent plus excess sludge) and the effluent separately were illustrated in Fig. 11. The trends indicated in the figure with dotted, dashed or straight lines described a moving average of 10 days. When none or only one measurement was done in 10 days, the average line can behave dynamic.

Both full-scale AGS installations showed rather constant effluent loading rates over time. The effluent TP loading rates were, on average, 0.67 ± 0.99 and 24 ± 26 kg d^{-1} in Utrecht and Garmerwolde. The TP loading rates (total TP in and out) were strongly fluctuating over time. On average, the total TP loading rate in and out were 10.2 ± 2.6, and 12.7 ± 6.2 kg d^{-1} for the installation in Utrecht. Overall, the TP-balance in Utrecht showed a phosphate loss of 2.5 kg d^{-1}. When the standard deviation of the loading rates was taken into account, however, the TP-balance of Utrecht can be considered closed. In Garmerwolde, the TP loading rates (total TP in and out) were 210 ± 84 and 180 ± 96 kg d^{-1}. Overall, the TP balance in Garmerwolde showed a phosphate accumulation of 30 kgTP d^{-1}. When the high standard deviation was taken into account however, the TP balance of Garmerwolde could also be considered closed.

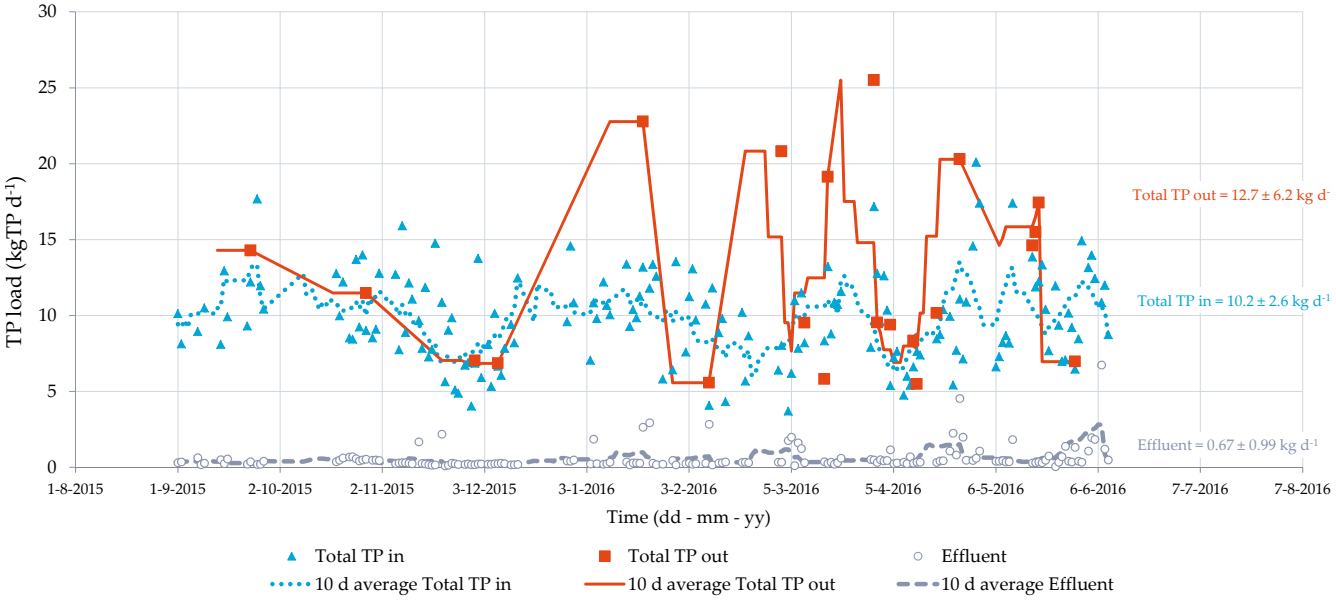
Overall, these results suggested that the fate of phosphate was dynamic in a full-scale AGS system, especially by the disordered influent and excess sludge loading rates. The sample frequency in Garmerwolde was increased between May and June 2016 the get more insights into these dynamics. It became evident that rain weather conditions had a large impact on the excess sludge loading rates. The TP balance during dry and rain weather conditions in Utrecht and Garmerwolde are shown in Addendum 1. The high excess sludge loading rates were only seen during rain weather conditions. The excess sludge samples contained significantly more sludge during the (first-flush of) rain events. Also the sludge content became constant during the sludge decant instead of exponentially decreasing (see Addendum 2). These results indicated that a phosphate loss occurred during the first-flush of a rain event. The mechanisms behind these observations leave room for further research.

Rain weather did not seem to increase or decrease the disorder of excess sludge loading rates in Utrecht. This trend could have been absent in Utrecht because the influent batches (m^3) stayed the same during rain weather conditions, thus keeping the exchange ratio the same. In Garmerwolde, however, the exchange ratio increased from 0.4 to 0.6 during rain weather events. Moreover, in Utrecht various operational changes were implemented during the research period, like adding clean water to the influent stream to increase the upflow velocity. This measure artificially lowered the influent TP loading rate. Also, excess sludge samples were taken by tapping a fraction of the excess sludge flow for ± 12 h to increase the representativeness of an excess sludge sample. This could have however, averaged the phosphate content over to many cycles to see the first-flush effects of a rain event. As rain events are not evenly spread over one day, the daily TP balance was to generic to disclose the dependency on weather conditions.

It should be noted that during the research period, various sludge extractions took place at the full-scale AGS installation in Utrecht and Garmerwolde. Sludge from the reactor was taken out and transported by truck. In total 36,315 and 8,349 kgTSS was extracted from Garmerwolde and Utrecht respectively, over the course of 7 months of the research period. These extractions were not taken into account in the TP balance, as the influent TP loading rates stayed the same. In Garmerwolde, a higher effluent TP loading rate was measured on the days of extractions, but only for 2 or 3 cycles. The extractions caused a loss of granules, which placed more load on the remaining granules to remove the same amount of phosphate. Until the biopolymer reserves of the PAOs were built up, the effluent phosphate loading rate can increase. The remaining granules also had more substrate available, which could have increased the biomass growth in the period right after the extraction. Some of this biomass growth can be suspended in flocs or small granules which end up in the excess sludge due to their slower settling properties. The excess sludge loading rate could therefore, also have increased. However, not enough days of extractions and excess sludge measurements were available to analyse this hypothesis.

In addition, it was investigated what caused the high standard deviation of the phosphate loading rates. The standard deviations of the discharge, total phosphate measurements and dry weight measurements in the influent, excess sludge and effluent are shown in Addendum 3. The standard deviation of the TP measurements (30 – 161 %) was higher than standard deviation of the discharges (7 – 44 %). Especially, the standard deviation of the effluent phosphate concentration was high (161 %), since it concerned very low numbers and fluctuations had a larger impact. This, however, does not impact the TP balance significantly. The standard deviations of the discharges in Garmerwolde (7 – 32 %) were slightly lower than in Utrecht (28 – 44 %). In Garmerwolde, however, the discharges concerned large amounts (35,000 m^3 d^{-1} instead of 1,200 m^3 d^{-1} in Utrecht) and therefore had a large impact on the TP balance. A more elaborate explanation on the errors and methods to mitigate the errors when performing a TP balance, including the protocol, are shown in Addendum 3.

Utrecht



Garmerwolde

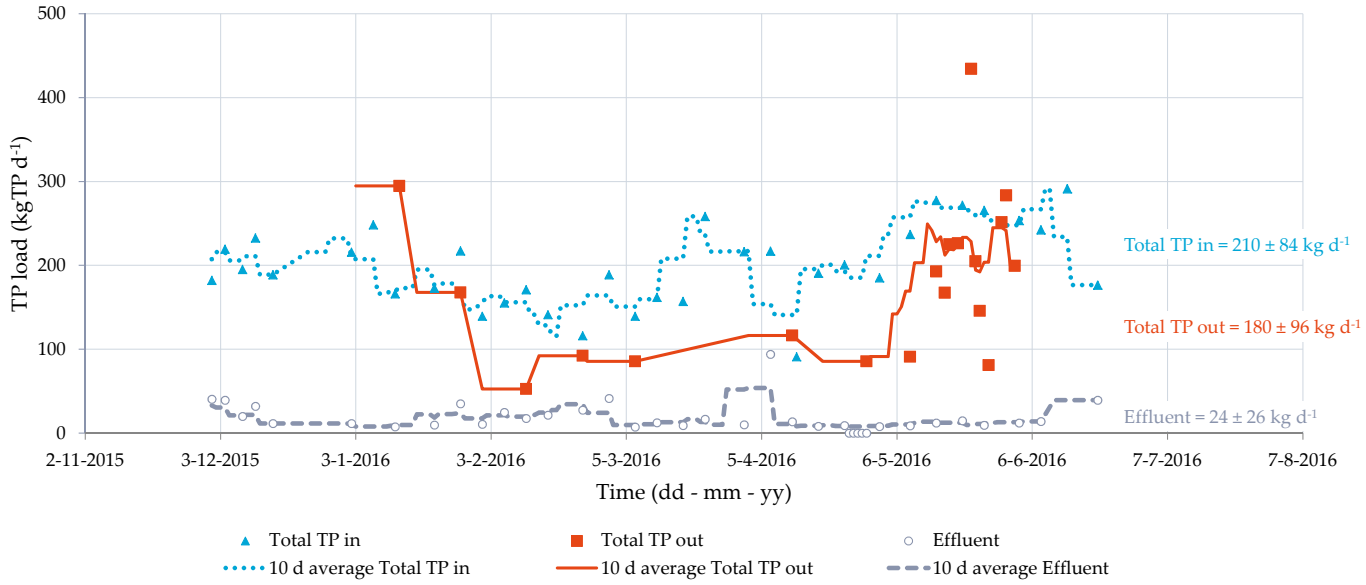


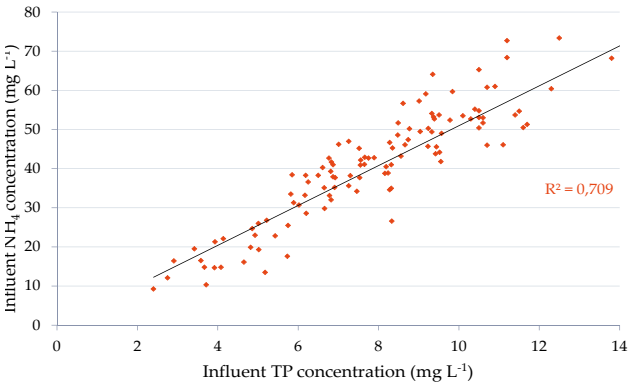
Fig. 11 Total phosphate balances of Utrecht (above) and Garmerwolde (below) with the total phosphate loading rate in (influent) and total phosphate loading rate out (effluent plus excess sludge). The effluent phosphate loading rate was illustrated separately in order to indicate the performance of the full-scale AGS systems with biological phosphate removal.

