A mechanistic model for anaerobic phototrophs in domestic wastewater applications: Photo-Anaerobic Model (PAnM)

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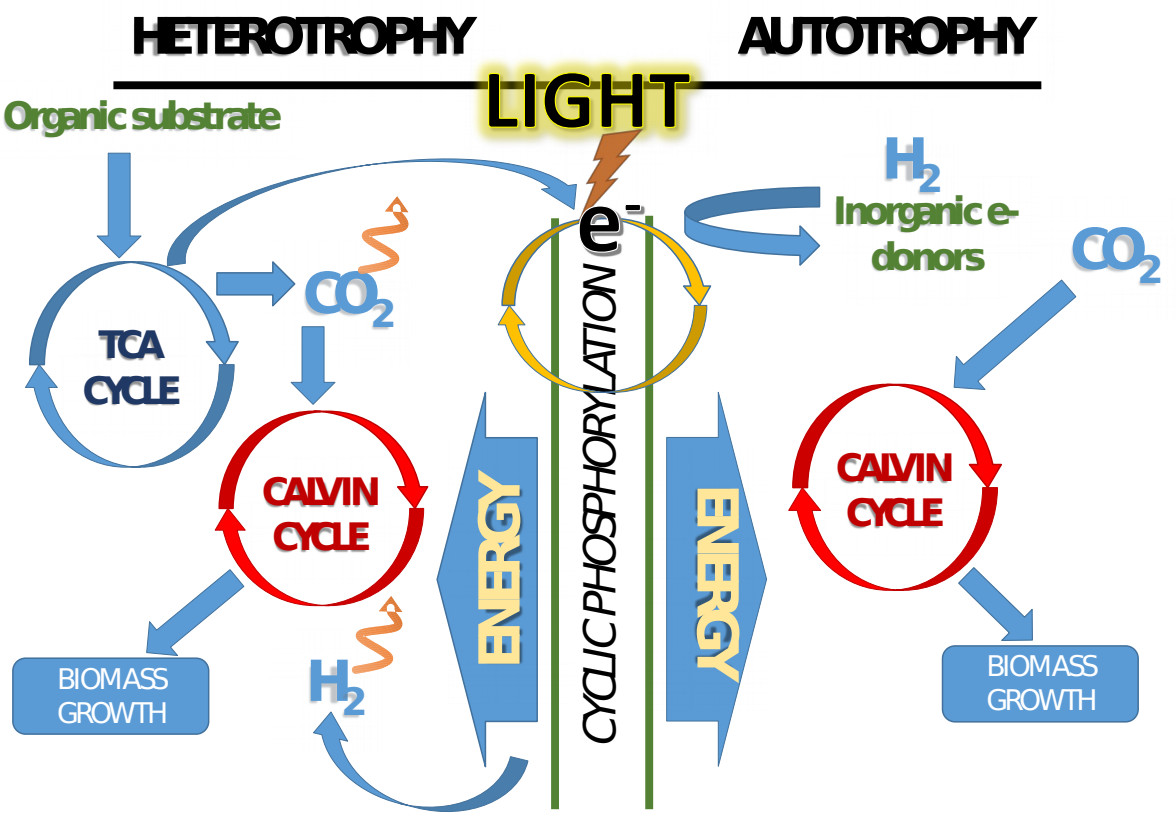
ABSTRACT

Purple phototrophic bacteria (PPB) have been recently proposed as a key potential mechanism for accumulative biotechnologies for wastewater treatment with total nutrient recovery, low greenhouse gas emissions, and a neutral to positive energy balance. Purple phototrophic bacteria have a complex metabolism which can be regulated for process control and optimization. Since microbial processes governing PPB metabolism differ from traditional processes

used for wastewater treatment (e.g., aerobic and anaerobic functional groups in ASM and ADM1), a model basis has to be developed to be used as a framework for further detailed modelling under specific situations. This work presents a mixed population phototrophic model for domestic wastewater treatment in anaerobic conditions. The model includes photoheterotrophy, which is divided into acetate consumption and other organics consumption, chemoheterotrophy (including simplified fermentation and anaerobic oxidation) and photoautotrophy (using hydrogen as an electron donor), as microbial processes, as well as hydrolysis and biomass decay as biochemical processes, and is single-biomass based. The main processes have been evaluated through targeted batch experiments, and the key kinetic and stoichiometric parameters have been determined. The process was assessed by analysing a continuous reactor simulation scenario within a long-term wastewater treatment system in a photo-anaerobic membrane bioreactor.