Total phosphate balance per cycle

The daily TP balance proved to be too generic to obtain specific insights into the dynamical phosphate processes of a full-scale AGS system. The online cycle measurements delivered data of every cycle, which made it possible to obtain a TP balance for every cycle. The influent ratio of ammonium: phosphate (NH_4 : TP) seem to correlate relatively well with a R^2 of 0.709 in Utrecht and 0.713 in Garmerwolde (see Fig. 12). The daily average phosphate influent concentrations measured by the water authority differed, on average, 29 % from the calculated phosphate concentration. Three manual influent phosphate measurements of a specific cycle however, only differed, on average, 8 % from the calculated phosphate concentration. These results indicated that the calculated phosphate concentrations gave a better indication of the real influent phosphate concentration in AGS installations than the daily averaged measurements by the water authorities. These results reaffirm that daily average concentrations are too generic to obtain specific insights in full-scale AGS systems. The comparison between the influent and the expected reactor ammonium concentration after feeding showed a ± 23% difference, on average. Apparently, the influent ammonium concentration was higher than the concentration measured in the reactor after feeding. The expected reactor concentration was corrected for the exchange ratio and residual ammonium concentration from the previous cycle. The exchange ratio was on average 29 ± 8 % and 41 ± 5.5 % in Utrecht and Garmerwolde respectively. The ± 23 % difference gave an indication on the amount of ammonium adsorption in Garmerwolde (see Fig. 13).

The fluctuations in the TP balance within one day became clear when looking at the results of the TP balance per cycle. On one day, the influent TP loading rate can shift between 7.34 and 0.17 kgTP cycle^{-1}. The cycle TP balance in Utrecht showed for total TP in and out 4.1 ± 2.2 and 3.7 ± 1.6 kgTP cycle^{-1} (of which 0.1 ± 0.3 kgTP cycle^{-1} was the effluent TP loading rate) (see Fig. 14). When the standard deviations were taken into account, the TP balance could be considered closed. The cycle TP balance in Garmerwolde, the cycle TP balance showed for total TP in and out 50 ± 24 and 25 ± 19 kgTP cycle^{-1} (of which 5 ± 10 kgTP cycle^{-1} was the effluent TP loading rate) (see Fig. 14). Even when the standard deviations were taken into account, the TP balance was hardly closed. The appearing TP gap in Garmerwolde can be explained by the fact that significantly more dry days were sampled than (the (first flush of) rain events. If the observed relation between rain events and higher total TP out is indeed true, the TP balance per cycle would show more accumulation of TP in the reactor than TP loss. Also, the sludge extractions described in *total phosphate balance per day* could have had a contribution to the observed gap: the decrease in sludge (and phosphate) content of the reactor gave

Utrecht



Garmerwolde

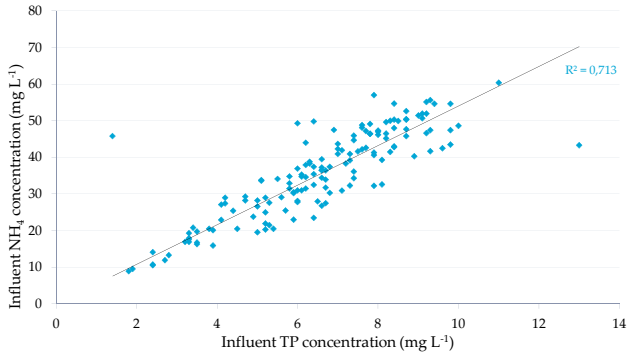


Fig. 12 Relation between influent ammonium and influent total phosphate concentrations of Utrecht (above, N = 113) and Garmerwolde (below, N = 148).

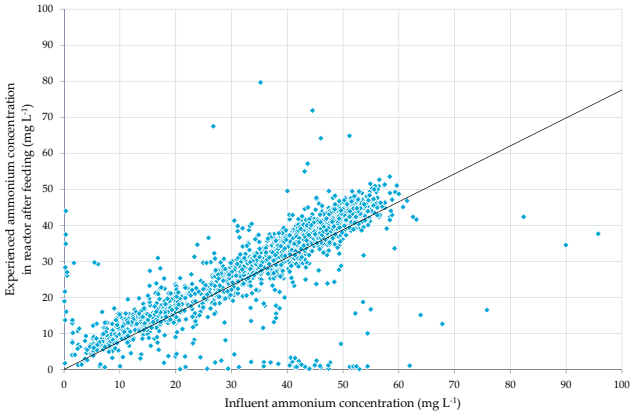
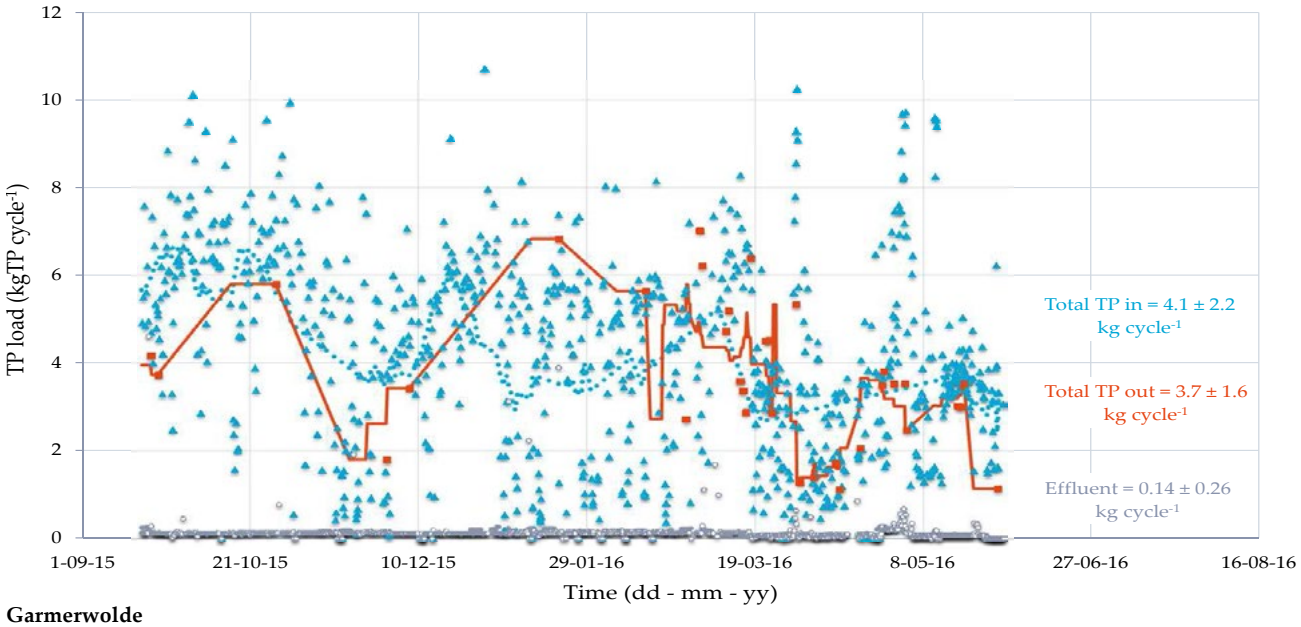


Fig. 13 Relation between the influent and the experienced reactor ammonium concentration after feeding showed ammonium adsorption in the anaerobic feeding phase of Garmerwolde (Royal Haskoning DHV 2016).

Utrecht



Garmerwolde

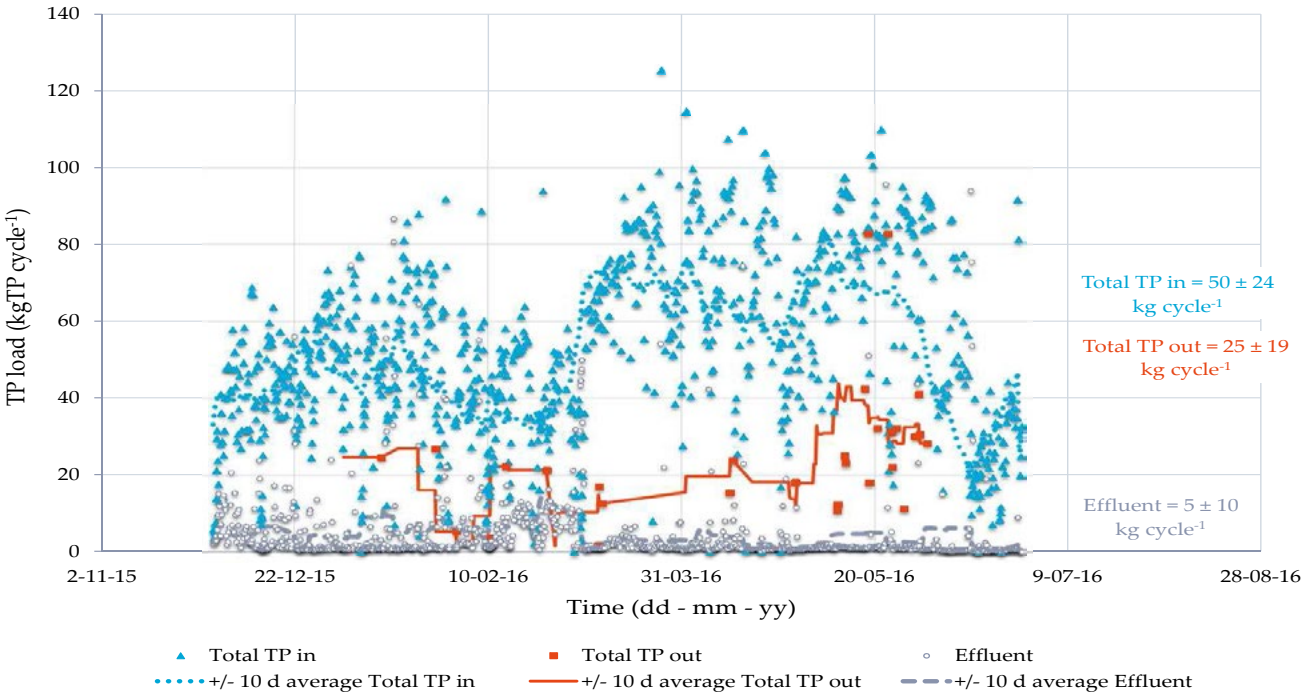


Fig. 14 Total phosphate balance per cycle of Utrecht (above) and Garmerwolde (below) with the total phosphate loading rate in (influent) and total phosphate loading rate out (effluent plus excess sludge). The effluent phosphate loading rate was illustrated separately in order to indicate the performance of the full-scale AGS systems with biological phosphate removal

room for a higher biomass yield and biological phosphate accumulation in the form of Poly-P.

A high standard deviation of the TP loading rates remained in the TP balance per cycle, as the cycles still contained dynamic (influent) concentrations. The trends however, are more distinguishable through the TP balance per cycle. For example, when the influent phosphate concentration was lower than average, the total TP out was also lower. In the first cycle(s) after a period of low influent phosphate concentration, when the phosphate concentration was on average again, the total TP out was considerably higher than average. When the phosphate concentration was particularly high for several cycles, the total TP out unexpectedly decreased. It is believed these observations are related to the biopolymer dynamics over various cycles. When the influent phosphate concentration was low for several cycles, the Poly-P content of the bacteria decreased, which made PHA accumulation more difficult in the preceding cycles. When the PHA content is not optimal, biomass growth can also not take place optimally. Since the excess sludge mainly contained small and therefore young granules, less sludge was found in the excess sludge and thus the TP loading rate out decreased. During the first cycles of recovered influent phosphate concentrations, the biopolymer contents can recover and biomass growth can significantly increase. This could be found back in the excess sludge content and therefore the TP loading rate out. When longer periods of high influent phosphate concentrations are experienced, it is believed that the Poly-P content of the bacteria already has optimised and more PHA is spent on maintenance than on biomass growth. This mechanism could explain the lower TP loading rates out during long periods of high phosphate concentrations. These observations leave room for further investigations, especially towards the role of the influent COD concentration in this mechanism.

Retention time based on solids or phosphate

The sludge retention time (SRT) is an important parameter in waste water treatment plants (WWTPs) to ensure sufficient biological conversions for low effluent concentrations. This parameter describes the sludge age, or in other words, the average time a particle spent inside the reactor. The SRT is also used to compare different WWTPs.

The calculated average SRT based on dry solids measurements was 27 ± 17 and 20 ± 9 days, in Utrecht and Garmerwolde respectively (see Fig. 15). The high standard deviations were caused by: the weather conditions, the influent concentrations, the difficulties to take a representative sample and the non-biomass related material in the TSS measurements (e.g., cellulose, leaves, twigs, sand particles). In Utrecht, 2 TSS measurements were not taken into account due to the reactor sludge extractions on the same day (see total phosphate balance per day). In Garmerwolde, no hinderance of the reactor sludge extractions was experienced during the measurements.

There are, however, some problems involved with SRT measurements in AGS systems. Firstly, microscopic pictures of sieved excess sludge samples from Utrecht showed a relatively high content of the non-biomass related material (see Fig. 16). Especially cellulose could be distinguished by the clear white fibres in the excess sludge sample. The cellulose could end up in the excess sludge if it easily binds to flocculent sludge or if it settles slowly by itself (see Outlook). Secondly, the usage of the SRT parameter for AGS system might be deceiving. During excess sludge decant, not all granule fractions are equally discharged (see Fig. 17 and Fig. 4 on page 13 for the granule distribution). The real SRT will be higher for the larger granules (> 0.6 mm) and lower for the smaller granules (< 0.4 mm). In fact, it is expected that some large granules (> 2 mm) remain almost forever in the reactor and that some small granules or flocs (< 0.2 mm) remain less than a day in the reactor. The range is so wide, that it is suggested to reconsider using the SRT parameter for full-scale AGS systems (see Discussion).

Instead of the SRT, it was proposed to consider the phosphate retention time (PRT) as a parameter to indicate the sludge age. The phosphate content of different granule fractions did not differ as much as the dry weight content between reactor and excess sludge. The TP content between large and small granules differed by approximately a factor two (see Fig. 3 on page 12). The dry weight content between reactor and excess sludge, however, differed by approximately a factor five: ≈ 2 g L^{-1} for excess sludge and ≈ 10 g L^{-1} for reactor sludge. Besides, the biological properties might differ per granule fraction, which is currently not taken into account by either parameter. Although the difference in TP content is still considerable, it would be a first step towards a more meaningful parameter for the sludge age in AGS systems. The difference between the calculated and the real experienced PRT of different fractions is smaller than the SRT. The phosphate retention times (PRT) were, on average, 18 ± 9 and 24 ± 14 days in Utrecht and Garmerwolde respectively (see Fig. 15). In Utrecht, the SRT had a significant higher standard deviation than the SRT (62 % instead of 49 %). This suggests that the PRT is a more reproducible parameter than the SRT. In Garmerwolde, the standard deviation of the SRT was artificially low (43 % compared to 59 % for PRT) as the involved TSS measurements at this installation were done by an independent lab without decimal accuracy (2 g L^{-1} instead of 2.00 g L^{-1}).

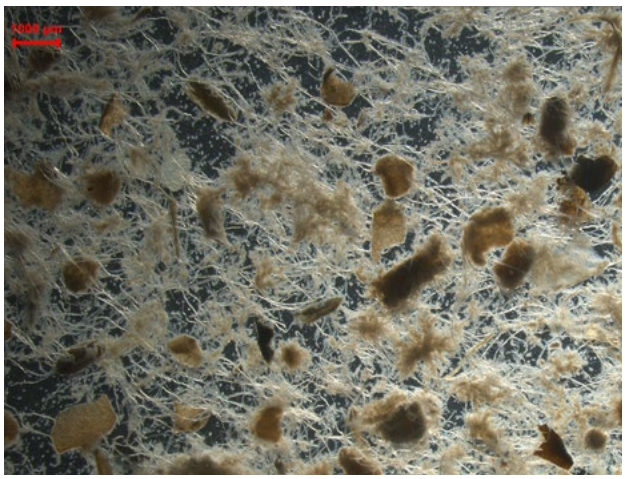


Fig. 16 Sieved (> 600 µm) and washed excess sludge showed clear cellulose fibres in Utrecht (Reproduced with authorization from M. Pronk (Unpublished)). Bars indicated 1000 µm.

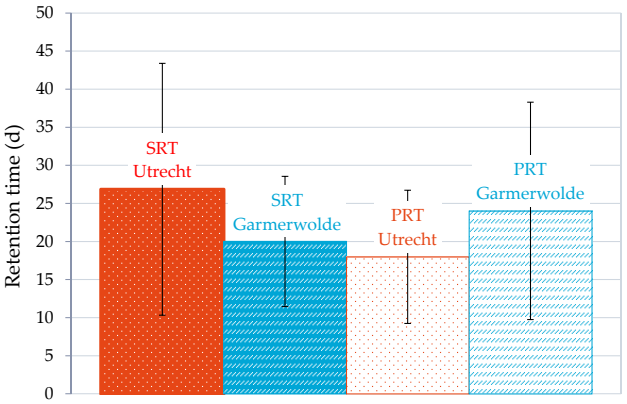
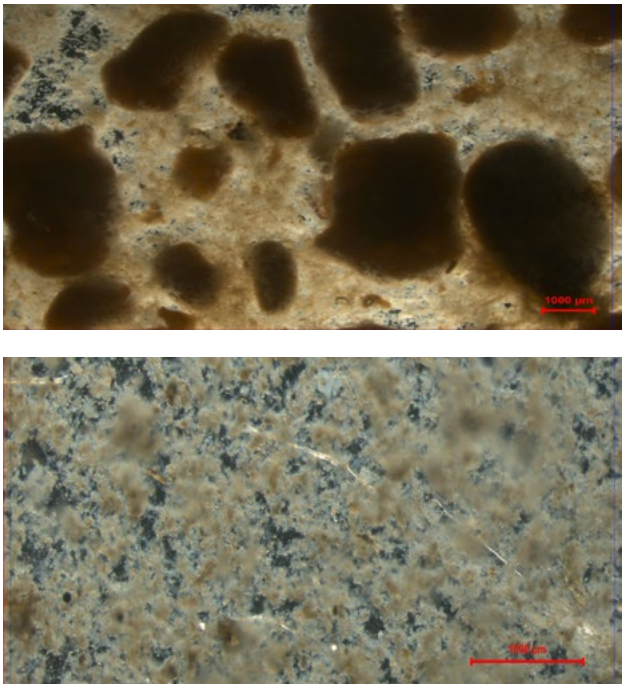


Fig. 15 Sludge retention time (SRT) and phosphate retention time (PRT) in Utrecht and Garmerwolde with standard deviations.