Key words: *Phototrophic bacteria, resource recovery, mechanistic modelling, Partition-Release-Recovery*

Graphical Abstract



HIGHLIGHTS

- A mechanistic model for anaerobic phototrophs has been developed: PAnM
- The model includes organic C and H_2 (as COD) and inorganic C, N and P.
- Microbial processes based on PPB metabolism were identified through dedicated experiments.
- Kinetic and stoichiometric parameters were determined in batch tests.
- Model was tested by simulating the process in a photo-anaerobic MBR

1 INTRODUCTION

Wastewater treatment is shifting focus to include the capture and recovery of organics and nutrients. This requires novel technological approaches. A key approach is the use of fast growing organisms to concentrate energy, nutrients, and trace compounds into the solid phase, and hence substantially reduce reactive removal of nitrogen and organics while enabling phosphorous recovery. One option is high-rate activated sludge, which can achieve 40% nitrogen removal in the primary stage through adsorption and assimilation (Jetten et al. 1997). Algae can also be used to partition to the solid phase, but a simultaneous

heterotrophic and photosynthetic mode is generally enabled by bacterial-algal associations that reduce organic substrate consumption efficiency (Muñoz and Guieysse 2006). Purple phototrophic bacteria (PPB) present a new partitioning approach, which has been shown to completely remove nitrogen to discharge limits when sufficient organic carbon is present without the need for pure cultures, and using infra-red (IR)light only as a driver for growth (Hulsen et al. 2014).

PPB grow phototrophically rather than photosynthetically, and do not use water as an electron donor to produce oxygen and organics. They are among the most metabolically versatile organisms on earth (Hunter et al. 2008). They grow heterotrophically using a wide range of organic compounds, both in presence and absence of light (photoheterotrophy and chemoheterotrophy) (Hunter et al. 2008). However, they can also grow autotrophically by using infrared light as the energy driver for CO_2 fixation, and with inorganic electron donors such as H_2, Fe^{2+}, S^{2-} or $S_2O_3^{2-}$ (cyclic anoxygenic photosynthesis) (Overmann and Garcia-Pichel 1998). Although they can grow in the presence of oxygen, they are extremely effective in anaerobic photoheterotrophic conditions (Gordon and McKinlay 2014, McKinlay and Harwood 2010). Their ability to recycle electrons during the cyclic anoxygenic photosynthesis gives them the ability to harvest and retain electrons, as well as a high energetic efficiency. They can even accumulate electrons in the form of reduced cofactors which enable the disposal of electrons. This can be done through two main strategies: (i) ATP-driven hydrogen production by ferredoxin oxidation in the hydrogenase/nitrogenase system at the end of the electron transport chain (ETC), and (ii) the increase of assimilative growth by re-fixation of CO_2 via the Calvin Cycle produced during heterotrophic metabolism (McKinlay and Harwood 2010). These metabolic features give them the possibility of growing and out-competing other heterotrophic microorganisms where light is present, including in low to medium strength wastewater systems with short hydraulic retention times (HRT) (Hulsen et al. 2014).

PPB have a number of additional metabolic functions useful in wastewater treatment systems. They are able to accumulate polymers such as poly-phosphate (poly-P) (Liang et al. 2010), polysaccharides (Klein et al. 1991), poly-β hydroxybutyrate (PHB) (Melnicki et al. 2009) and other poly-3(hydroxyalkanoates) (PHA) (Brandl et al. 1991). Under an excess of organics

and available energy, and in the absence of mineral nitrogen, they generate hydrogen and fix nitrogen as ammonia (Basak and Das 2007).

PPB have been assessed for wastewater treatment, particularly for processing swine (Kim et al. 2004), latex rubber-sheet (Kantachote et al. 2005), tofu (Zhu et al. 1999), and sugar refinery wastewaters (Yetis et al. 2000). However, most of these studies were focused on hydrogen production rather than organics removal or nutrient recovery (Fang et al. 2005, Lee et al. 2010, Tao et al. 2008). They have also been applied to domestic wastewater (DWW) in batch and continuous operation to completely remove nitrogen to discharge limits (Hulsen et al. 2014). This process enables a single-step treatment of wastewater with HRT and effluent qualities similar to those of activated sludge processes without destroying nitrogen and phosphorus.

Modelling is used to design, benchmark, and analyse wastewater treatment systems, with the IWA Activate Sludge Model (ASM) family models being the most widely used for conventional activated sludge processes (Henze et al. 2006). The IWA anaerobic digestion model no. 1 (ADM-1) is the analogous model for domestic and industrial anaerobic systems (Batstone et al. 2002). The IWA Models, and wastewater modelling in general has generally applied first order hydrolysis for solids transformation (including decay), Monod for uptake kinetics and inverse Monod (non-competitive) for inhibition functions, with a COD basis for organics and molar basis for inorganic compounds. Development of new technologies such as PPB requires development of a similar mechanistic model.

There are complex metabolic models based on PPB metabolism primarily focused on the electron transport chain (Golomysova et al. 2010, Klamt et al. 2002). Due to their complexity, these models are motivated more by a need for a mechanistic understanding of the underlying process rather than field applications. These models are therefore unsuitable for a wastewater model.In particular, they include components which can’t be measured readily, making validation difficult. They also lack capability outside the core application area. There has also been work done on modelling PPB to describe hydrogen production (Eroglu 2008, Gadhamshetty et al. 2008, Obeid et al. 2009),. In contrast, due to the domestic wastewater matrix, the key growth modes are photoheterotrophy (principal) as well as chemoheterotrophy and photoautotrophy. . Biochemical processes relevant to complex substrates such as solids hydrolysis and biomass decay must be considered as well. Therefore,

this work aims to propose a mechanistic model for mixed culture PPB as a partition agent in DWW treatment with adaptability to treatment of industrial wastewaters.

2 Materials and Methods

2.1 Model Description

The model was developed to be unit-compatible with the IWA ASM and ADM series (Batstone et al. 2002, Henze et al. 2006). Therefore, units of mgCOD L^{-1} (or gCOD m^{-3}) for both soluble and particulate organics were chosen. Nutrient units are in mgN L^{-1} and mgP L^{-1}, respectively, with inorganic carbon (IC, HCO_3^-) in molC L^{-1}.

Monod kinetics is uniformly applied for biological growth processes, with first order kinetics for hydrolysis and decay. Monod or non-competitive inhibition has been applied for limiting or inhibitory expressions respectively. Due to a lack of functional differentiation within the PPB clade, and limited evidence to the contrary, only one biomass component has been selected (PPB). Other biological groups present in ASM and ADM1 models (e.g., hydrogen utilising methanogens, denitrifiers or fermentative bacteria) could be readily included. As in the ASM/ADM models S_i is used for soluble compounds, and X_i for particulate compounds, where subscript i denotes the compound.

The model does not currently include poly-P or other polymer accumulation, since this occurs mainly in static growth mode (Hiraishi et al. 1991, Liang et al. 2010) complexity. Likewise, nitrification/denitrification processes are not included, since they can only occur in aerobic conditions where ammonia can be oxidized to nitrite or nitrate. Therefore, N and P are removed by assimilative growth only.

In the presence of organic substrates and IR light, photoheterotrophy through the tri-carboxylic acid (TCA) cycle is assumed to dominate. Two major mechanisms of electron disposal by PPB are considered. Firstly, the production of CO_2 (S_{IC}) is a key feature of PPB biomass under growth conditions (McKinlay and Harwood 2010) and is important for closing the C balance. The oxidation state of the organic compound determines if the biomass fixes CO_2 for substrate uptake and electron balance (in the case of reduced substrates such as propionate, butyrate or valerate), or the uptake produces CO_2 (in the case of oxidized

substrates such as acetate, succinate or ethanol) (McKinlay and Harwood 2011). In the latter case, the biomass disposes of excess of electrons by re-fixing the CO_2 produced in the TCA cycle. As a consequence, there is usually limited consumption or production of CO_2 in domestic wastewater. A theoretical explanation of this mechanism is explained in Supplementary Information (SI). The other major mechanism of electron disposal by PPB is H_2 production via the nitrogenase complex. In static growth mode, the PPB biomass is able to use the excess of electrons for redox balance at the end of the ETC. The ferredoxin complex is the carrier for this process, but the biomass needs energy in the form of ATP (Golomysova et al. 2010). However, this process is inhibited in presence of NH_4^+, a strong inhibitor of the nitrogenase activity (Rodionov et al. 1986). Indeed, H_2 production is inhibited in a DWW fed situation due to (i) presence of ammonium and (ii) disposing of electrons by CO_2 re-fixation which promotes growth (see SI for more details). Therefore, it can be deduced that CO_2 production and re-fixation into the Calvin Cycle is the major electron sink in PPB metabolism. In the absence of organic substrates, autotrophic growth is the sole growth mode, using reduced inorganic compounds other than water as electron donor (anoxygenic photosynthesis). In the interest of model simplification and considering domestic wastewater contains generally low sulfur levels, the sulfur cycle has been omitted. It is however possible to add sulfate reduction into the model with subsequent sulfide utilisation as an electron donor for autotrophic PPB growth. This would require the addition of another biomass component (PPB cannot perform sulfate reduction). PPB can perform chemoheterotrophy at a lower rate, providing H_2 (S_{h2}) for photoautotrophy (Golomysova et al. 2010).