Utrecht



Garmerwolde

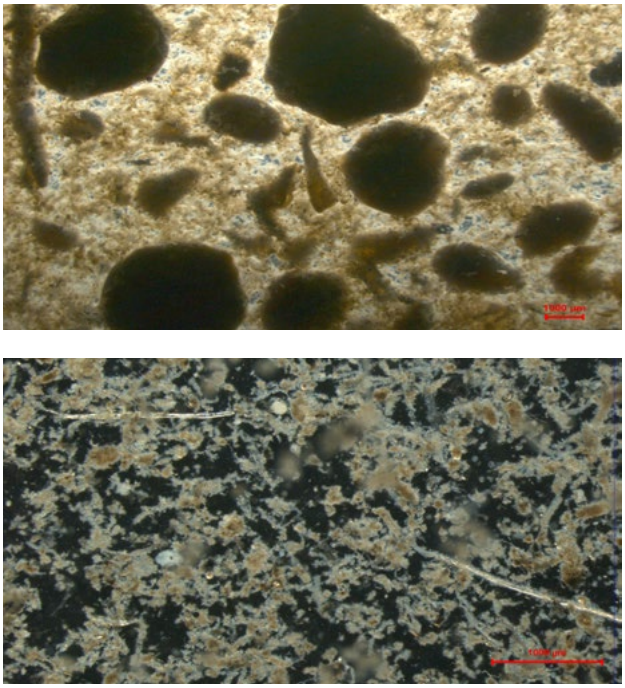


Fig. 17 Pictures of reactor and excess sludge of Utrecht (above) and Garmerwolde (below). Bars indicated 1000 µm.

4. Discussion

Biological induced phosphate precipitation in full-scale AGS systems is considerably different from lab scale reactors

Where multiple authors indicated phosphate precipitation in granule sludge lab reactors (Barat et al. 2011, Mañas et al. 2011, Lin et al. 2012, Winkler 2012, Li et al. 2014), this study showed that in full-scale AGS installations, the biological induced precipitation is sporadically, site-dependent and had no crucial contribution to the overall phosphate removal. These results are in line with the results from Yilmaz et al. (2008) and Carlsson et al. (1997), who also indicated minor or no phosphate precipitation contribution to the overall phosphate removal. These results are however in contrast with the results from lab reactors results of Mañas et al. (2011) and (Barat et al. 2011) who found that significant Calcium phosphate precipitation inside aerobic granules.

Operational and influent conditions in full-scale installations were in general considerably different compared to previous studies focusing on laboratory scale reactors with synthetic influent. For example, higher medium pH or calcium influent concentration were used, which both are important parameters for phosphate precipitation (Song et al. 2002, Yilmaz et al. 2008). Also, the influent COD concentration in lab-reactors is often pure (sodium) acetate (Barat et al. 2011, Lin et al. 2012), whereas in full-scale installation the acetate-COD is only 3.7 - 19.7 % of the influent COD (Chen et al. 2004, Stubbé et al. 2016). Using acetate as the carbon source might influence the phosphate precipitation as the phosphate release is enhanced when easily degradable volatile fatty acids are used (Hollender et al. 2002, Guerrero et al. 2011). Influent phosphate and calcium concentrations in lab experiments described in Barat et al. (2011), Mañas et al. (2011) and Li et al. (2014) were 50 - 300 % and 17 - 50 % higher than the average concentrations of full-scale installations found in this study. The large difference in phosphate concentration between lab tests and practice could indicate that this could be the limiting factor of phosphate precipitation to contribute to the overall phosphate removal in the full-scale AGS reactors. Also, the pH profile during the cycles differed considerably between the lab reactors and the monitored full-scale reactor in Utrecht and Garmerwolde (Stubbé et al. 2016). In the lab reactors a pH of ± 8 during aeration was reached, whereas in full-scale the pH during aeration did not exceed ± 7.3 (Stubbé et al. 2016).

The changing XRD results in time indicated the option that the precipitation process in full-scale AGS systems could be very dynamic. At least three different precipitation pathways could take place:

1. supersaturation might occur in the aqueous phase, leading to homogeneous precipitation and possible biomass formation around it as observed by Wan et al. (2015),
2. supersaturation occurs in the exopolysaccharides (EPS) structure, which provides nucleation sites as also suggested by Yilmaz et al. (2008), Lin et al. (2012) and Dupraz et al. (2005), leading to heterogeneous precipitation, but dissolution occurs in the following aerobic phase as suggested by Carlsson et al. (1997) and Serralta et al. (2004),
3. supersaturation occurs in the EPS structure, leading to heterogeneous precipitation, but the granules break into aggregates as stated by Lemaire et al. (2008) and Nor-Anuar et al. (2012) which leads to partly dissolution of the precipitates and partly biomass grows around the broken pieces again.

The first pathway requires high levels of supersaturation to overcome the critical size of a stable nucleus (Crutchik et al. 2016). It is highly doubted whether the saturation indices in full-scale AGS would be able to reach those levels. The second pathway can already partly be found in literature: ammonium adsorption is shown for aerobic granular sludge (Bassin et al. 2011), but the possible contribution of struvite formation for the disappearance of ammonium was not taken into account. The second pathway is further elaborated in Stubbé et al. (2016), which shows precipitation and dissolution of phosphate minerals inside aerobic granules under normal full-scale conditions. The third path is difficult to quantify, since the effect of granule breakage on precipitate formation or dissolution has not been studies before, to the authors knowledge. There remain many unknown variables that influence phosphate precipitation in full-scale AGS systems. To investigate the possibilities of enhancing biological induced phosphate precipitation, there is a need for a model that provides more detailed insights into the decisive parameters.

Total phosphate balance

Mass balances are suggested in previous works to improve modelling operational conditions, design and benchmarking of conventional activated sludge systems (Puig et al. 2008, Meijer et al. 2002). The measurements done on full-scale waste water treatment plants are known to contain gross errors. Without proper verification, the data would lead to unjustified adaptations and a loss of valuable trend analyses (Meijer et al. 2002). This study applied the total phosphate balance on a full-scale granular sludge system. As aerobic granular sludge systems are more dynamic than conventional activated sludge systems, there is an even greater need for data reconciliation (Meijer et al. 2002). The error mitigation applied in this study is more elaborated in Addendum 3. Although it was found that the total phosphate balance and phosphate measurements in general can potentially contribute to the process control of full-scale AGS systems, the difficulties of obtaining representative, reproducible data should not be underestimated. More developed, creative methods to

further eradicate errors on these measurements would be a first step to exploit the phosphate potential to contribute to the process control in waste water treatment systems.

The relation of the total phosphate balance with the solid fraction was firstly proposed by Meijer et al. (2001). This proved to be justified over a relative long period of time; around three times the SRT. As this study was done over a period of 6 months (more than six times the SRT), even more data was acquiered. The SRT however was suggested to be better estimated based on the total phosphate measurements, as done by Bdrjanovic et al. (2000). The advantages of phosphate over other mass balances are multiple: no volatile gasses are involved in the conversions, it is relatively simple to measure, the phosphate concentration is often above detection limit and mostly the total phosphate concentrations of the influent and effluent are already available (Meijer et al. 2002, Puig et al. 2008).

Phosphate measurements can be a promising tool for process control of AGS systems

Phosphate can contribute to the process control of AGS systems in four ways that are further elaborated:

A. the online phosphate cycle measurements provide insights in the dynamic relation between the biopolymer content of the PAOs (PHA, Poly-P and glycogen) and oxygen levels in the reactor.

B. the reactor total phosphate measurements can indicate the phosphate saturation levels of the PAOs.

C. The total phosphate balance could be used to estimate the biomass production and yield.

D. The total phosphate balance could also be used to provide an alternative for the more conventional SRT.

The first method is straightforward and can be applied directly; when the phosphate concentration profile in the online measurements does not decrease in a straight, linear line until the minimum, oxygen might have been oversaturated in previous cycles (see black arrows in Fig. 18). When too much oxygen is available during the aeration in previous cycles, the internally stored PHA biopolymer becomes depleted (Stubbé et al. 2016). The only available PHA in the next cycle is the freshly produced PHA during the next cycle's feeding phase. In that case, the available PHA is not enough for complete phosphate uptake and Poly-P storage, and therefore leads to slower rates in the next cycle until PHA reserves are restored. This shortage of biopolymer content is expressed in the phosphate online cycle measurements by a decreasing slope in the phosphate uptake rate (see Fig. 18).

The second method is of importance to prevent the phosphate effluent concentration to exceed the effluent requirement. However, this process control method is still in the development phase and the mentioned TP content should be

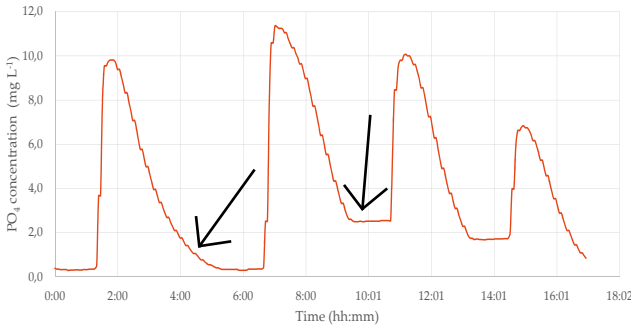


Fig. 18 Real time phosphate profiles that indicate oxygen oversaturation (left arrow) and saturation (right arrow).

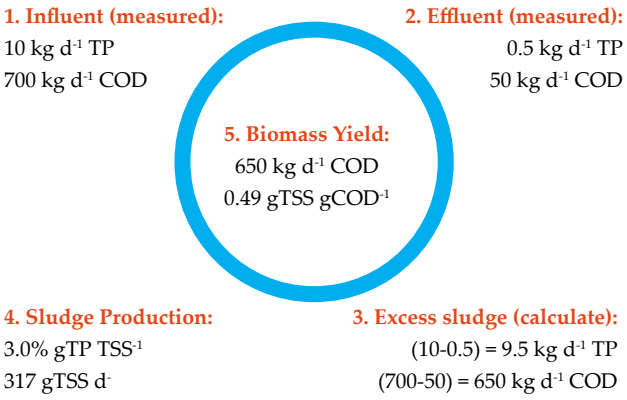


Fig. 19 Method to determine the biomass production and yield based on the total phosphate balance of full-scale aerobic granular sludge systems. Numbers are fictitious.

taken as an indication alone. When the Poly-P saturation level is reached, the PAOs cannot take up more phosphate than they have released, thus leaving the nett phosphate release and uptake the same (Wentzel et al. 1989, Majed et al. 2009). The luxurious uptake, in that case is non-existent. To prevent this, the saturated, bigger granules should be discharged out of the system, leaving more substrate for the unsaturated granules that can perform the needed luxurious phosphate uptake.

During this research, it became evident that the full-scale AGS system in Garmerwolde was experiencing phosphorus saturated PAO. The phosphate effluent concentration increased while COD was still removed, possibly for biomass maintenance purposes and denitrification. It is proposed that the total phosphate (TP) measurements of the sludge in the reactor can provide an indication of the phosphate saturation level that can be reached in aerobic granules. As the XRD, XRF and SEM-EDX results indicated that phosphate precipitates are not significantly present in the granules, the reactor TP content is very closely related to the PAO saturation level. Instead of using the SRT as a parameter to estimate the required time of the bacteria in the tank, the reactor TP content could indicate which granule fraction should be discarded as excess sludge. The discharge should actually happen before the saturation levels are reached, since the system needs time to build up the required biopolymers for the luxurious phosphate uptake. This hypothesis could unfortunately not be tested during this study, as other operational activities at the installation in Garmerwolde interfered with measurements. More data needs to be acquired to estimate when and which fraction exactly should be discharged.

The third method consists of the total phosphate balance contributing to the process control of AGS systems, by estimating the biomass production and yield estimation. This method can be applied directly if the measurement error depicted in Fig. 6 on page 13 can be accepted. If not, extra data should be acquired while limiting the errors as suggested in Addendum 3. Since dry weight measurements have various uncertainties and risks for human errors (see section *error mitigation*), the TP measurements are more reliable. Although nitrogen and carbon dioxide could also be used to estimate the biomass yield (Selna et al. 1979, Tchobanoglous et al. 1991), phosphate has the advantage of a lower volatility. The steps to estimate the biomass production and yield are illustrated in Fig. 19. The TP of the influent and effluent are part of the water authorities' required analyses of the water quality (step 1a). These TP values are used to calculate the TP excess sludge loading rate, assuming no major TP-accumulation occurs in the reactor on a daily basis (step 2a). The averaged TP content of the excess sludge of this research is used to calculate the excess sludge TSS concentration, which gives the sludge production (step 3a). The sludge production is equal to the biomass production when the biomass content of the reactor is constant.

If the TP-balance is expanded with COD measurements, the observed biomass yield can be calculated. The COD measurements of the influent and effluent are also part of the water authority's required analyses (step 1b). The COD values are used to calculate the removed COD loading rate (step 2b); dividing the removed COD by the excess sludge TSS concentration gives the biomass yield (step 4). A lower yield is beneficial for operating costs (Mayhew et al. 1998, Low et al. 1999), but disadvantageous for reactor start-up durations. The reported biomass yields of aerobic sludge range from 0.2 $gCOD_{biomass}$ $gCOD_{substrate}^{-1}$ to 0.48 mgMLSS $mgCOD^{-1}$ (Tay et al. 2001, Mosquera-Corral et al. 2005). The daily TP balance of this study obtained a biomass production of 424 ± 267 and 6,774 ± 4,075 kgTSS d^{-1} following the method described in Fig. 19. The observed biomass yield was 0.42 ± 0.14 and 0.61 ± 0.13 kgTSS $kgCOD^{-1}$ in Utrecht and Garmerwolde based on the COD measurements of the water authorities. Average yields of 0.42 - 0. 6 kgMLSS $kgBOD^{-1}$ in activated sludge systems were found with the conventional method based on the TSS measurements (Mayhew et al. 1997, Tchobanoglous et al. 1991). In other AGS systems, an average yield of 0.4 - 0.6 Cmol $Cmol^{-1}$ was estimated (Beun et al. 1999).

The fourth and last method for phosphate to contribute to the process control of full-scale AGS systems, is more elaborated in the next section.

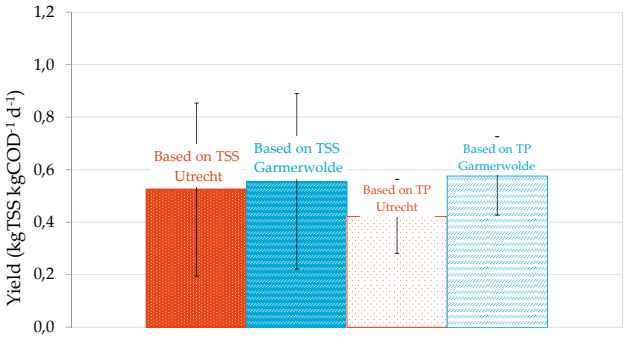


Fig. 20 Yield calculation based on total suspended solids (TSS) and total phosphate (TP) measurement for the full-scale AGS systems in Utrecht and Garmerwolde with standard deviations.

The sludge retention time is a deceiving control parameter for AGS systems

During the course of this research, it became evident that the sludge retention time (SRT) is a deceiving parameter for AGS systems where selective excess sludge withdrawal takes place. The AGS process, as a batch system with selective excess sludge discharges, is fundamentally different than activated sludge systems. An activated sludge system is operated as a continuous system and excess sludge consists of averaged reactor sludge. The SRT is, originally, the estimated average time of a bacteria spend in a purification system (Walker 1971). In AGS systems, the calculated SRT is only true for an exceptionally small fraction of the sludge in the reactor; the excess sludge has none of the characteristics of the granules present in the reactor in terms of sludge volume index, dry weight, size (see Fig. 17 on page 23) or granule fraction distribution (see Fig. 4 on page 13). It is even studied that different types of bacteria (PAO and GAO) have different SRTs in AGS systems (Winkler et al. 2011). The bigger granules in an AGS system experience a lot higher SRT (towards infinite) than the smaller granules or flocs (estimated to be less than a day). For the reasons mentioned above, the SRT is better not used as a control or design parameter for AGS systems.

Originally, the SRT was an important control parameter to design and operate wastewater treatment plants in order to comply with nitrogen effluent regulations (STORA 1988, Tchobanoglous et al. 1991, Grady Jr et al. 2011). Additionally, the SRT is used to compare different purification systems, although this is purely from an engineering point of view. For AGS systems, the SRT could still serve as a parameter to compare different purification systems. The authors propose however to release the historically importance of the SRT as a control parameter for AGS systems.

Instead, the phosphate retention time (PRT) is proposed as an additional parameter to indicate the sludge age inside an AGS system. The phosphate content is more evenly distributed over the granule fractions than the solid content is. The average retention time based on the total phosphate would therefore give a more meaningful value for the average time a particle spents in the reactor. Besides, the smaller standard devaition of the PRT compared to the SRT suggests that this estimate is also more accurate. This was also stated by Brdjanovic et al. 2000. So far, the PRT is not calculated or used in practise for full-scale aerobic granular sludge systems. To help the transition, a first small step could be to only mention the SRT of a certain granule fraction instead of the whole reactor. This would however, become very laborious and redundant in time.

5. Conclusion

The phosphate precipitates in full-scale AGS systems were investigated with different analyses: XRD, XRF, SEM-EDX and extractions. The results pointed out that the phosphate precipitation process in full-scale AGS systems is dynamic and site-dependent; the types of precipitates differed over time and per installation. It seemed that the contribution of the precipitation to the overall phosphate removal was minimal. At times the precipitates were completely absent. There are still too many variables to estimate the amount and behaviour of (amorphous) precipitates in AGS systems, when present.

Phosphate has the potential to contribute significantly to the process control of full-scale AGS systems. Online cycle measurements in the tank and total phosphate (TP) measurements of the reactor sludge can prevent high effluent phosphate concentration by indicating possible biopolymer depletion and saturation levels. The TP balance could make a significant contribution by estimating the biomass production and yield. The next step to exploit the potential of phosphate as control parameter is to minimize the errors in the measurements for the TP content of the excess sludge.

6. Outlook

Biological phosphate content determination

During the extraction method of De Haas et al. (2000), another method was tested with ethylenediaminetetraacetic acid (EDTA). vAlthough this method is not verified, it showed potential of releasing the polyphosphates from the biomass without immediate precipitation with present cations in the solution. EDTA has the characteristic of binding cations, which prevents precipitation. Additionally, the biologically-bound phosphate can be quantified by a phosphate-release test (Waterbeheer 2001). Although the biomass tends to keep a certain polyphosphate portion in presence of excess acetate, this would give a first indication (Wentzel et al. 1989). The extraction and release test can be performed on different granule fraction to compare the differences with the reported TP content in this study.

In addition, the XRF metal ratios can be an useful method to indicate the likelihood of possible phosphate bindings. The extent of the measurements however, should be increased to provide actual evidence. These extensions could consist of, firstly analysing granules saturated with Poly-P and after releasing phosphate in order to how much of the actual metals in Poly-P ($Mg_{0.36}K_{0.28}H_2PO_4$) come in the solution. Afterwards, the metal ratio can be corrected for Poly-P contribution and see how much is left for the chemical removal of phosphate. Secondly, the real experienced K_p can be calculated based on averaged concentrations, speciation, temperature and pH.

Cellulose in AGS systems

In the excess sludge pictures in Fig. 17 on page 23, cellulose can be distinguished by the one or two clear white, straight fibres. A photo of sieved excess sludge depictures the cellulose even better (see Fig. 16 on page 23). Cellulose embodies between 30 - 50 % of the suspended solids in the waste water of western countries (Ghasimi et al. 2016). Previous works show that cellulose is not 100 % converted in activated sludge systems in the Netherlands (Verachtert et al. 1982, Ruiken et al. 2013). It is suggested that only 30 - 70 % of the cellulose is degradated in conventional waste water treatment plants (Tchobanoglous et al. 1991). In aerobic granular sludge systems, a relatively large fraction of the cellulose could end up in the excess sludge due to its (slow) settling properties or binding properties to flocculent sludge. It should be investigated if the cellulose concentration in the excess sludge of AGS systems is higher than in the reactor. This could cause a serious discrepancy in the TSS measurements. This would cause a discrepancy in the TP content (gTP $gTSS^{-1}$) between the excess and reactor sludge measured in this study. Cellulose recovery technologies could take advantage of this relatively high content of cellulose in the excess sludge stream of AGS systems.