Transforming these mechanisms to a model enables the following key processes (Figure 1):

(i) *Photoheterotrophy on acetate (S_{ac}) (acetate uptake):* This involves acetate assimilation by PPB in the presence of infra-red radiation Acetate is treated separately from the other substrates due to differences observed during batch tests. Due to an imbalance in substrate-biomass carbon oxidation state, this process also results in production of CO_2.

(ii) *Photoheterotrophy on other organics (S_s) (photoheterotrophic uptake):* These include all soluble organics that PPB can assimilate for growth in the presence of infra-red radiation. Compounds include VFAs excluding

acetate, alcohols, and some sugars. These have been lumped into a single soluble substrate. Similar to (i) this results in the uptake of CO_2.

(iii) *Chemoheterotrophy (chemoheterotrophic uptake):* This process involves the assimilative consumption of any organic in dark conditions that can be metabolized through either fermentation or anaerobic oxidation processes. All these processes have been joined as one process for a shake of simplicity. This process involves H_2 and acetate as end products. Acetate is not further oxidised through chemoheterotrophy due to a lack or very limited terminal electron acceptors such as Fe(III) and sulfate (Finneran et al. 2003).

(iv) *Photoautotrophy (autotrophic uptake):* This process involves assimilative CO_2 fixation by PPB in the presence of infra-red radiation using H_2 as the electron donor. Other electron donors such as Fe^{2+}, S^{2-} and $S_2O_3^-$ have been omitted but could be included.

(v) *PPB cell death (decay)*: This process involves the deactivation of PPB by cell death. Ammonium, phosphate and inorganic carbon are released and the biomass is converted into biodegradable organic particulates (X_S) and particulate inerts (X_I).

(vi) *Hydrolysis and particulate fermentation (hydrolysis):*. The decomposition of biodegradable particulates into organics (S_{ac} and S_S), ammonium, phosphate, hydrogen and inorganic carbon is addressed as a sole process for simplicity. Both soluble and particulate inerts are also products of this process. A breakdown of particulate fermentation could be incorporated into the model *e.g.* for processes with long solids retention times (SRT).

The model is presented in Petersen matrix notation in Table 1. Kinetic parameters were generally obtained from the batch experiments, or from the literature in specific cases as described below. The saturation constant for hydrogen consumption by photoautotrophic process ($K_{S,h2}$) light limitation ($K_{S,E}$) and inhibition by ammonia ($K_{I,FA}$) were set arbitrarily low since affinity is high (Chen et al. 2008, Uyar et al. 2007). Stoichiometry was determined by both theoretical calculations from literature, and experimentally. The model is balanced over COD, C, N and P. HCO_3^-, NH_4^+ and PO_4^{2-} have been used for closing C, N and P balances, respectively.

Extra information regarding all aspects of model development and implementation can be found in the supplementary information (SI) and codes can be found on the UQ repository (LINK). SI1 includes the description of model components, full kinetic parameters and stoichiometric coefficients. The determination and calibration of stoichiometry is included in SI2, and SI4 contains the full list of model equations.

2.2 Batch Experiments

Batch experiments were done to identify parameters based on the developed model. Detailed experimental methods are provided in the SI.. The inoculum was sourced from a lab-scale continuous photo-anaerobic membrane bioreactor (PAnMBR) described by (Hülsen et al. 2016b) operated over 300 d. Domestic wastewater was collected from the Taringa wastewater lift station (Brisbane, Australia) with an average strength of 572 mgCOD L^{-1} and soluble COD of 241 mgCOD L^{-1}, 63 mgN L^{-1}, and 9 mgP L^{-1}.

Where wastewater was not the medium, synthetic Ormerod medium was used at pH 7.5 as described previously (Hulsen et al. 2014).

Metabolic growth batch tests: All batch tests were done in 100mL working volumes (160 mL serum flasks) in triplicate, inoculated from the PAnMBR reactor. The headspace was flushed with N_2 and experiments were carried out at 20℃ in an orbital shatter at 150 rpm (Edwards Instrument Company). The array of flasks was irradiated with 150W lamps using UV-VIS absorbing foil as described elsewhere (Hulsen et al. 2014). All experiments were accompanied blank samples with no substrate, and by positive and negative controls where necessary. A summary is provided in Table 2.

Hydrolysis and biomass decay: The inoculum (0.5 L) was collected as per the above method(2.1 g VSS L^{-1}). The biomass was centrifuged in 50 mL Falcon tubes and the pellet resuspended again in NaCl 0.2 M three times. Biomass was then placed in 0.5 L of NaCl 0.2 M and was divided into two 0.25 L Schott bottles, which were subsequently flushed with N_2 and magnetically stirred at 200 rpm. The bottles were operated for 30 d.

One of the bottles was covered with aluminium foil to avoid phototrophic activity, and was used for the hydrolysis analysis. Liquid sampling was performed twice a week to analyse volatile fatty acids (VFAs), NH_4-N, PO_4-P, total inorganic carbon

(TIC) and pH. Headspace was analysed for CH_4, H_2 and CO_2. TSS/VSS, TKN and TP was analysed every 7 d.

The other bottle was illuminated as indicated above without feed, and biomass samples were taken every 7 d to assess activity (determining decay coefficient). Activity tests were done as above with 100 mgCOD L^{-1} of acetate and 10 mg NH_4-N L^{-1}.

Calculation of Specific Phototrophic Activities (SPA). Non-linear parameter estimation is generally used to determine parameters as described in 2.4.2, but specific phototrophic activity was also determined by linear regression of substrate concentration over a minimum of four points through the region of maximum consumption divided by biomass concentration.

2.3 Analytical methods

Total COD (TCOD) and soluble COD (SCOD) were determined by COD cell tests (Merck, 1.14541.0001, Darmstadt, Germany). Dissolved NH_4 -N, NO_2-N and PO_4-P were determined by a QuikChem8000 Flow Injection Analyzer (FIA) (Hach Company, Loveland, USA). Temperature and pH were measured using an Oakton pH 11 Series (Vernon Hill, IL, USA). TSS and VSS were determined by filtration according to standard methods, where TSS were calculated after drying the sample in an oven at 105 ± 2 °C and VSS were calculated after burning it in a furnace at 550 ± 5 °C (APHA. 1998). Illuminance (W m^{-2}) was measured with an IR light sensor (PAS Port™, Roseville, CA, USA). VFA samples were analysed by gas chromatography (Agilent Technologies 7890A GC System, Santa Clara, CA, USA) equipped with a flame ionisation detector (GC/FID) and a polar capillary column (DB-FFAP). Gas samples were analysed by GC (2014 Shimadzu, Kyoto, Japan) with thermal coupled detector (TCD) (Tait et al. 2009). TKN and TP were determined using sulfuric acid, potassium sulfate and copper sulfate catalyst in a block digestor (Lachat BD-46, Hach Company, Loveland, CO, USA) (Patton and Truitt 1992). TIC was analysed by using a total organic carbon (TOC) analyser (Shimadzu TOC-L CSH TOC Analyser with TNM-L TN unit) coupled to a near infrared detector (NIRD) for measuring the CO_2. All soluble constituents were determined after filtering with a 0.45 µm membrane filter (Millipore, Millex®-HP, Merck Group, Darmstadt, Germany).

2.4 Data analysis

2.4.1 Data handling

Biomass concentration was calculated in g VSS L^{-1}, and it was further transformed into COD by using the COD relationship calculated from the biomass equation $CH_{1.8}O_{0.38}N_{0.18}$ (McKinlay and Harwood 2010) (1 g biomass expressed as VSS = 1.78 g COD).

Biomass yields (*Y*) were calculated accounting for the initial and final biomass concentration (in g VSS L^{-1}) based on substrate consumption. Biomass concentration was further transformed into COD and then yields are expressed as $mgCOD_{biomass}$ $mgCOD^{-1}$.

2.4.2 Statistical analyses

All parameters were estimated from triplicate batch/measurements by minimisation of residual sum of squares (J=RSS). Parameter uncertainty was determined using two-tailed *t*-tests calculated from standard error in parameter value, obtained from the Fisher information matrix. Where parameter optimisation problems involve multiple parameters (k_M, K_S), parameter uncertainty surface ($J=J_{crit}$) has also been assessed as described in (Batstone et al. 2003) . Confidence intervals (at 95%) were also calculated based on two-tailed *t*-tests from parameter standard error, as above, and used for statistical representative comparisons. Error bars in experimental data represent 95% confidence intervals in mean based on a two-tailed *t*-test (5% significance threshold). Uncertainty of the slope for the analysis of SPA was analysed in Excel using Data Analysis. Standard error in slope was subsequently converted into 95% confidence interval. All statistical analyses were done with a 5% significance threshold.

2.5 Simulation of a continuous PAnMBR

The resulting kinetic expressions were used in the development of a continuous PAnMBR model. As previously demonstrated, the concentration of the bioavailable SCOD in medium strength domestic wastewater is insufficient for the system to achieve total nitrogen and total phosphorous discharge limits (Hülsen et al. 2016b). To achieve full removal, additional SCOD is required.

The goals of the simulation were the following: a) to highlight the requirement of additional SCOD to achieve total nutrient removal, and b) to demonstrate that the inclusion of a primary clarifier before can lead to a product enriched in PPB.