Phosphate precipitation model

The XRD measurements showed a variety of phosphate precipitates in full-scale AGS systems, which could indicate a dynamical system of dissolution and precipitation within or over the cycles. It is interesting to see if a model could help understand the precipitation dynamics (Barat et al. 2011, Mbamba et al. 2016). Which operational factors lead to phosphate precipitation? Under which conditions would magnesium phosphate precipitates dominate over calcium phosphate precipitates? Insights in these questions can have practical applications towards recycling phosphate from wastewaters (De-Bashan et al. 2004).

In the model, it is advised to include the iron chemistry. The results of this study (XRD, XRD and microscopic pictures) indicate that iron can residue longer than expected in the granules after iron dosing is stopped. As the iron-phosphate bindings are relatively strong, this could have a serious impact on the fate of phosphate in full-scale AGS systems.

Dynamic excess sludge regime

The TP-balance over individual cycles in Garmerwolde showed a relation with rain weather conditions. In order to investigate the effect of rain on the phosphate precipitation further, it could be possible to limit or halt the excess sludge discharge during the first-flush and measure the mineral content subsequently. This dynamic excess sludge regime can be coupled with a sporadically increased or mixed excess sludge discharge when phosphate saturation levels are reached.

To accomplish this, first more data is needed for establishing a clear threshold of phosphate saturation. Additionally, the granule fraction that should be discharged to most effectively diminish the phosphate saturation level can be investigated. Even the height of the excess sludge withdrawal can be dynamically altered in future reactors.

7. Acknowledgements

You'll never work *alone* – Frank Sinastra

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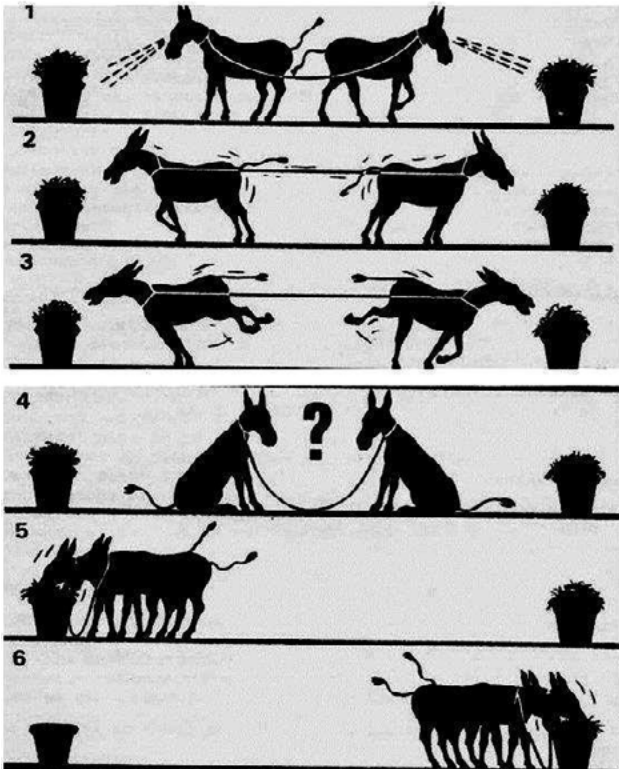

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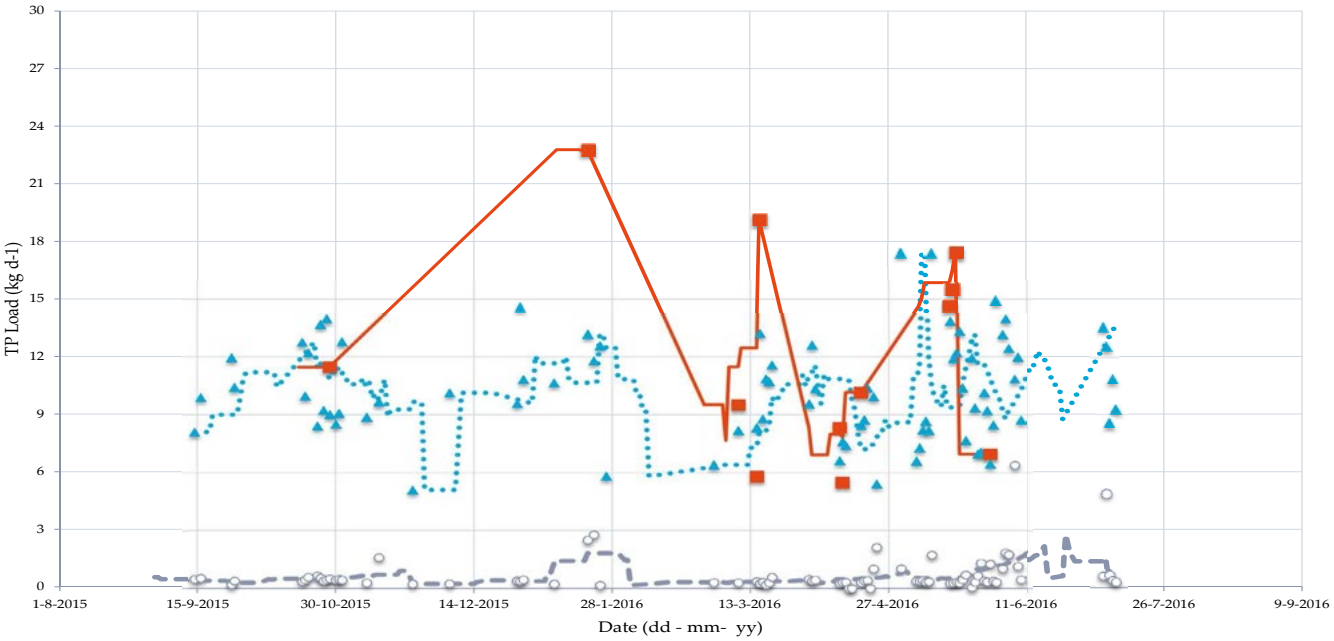
Addendum 1

Dry and rain weather conditions influence on the total phosphate balance per day in Utrecht and Garmerwolde

The small difference between the dry and rain weather loading rates in Utrecht can be explained by the distribution of the influent over the Nereda and conventional treatment plant. The discharge towards the AGS is always kept constant, causing the rain weather loading rate to change only by a difference in TP concentration. The large difference between the dry and rain weather loading rates in Garmerwolde can be explained by the sewer system design. In Garmerwolde, the long sewer system consists of multiple pumping stations with the necessary retention tanks where at a certain water level, the pumping velocity increases (Heeringen et al. 2015). Such a sewer system leads to a wide range of retention times (1 - 12h) between the nearest and furthest point of the system, spreading the effects of a rain event over time. Besides, in the pumping stations, dilution takes place during the first-flush of a rain event. A rain event, therefore, reaches the treatment plant in Garmerwolde with a diluted first-flush and spread in time. This leads to an extended period of time with higher loading rates than other treatment plants with a pronounced first flush at the start of a rain event. This phenomenon affected the measurements, as the frequency of the samples was not sufficient to average the fluctuating concentration during the extended rain event. Thus, this increased the chance that a sample day measured an above-average loading rate in Garmerwolde. At the same time, when the pumping velocity at the pumping stations is back to dry weather conditions, the very diluted concentrations of the rain event are still present in the sewer. The chance to measure a lower concentration demarked as a dry day is also artificially increased due to this sewer design. This explained the larger difference between dry and rain weather influent loading rates in Garmerwolde compared to Utrecht.