Dynamic influent data was simulated according to the influent generator model developed by Gernaey et al. (2011), and adapted to the typical concentrations of primary influent reported by Hülsen et al., 2014. Based on the average influent characteristics and an HRT of 12 h, volumetric loading rate (VLR) of 1400 ± 12 mg COD L^{-1} d^{-1} and a solid retention time (SRT) of 3 d, a reactor volume of 670 m^3 was applied. An ideal primary clarifier was included, with a solids removal efficiency of 60%±3% Tchobanoglous et al. (2003).

Simulation and subsequent data processing were done in Matlab (MATLAB R2015a, The MathWorks Inc., Natick, MA). As the system of equations is stiff, the system of ordinary differential equations was solved by ODE15s. The case was simulated for 600 days with 3 stages of differing SCOD concentrations. The dynamic influent after settling was applied directly during Stage I until day 300. During Stage II (days 300-450), acetate was added to the optimum COD/N/P ratio of 100/7.1/1.8 based on the limiting nutrient (N or P). During Stage III, acetate addition was ceased. This was to assess process response to a sudden change, and to demonstrate that the sytstem requires wastewater with a specific COD/N/P ratio. . State equations were implemented in a fixed volume, completely mixed membrane bioreactor.

The results from the simulation were balanced over COD, N, P and C, and have been included in the SI.

The Matlab function and run files, along with their supporting datasets, have been uploaded to http://espace.library.uq.edu.au/view/UQ:412280.

3 Results

The sludge used for all the experiments came from a lab-scale PAnMBR (Hülsen et al. 2016b). Most of the microorganisms are related with α-*proteobacteria,* PPB accounting for more than 70% of the total gene copies detected by the pyrosequencing technique. The genus *Rhodobacter* ssp. is the most abundant, representing more than 60% of the microbiota (Hülsen et al. 2016b). The presence of photosynthetic organisms such as microalgae and cyanobacteria accounts for less than 1% of total gene copies. Therefore, the biomass can be considered as PPB-dominant biomass.

3723.1 Growth Processes

373Photoheterotrophy was assessed with VFAs and ethanol as substrate (Fig 2a). All 374substrates were completely consumed during the experiment, and overall yields 375were similar in all cases, with an average biomass yield of 1.13 ± 0.21 mg 376$COD_{biomass}$ mg^{-1} COD. More details are provided in the SI. As can be seen in Figure 3772b, uptake rates of substratesexcluding acetate were similar, with a k_M of 1.3 ± 3780.1 (mgCOD $mgCOD^{-1}$ d^{-1}), and undetectable K_S.Acetate had a significantly higher 379k_M (2.4 ± 0.2 mgCOD $mgCOD^{-1}d^{-1}$) and detectable, albeit low, K_S of 20± 4 mgCOD 380L^{-1}. This essentially means that growth (uptake) is faster on acetate, but with a 381lower affinity such that acetate uptake is faster at the beginning of the batch, 382but slower at the end.

383The analysisof chemoheterotrophic metabolism by PPB was conducted by using 384acetate and ethanol as substrates in dark conditions (Figure 2c). PPB biomass 385was much less effective in dark conditions compared with light conditions 386(biomass yield 0.5 vs 1.1 mg $COD_{biomass}$ mg^{-1} COD in dark and light conditions, 387respectively). Biomass yield in dark conditions is relatively high compared to 388typical values reported in literature for dark fermentation and anaerobic 389oxidation processes, which are rarely greater than 0.2 mg $COD_{biomass}$ mg^{-1} COD 390(Batstone et al. 2002). The occurrence of energy storage (particularly poly-P) 391may have a significant role here due to batch operation (Liang et al. 2010). The 392maximum uptake rate, is approximately half that of photoheterotrophy (Figure 3932d), though with again, extremely low K_S values. While chemotrophic growth is 394not dominant under photoheterotrophic conditions, it can be very important to 395consider in reactor design (e.g., where there is insufficient light), and also for 396balancing COD, C, N and P.

397Analysis of photoautotrophy was done with $NaHCO_3$ as C source and Na_2S as 398electron donor in 5-fold stoichiometric excess (see Table 2) (Figure 2e). The 399biomass had a yield of 36,000 mg $COD_{biomass}$ mol^{-1} C comparable to the value on 400acetate (31,560 $mgCOD_{biomass}$ mol^{-1} C). However, maximum uptake rate was far 401lower at $3.4\pm0.2\times10^{-6}$ molC mgCOD d^{-1} (compared to $75\pm 2\times10^{-6}$ molC mgCOD 402d^{-1} on acetate) (Figure 2f). Photoautotrophy needs to be considered for when 403there is an excess of bicarbonate and electrons from inorganic sources in the 404wastewater. It is also important to consider photoautotrophy in order to close 405mass balances. This case is particularly relevant in light deficiency, where

fermentation and anaerobic oxidation processes may become important and hence H_2 is available as a major electron source for PPB.

Nutrient limitation experiments for N and P were used to determine saturation coefficients for N and P. K_S values were extremely low such that the N and P regulation became a switch function (data shown in SI). Biomass assimilated nutrients at a COD/N/P ratio of 100/7.1/1.8, which is higher than conventional aerobic bacteria and much higher than other anaerobes (Tchobanoglous et al. 2003). These values are in line with previous works (Hulsen et al. 2014). However, PPB were able to grow at a lower rate once the nutrients were completely consumed (42% lower than in full nutrient conditions), likely due to fixation of headspace N_2 (Hunter et al. 2008) (inhibited in the presence of ammonium). Also, PPB can accumulate polymers such as poly-P (Liang et al. 2010) as well as PHA (Melnicki et al. 2009), which can be used in static growth mode. Since the model developed here is sustained on biomass growth in presence of nitrogenase inhibiting ammonium , nutrient limitation for growth must be included.

3.2 Endogenous processes – hydrolysis and decay

Hydrolysis and decay are considered as transversal first order biochemical processes in most models (Batstone et al. 2006, Henze et al. 2006, Szilveszter et al. 2010). These could be considered separately, since phototrophic growth can be restricted in the absence of irradiance, and decay can be determined directly by measurement of phototrophic activity following periods of irradiation without substrate. Figure 3 shows the time series of the SPA values (on acetate) calculated for the PPB biomass during starvation. Biomass activity reduced according to a first order model with decay coefficient of 0.09 ± 0.02 d^{-1}. Hydrolysis was assessed in dark conditions with substrate present, to avoid reassimilation of products by PPB. Therefore, hydrolysis products (organic C sources as COD, inorganic C as HCO_3^-, N as NH_4^+ and P as PO_4^{3-}) could be measured and were directly correlated with first order kinetics of the hydrolytic process. Hydrolysis also followed a first order model with a hydrolysis coefficient of 0.071 ± 0.002 d^{-1} (Fig 4). It should be noted that hydrolysis is substrate specific, and is highly situation specific (Batstone et al. 2015), but that a value of close to 0.1 d^{-1} is comparable with hydrolysis kinetics under anaerobic conditions, but much lower than that for aerobic processes (Henze et al., 2006).

4 Discussion

4.1 Parameter values vs pure culture PPB

A full list of parameter values can be found in the SI, whereas Table 3 shows parameters determined from the literature in comparison with those reported here. Parameters were calculated on the basis that (i) protein composition of PPB is in all cases 60% of dry weight (McKinlay and Harwood 2010), (ii) 1 g VSS = 1.78 g COD and (iii) PPB biomass equation is $CH_{1.8}O_{0.38}N_{0.18}$ (McKinlay and Harwood 2010).

In general, biomass yields calculated here are in line with values reported in the literature (Table 3). The only exception is the biomass yield for autotrophic growth, where no relevant values have been found and only indirect calculation can be performed. Wang et al. (1993) reported biomass growth and CO_2 fixation in *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* using different electron sources (H_2, thiosulfate, sulphide and malate) and the biomass yield values extracted from their activities vary considerably with an average value of 84,000 mg COD mol^{-1} C fixed. These values, however, did not consider re-fixation of CO_2 from malate that may underestimate considerably real CO_2 usage for growth in the Calvin cycle (McKinlay and Harwood 2011).Therefore the biomass yield differs from the value reported here (36,100 ± 850 mg COD mol^{-1} C fixed). The value determined during this study is however very close to the theoretical maximum yield for carbon dioxide fixation of 39,840 mg COD mol^{-1} C, and as such, is a reasonable value.

However, specific uptake rates were substantially different to the literature values depending on the growth mechanism, which may be due to use of pure cultures in contrast with mixed cultures used in the present work. Generally, chemoheterotrophic parameters, pure cultures have an activity close to two orders of magnitude higher than the mixed culture in this work. This results in activities simular to those of typical fermentative bacteria. An example is found in (Schultz and Weaver 1982) where the growth rates of *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata* were studied on several chemoheterotrophic substrates in the dark. The authors used trimethylamine-N-oxide as accessory electron acceptor on fructose, glucose and succinate, likely removing electron management as a major limitation. Photoheterotrophic parameters also divergeddepending on the substrate. While acetate uptake rates were similar to the values reported here (Golomysova et al. 2010, McKinlay and Harwood 2011),