Utrecht Dry weather



Utrecht Rainy weather

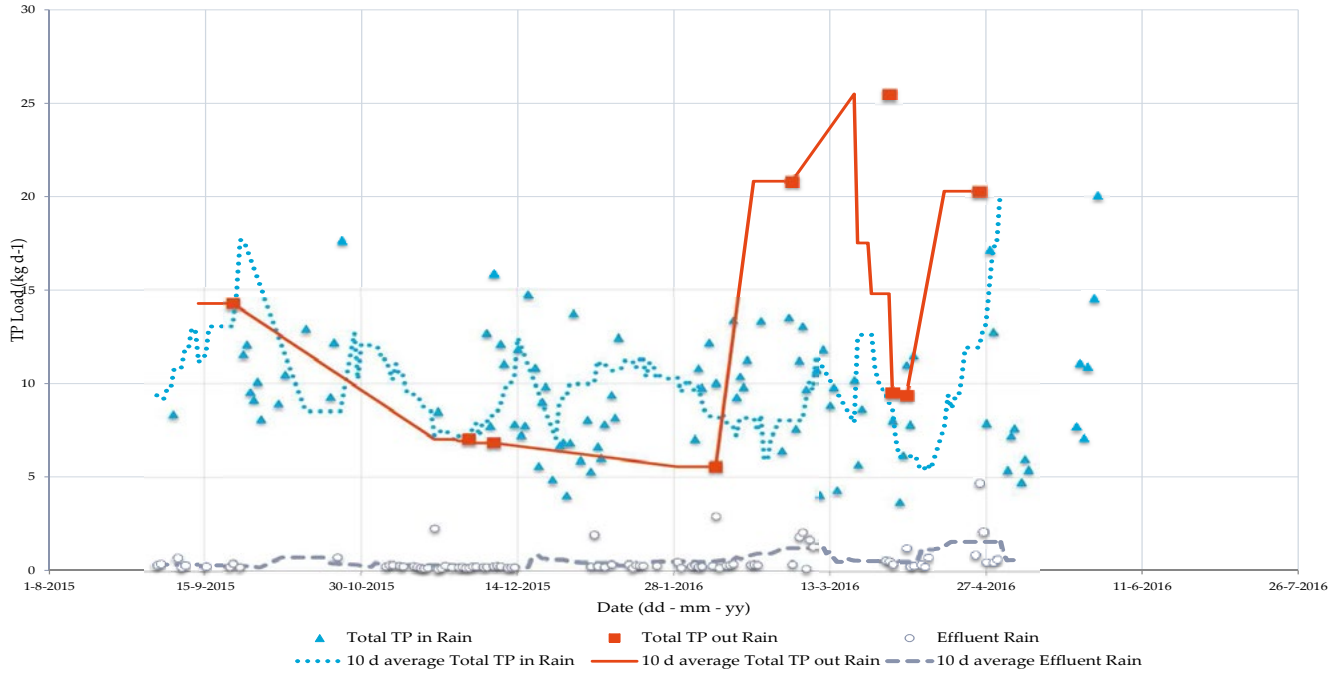
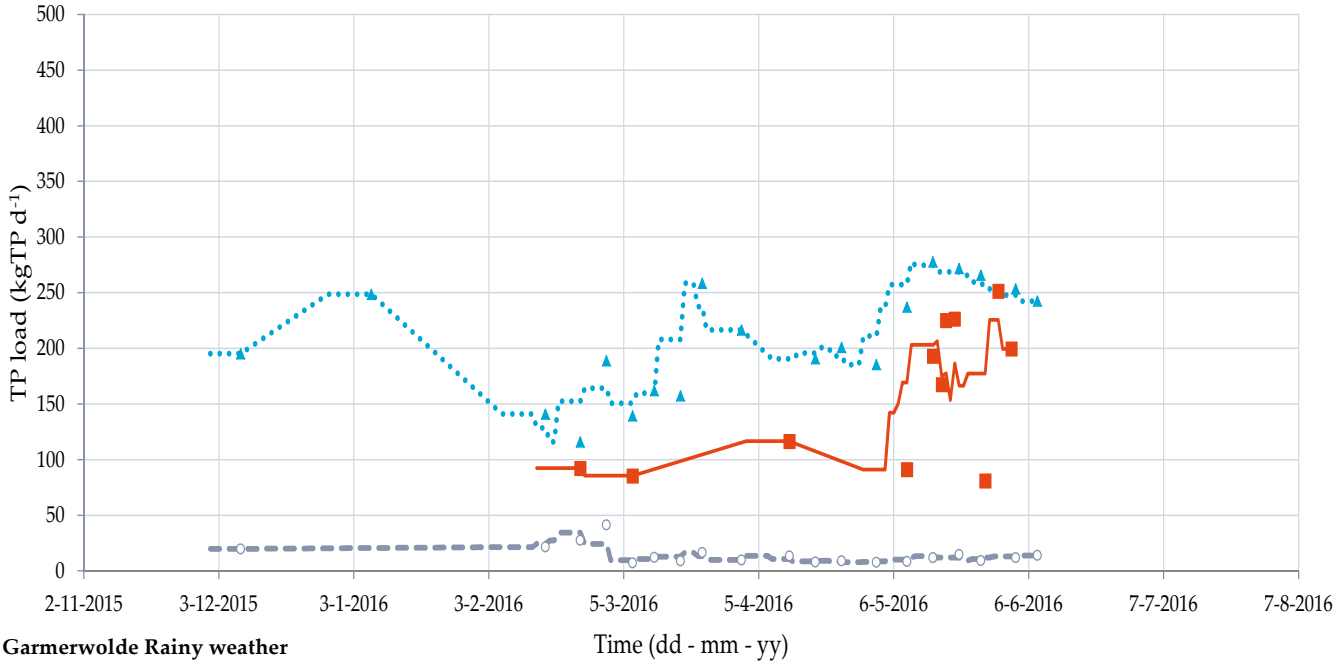


Fig. 21 Total phosphate balances of Utrecht during dry weather conditions (above) and rainy weather conditions (below) with the total phosphate loading rate in (influent) and total phosphate loading rate out (effluent plus excess sludge). The effluent phosphate loading rate was illustrated separately in order to indicate the performance of the full-scale AGS systems with biological phosphate removal.

Garmerwolde Dry weather



Garmerwolde Rainy weather

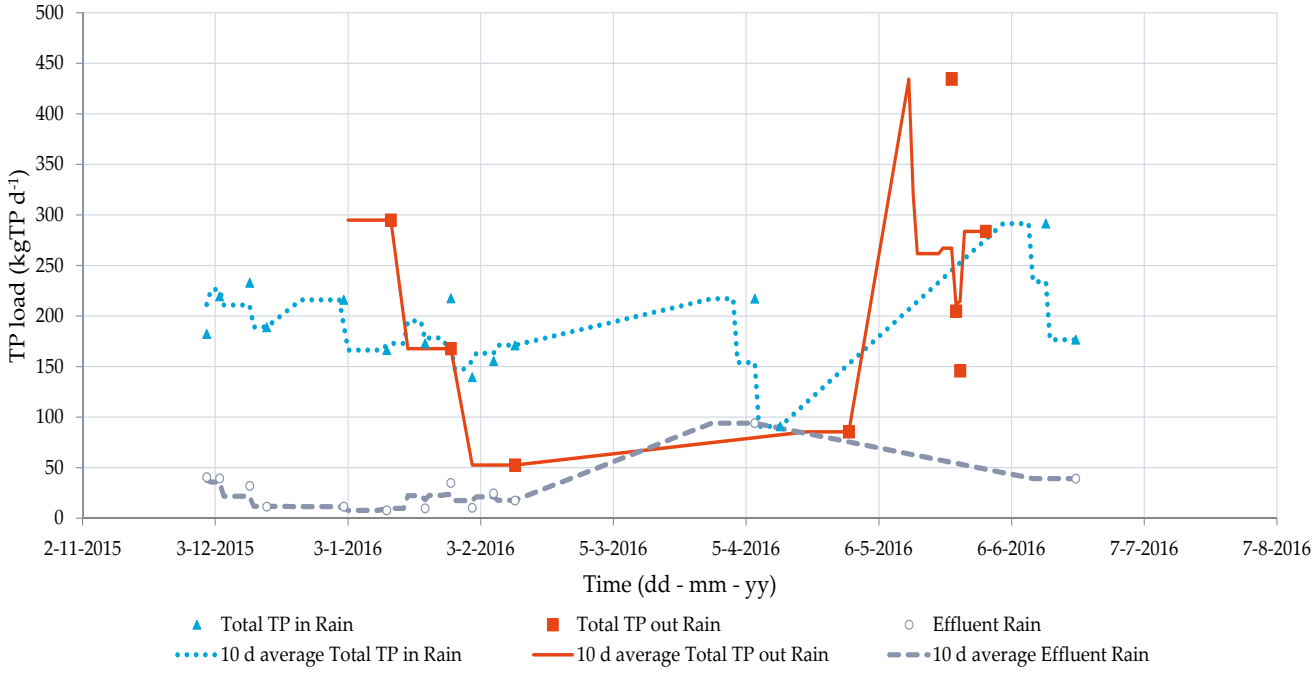


Fig. 22 Total phosphate balances of Garmerwolde during dry weather conditions (above) and rainy weather conditions (below) with the total phosphate loading rate in (influent) and total phosphate loading rate out (effluent plus excess sludge). The effluent phosphate loading rate was illustrated in order to indicate the performance of the full-scale AGS systems with biological phosphate removal.

Addendum 2

Excess sludge samples during dry (top) and rain (below) weather conditions in Garmerwolde

During dry weather conditions, the first (# 2 – # 5) excess sludge samples contained more sludge than the last samples (# 6 - # 8) (see Fig.23). Also, the sludge was floating due to nitrogen gas produced by denitrification. During rain weather conditions, the excess sludge samples all contained significant amounts of sludge (# 1 - # 8) and no denitrification occurred in the sample bottles. This explains the dynamics in excess sludge TP loading rates: more sludge, thus TP, was removed during rain weather conditions.

Fig. 23 Excess sludge samples during dry weather conditions (above) and rain events (below) showed differences in sludge content and sludge distribution over the bottles.

Addendum 3

Error inventory

The standard deviations of the discharges, total phosphate measurements and dry weight measurements in the influent, excess sludge and effluent are shown in Table 4. The standard deviation of the TP measurements (30 – 161 %) was higher than standard deviation of the discharges (7 – 44 %). Especially, the standard deviation of the effluent phosphate concentration was high (161 %), since it concerns very low numbers and fluctuations have a higher impact. This, however, does not impact the TP balance significantly. The standard deviations of the discharges in Garmerwolde (7 – 32 %) were slightly lower than in Utrecht (28 – 44 %). In Garmerwolde, however, the discharges concerned large amounts (35,000 m^3 d^{-1} instead 1,200 m^3 d^{-1} in Utrecht) and therefore have a large impact on the TP balance.

An inventory was made of the errors in the TSS and TP measurements in order to locate the mayor contributor to the observed standard deviation in the TP balance. The fluctuating influent TP loading rates can be caused by several factors. The type of error was separated in three groups; the analytical, sample and external error. The analytical error was caused by the inaccuracy of (handling) the equipment (e.g., pipetting) on duplicate or triplicate samples. The sampling errors were caused by the sampling method, timing and preparation of at least two different samples at the same time before analysis. The external errors between samples of various days were considered to be caused by sudden discharges of industrial wastewater, farmers (seasonal agricultural manure leakages), restaurants, heavy rainfall or operational changes. The errors are accumulative in scale: the external errors also contain the sample and analytical errors and the sample error also included the analytical error

As it turned out, the overall standard deviation was between 29 – 56 %, including factors such as operational conditions, sampling and analytical errors. For full-scale installations, an overall deviation of ± 30 % is common in practise, as expressed by the operators with at least 15 years of experience. The standard deviation caused by taking a sample (7 - 22 %) was slightly higher than the analytical standard deviation (2 – 16 %). In Utrecht, the standard deviations of the TP measurements were slightly lower than the standard deviations of the TSS measurements (5 – 43 % compared to 8 – 47 %). In Garmerwolde, the standard deviation of the TSS measurements was artificially low as the measurements were done in the independent lab of the water authority and they express the TSS without decimal accuracy (2 g L^{-1} instead of 2.00 g L^{-1}). Overall, the standard deviations were higher in Garmerwolde than in Utrecht. This can be explained by the differences in the sewer systems (see the explanation in *dry and rain weather conditions influence the TP balance in Utrecht and Garmerwolde).*

Table 4 Standard deviations of discharge, total phosphate and total solid measurements in the influent, excess sludge and effluent during the total phosphate balance data acquisition

	Discharge St. Dev. (%)		
	Influent	Excess sludge	Effluent
Utrecht			
Overall average	35	44	41
Dry weather	28	42	35
Rain weather	39	38	43
Garmerwolde			
Overall average	30	22	32
Dry weather	7	12	7
Rain weather	26	20	28
	Total phosphate St. Dev. (%)		
	Influent	Excess sludge	Effluent
Utrecht			
Overall average	30	38	148
Dry weather	29	51	136
Rain weather	30	41	161
Garmerwolde			
Overall average	36	73	42
Dry weather	-	72	40
Rain weather	-	70	52
	Dry weight St. Dev. (%)		
	Influent	Excess sludge	Effluent
Utrecht			
Overall average	-	47	124
Garmerwolde			
Overall average	-	33	30

Table 5 Standard deviations of reactor and excess sludge measurements during the total phosphate balance data acquisition

	External error (%)		Sample error (%)		Analytical error (%)	
	TSS	TP	TSS	TP	TSS	TP
Utrecht						
Reactor	29	32	10*	11*	2*	9*
Excess sludge	47	43	12	7	8	5
Garmerwolde						
Reactor	35	53	-	22*	9*	16*
Excess sludge	53	56	-	-	4	7*

*Based on two samples

Error mitigation

In order for the TP balance to contribute to the process control of AGS systems, the measurements and sampling should be done adequately. The experience obtained through this research to make an adequate TP-balance is summarized in table 6. The representativeness of a measurement is defined as the ability to obtain a value as close as possible to the real value. The reproducibility of a sample is defined as the ability to obtain the same value again from a different sample.

First of all, the preparation described in table 6 prevents errors when interpreting the results later-on. Also, a good preparation indicates where additional measures or estimations are necessary to compensate a discrepancy, like the sewer system in Garmerwolde needed. Secondly, the reproducibility of the measurements should be closely monitored. Throughout this study, we found that good reproducible measurements were the hardest to obtain. The AGS reactor is in both space and time extremely heterogeneous: during one cycle and throughout one week, also including the weather influences. For example, the dry weight measurements of the excess sludge were problematic because of the difficulty to take a representable sample; not only do the discharge and the sludge concentration decrease during the excess sludge, but also the operating conditions (blower capacity, rain, e.g.) can change the excess sludge content from day to day. The representativeness of the measurement was less of a problem, but this error could be diminished to a minimum level by the measures mentioned in table 6. Especially the excess sludge is inadequately investigated; we suggest that the routine analyses of the influent and effluent concentrations include also the excess sludge prior to utilizing the TP balance for process control purposes as outlined before.

Lastly, longitudinal data is necessary to distinguish a trend and diminish the effect of outliers. In order to interpret the data adequately, the observations of the local operator are essential. It is therefore no luxury, but a necessity to have open communication with the operator. If longitudinal data is not an option, it is preferred to sample 2 or 3 weeks full – time, including a rain event. In that way, the dynamics of the weather conditions are distinguished. For now, no seasonal changes are seen in the TP balances. Also, the TP balance is preferred per batch instead of per day, as the daily measurements are to generic to be accurate in a full-scale system.

Table 6 How to make an adequate TP balance of a full-scale aerobic granular sludge system installation

	Description	Explanation
Preparation	*Inventory of the sewer system*	
	Relative level to groundwater table	for clay particles and groundwater (Al, Fe) intrusion
	Retention time	for delay and dilution of rain events
	Industrial water discharges	to estimate the raw wastewater composition
	Inventory of the treatment plant	
	Location of influent and effluent sampling points	for possible buffering of pre/post-treatment steps
	Precision and accuracy of sensors	for inventory of measurement error
	Decide how to determine a 'dry' day from rain conditions	to distinguish the different cycles times and higher discharge
External factors	*Ensure good information*	
	Maintain open communication with the operator	to be well informed on special events, to make adequate decisions
	Take measurements during consecutive days	to diminish the effect of outliers
	Take measurements during both dry and rain conditions	to diminish the effect of outliers
	Investigate online profiles from the same cycle (P-release, Q)	to investigate extra external influences on the data obtained
Reactor Sampling	*Ensure representative samples*	
	Take enough sample volume	a sample of ≥ 1 L is necessary
	Take a sample when the reactor is completely mixed	when the blowers are at 100 % for 20 min
	Ensure reproducible samples	
	Transfer the sludge a minimal amount of times to another beaker	to reduce the number of additional introduced errors
	Take the sample always from the same location in the reactor	to avoid the influence of unevenly distributed blowers
Excess sludge Sampling	*Ensure representative samples*	
	Tap continuously a fraction of the stream	Periodic samples are less representative due to the decrease in TSS concentration and discharge during sludge decant
	Mix the tapped excess sludge before taking a sample	To capture the average excess sludge TSS concentration
	Ensure reproducible samples	
	Transfer the sludge a minimal amount of times to another beaker	to reduce the number of additional introduced errors
Analysis	*Ensure representative sample*	
	Crush the complete sample before analysis	To homogenize the sample
	Stir the sample during pipetting	To ensure a representative sub-sample
	Ensure reproducible samples	
	Analyse the samples within 48 h of sampling	To reduce the lysis of biomass
	Store the sample in the meantime at 4°C	To reduce the activity and the lysis of the biomass

Total Phosphate in Aerobic Granular Sludge Protocol

Name: _____
Date: _____
Place: _____

Sampling

Reactor sample:

- Take ±1h after the start of the aeration phase (maximal O_2 set-point), two samples of ±1 L. *This can be done with a bucket, rocket or equivalent equipment.*
- Put each samples in a ±1 L container
- Label the containers (name, date, place, time, reactor number) (container #**1** and #**2**)
- Write down time of sampling, reactor number and volume in table 1
- Store container cool (2-4°C) until dry weight and total phosphate measurement

Excess sludge sample:

- Take a mixed liquor sludge sample during decant.

  This can be done by tapping a fraction of the excess sludge stream during the whole decant period or with time steps (±1 L every 30s for the whole decant period and mix all samples together)
- Shake or stir the >12L obtained mixed liquor sludge sample
- Take ±1 L mixed sample from the mixed liquor and put in a container (#**3**)
- Take another 100-500ml mixed sample from the mixed liquor and put in a container (#**4**)
- Label both containers (name, date, place, time, reactor number)
- Write down time of sampling, reactor number and volume in table 1.
- Store container cool (2-4°C) until dry weight and total phosphate measurement

Dry Weight

Preparation:

- Take two aluminium cups and mark them with balpen

  only engraved letters remain after high temperatures
- Put two coffee filters in the oven at 105°C for 30 minutes
- Put the aluminium cups in the oven at 550°C for 30 minutes
- Take the filters and cups out of the oven and let them cool down for 5-10 s
- Weigh the filters and cups on a micro balance
- Put the filters on a (vacuum) filter unit
- Filter the content of container #**1** and #**3** over their respective filters
- Put the filter with sample in their respective aluminium cups
- Weigh the filter + sample + aluminium cups on a micro balance (WW #1 and #3) (table 2)

Drying and Burning:

- Put the filter + sample + cups in the oven at 105°C for 24 hours
- Take the filter + sample + cups out of the oven and weight them on a microbalance (DW #1 and #3) (table 2)
- Scrape some of the dry weight of the filters and put them in the same aluminium cup back
- Label the filter with pen (name, date, sample type, time) and store in a closed plastic bag
- Weigh the scraped dry weight and aluminium cup (DWA #1 and #3) (table 2)
- Put the scraped sample + cups in the oven at 550°C for 2 hours
- Take the sample + cups out of the oven and weight them on a microbalance (VSS #1 and #3) (table 2)

Total Phosphate

Preparation:

- Label two measuring jars (1L) with 'reactor sample' and the other with 'excess sludge'
- Empty containers **#2** and **#4** in their respective measuring jar
- Put the measuring jars on a stirring device and mix the sample
- Blend or crush both samples

Dilution:

- Label two plastic tubes (50ml) with 'reactor sample' and the other with 'excess sludge'
- Put one plastic tube on the balance and tare the balance to zero
- Choose an appropriate dilution (for Dinxperlo: reactor 25x and excess sludge 8x)
- Fill amount of sample into the plastic tube by aiming for the weight displayed below
- Note the measured weight [g] of sample in table 3
- Fill with water (1:milliQ, 2:demi, or 3:tapwater) by aiming for the weight displayed below
- Note the total weight of sample + water [g] in table 3
- Repeat **dilution** for plastic tube 'excess sludge'

Hach Lange test:

- Use sample from the diluted samples in the plastic tubes 'reactor sample' and 'excess sludge'
- Perform the Hach Lange test according to the protocol display on the box. Preheat the heating block for about 30 minutes. When new heating block is used, the pre-heating time is included in the program (so no pre-heating is possible).
- Multiply the TP concentration with the chosen dilution and note in table 3

Total Phosphate in Aerobic Granular Sludge Protocol

Name: _____ Date:_____ Place: _____

Table 1 Sampling

	Reactor Sample Container #1	Container #2	Excess Sludge Sample Container #3	Container #4
Location				
Time				
Reactor #				
Volume				

Table 2 Drying and Burning

	Reactor Sample Container #1	Excess Sludge Sample Container #3
Weight Filter (g)		
Weight cup (g)		
WW (g)		
DW (g)		
DWA (g)		
VSS (g)		

Table 3 Total Phosphate

	Dilution	Reactor Sample Container #2 Aiming for:	Measured:	Excess Sludge Sample Container #3 Aiming for:	Measured:
Weight Sample (g)	8	2		2	
Weight total (g)		16		16	
Weight Sample (g)	25	2		2	
Weight total (g)		50		50	
Weight Sample (g)	50	2.00		2.00	
Weight total (g)		100.00		98.00	
Weight Sample (g)	100	1.00		1.00	
Weight total (g)		100.00		99.00	
Total Phosphate (mg/L)					
TP multiplied with dilution (mg/L)					