those obtained from other organics, such as malate (Gadhamshetty et al. 2008, Klein et al. 1991), lactate + malate (Obeid et al. 2009), or butyrate (McKinlay and Harwood 2011) were almost one order of magnitude higher. These parameters were obtained in hydrogen production studies. Under these situations, the substrate uptake is optimized for biogenic H_2 by dislocating catabolism from anabolism due to excess of electrons. This increases considerably the substrate uptake rate while minimising yield (Basak and Das 2007). In this work, the μ_{max} for photoheterotrophic metabolism was calculated to be 1.54 d^{-1}, which corresponds to a doubling time of 0.45 d. It is similar to those reported by McKinlay and Harwood (2011) (0.27-0.44 d), and generally aligns well with purple phototrophic bacteria (Hunter et al. 2008). The use of pure cultures promotes specific uptake rates to the detriment of substrate affinity. This leads to increased k_M and K_S parameters, a typical behaviour of r-strategist microorganisms (Dorodnikov et al. 2009).

Hydrolysis and decay rates dependent the particulate substrate hydrolysing, and the system redox conditions. In general, for a given material, the hydrolysis coefficient increases from anaerobic to anoxic, and from anoxic to aerobic (Henze et al. 2006). The biomass decay and hydrolysis constants found in literature were obtained in aerobic photoheterotrophic processes (Huang et al. 1999, Huang et al. 2001),.This explains considerably higher values than those calculated here.

Compared with previous analyses, this study is focused on mixed culture photoheterotrophic metabolism. The biomass seems to be a K-strategist which promotes substrate affinity over uptake, a microbial strategy in low-strength systems as domestic wastewater with low hydraulic retention times (less than 12 h). Such behaviour is useful for out-competing other fast-growing microorganisms. It is clearly effective when compared to the slow growing methanogens, which are the only competitors for acetate under anaerobic conditions with low concentrations of sulfate or reduced metals (Dorodnikov et al. 2009). Indeed, PPB microorganisms have been demonstrated to prevail and dominate in continuous PAnMBR reactors treating real domestic wastewater without previous inoculation, both in mesophilic (Hülsen et al. 2016b) as well as in psychrophilic (Hülsen et al. 2016a) conditions.

4.2 Model application

The model was tested in a realistic scenario, with influent profile generated using the BSM influent generator (Gernaey et al. 2011). Detailed information about the simulations is provided in the SI.

4.2.1 Fate of C, N and P

The model indicates different SCOD removal efficiencies for particular periods of operation. In general, adaptation to seasonal periods of variable wastewater composition is rapid, as can be shown in input values from Figures 5 and 6. For periods (I) and (III), which correspond to no additional acetate in the system (average inlet SCOD of 290.2 ± 0.5 mg COD L^{-1}), the mean SCOD removal efficiency is 87% (Figure 5a) The remaining SCOD in the system can be almost entirely attributed to the presence of non-biodegradable SCOD, accounting for 97% of the effluent SCOD. During period (II) acetate was added to agree with the COD/N/P requirements for PPB. Average SCOD removal efficiency remained almost invariable at around 87% due to optimized COD/N/P conditions. As in the Stage I, almost all the remaining SCOD corresponded to soluble inerts. The model, however, is not able to reproduce the PPB behaviour under a high excess of inlet SCOD concentration since it is based on assimilative mechanisms only and accumulation processes are not included, as e.g. PHA or glycogen. The PPB biomass is able to accumulate these compounds (Brandl et al. 1991, Melnicki et al. 2009), and so SCOD removal efficiencies are expected to be higher and less dependent on nutrients in real cases (Hülsen et al. 2016a, Hülsen et al. 2016b). An upgraded model including accumulative mechanisms is therefore needed for high COD:N ratio wastewater. However, this model is suitable for normal DWW treatment operation, where N and P are generally in excess.

Nutrient assimilation was directly linked with biomass growth. The optimum assimilative COD/N/P relationship has been calculated to be 100/7.1/1.8 from batch experiments. Therefore, periods with non-optimal ratios are expected to have higher effluent nutrient concentrations. Under normal situation (periods (I) and (III)), with no additional acetate, nutrients were not completely removed and ammonium and phosphate efficiencies were 47% and 59%, respectively (Figures 5b and 5c, respectively). This justifies the need for extra SCOD addition, as has been previously described experimentally (Hülsen et al. 2016b). Phosphorus was almost completely removed during C and N sufficiency during period (II), with removal efficiencies of 93%. However, depletion of P prevented a high N

removal due to nutrient imbalance, and so N removal efficiencies during these period averaged 74%. Again, accumulative mechanisms may have a key role here, as PPB are able to accumulate poly-P (Liang et al. 2010). This mechanism is quite complex and has not been properly defined, particularly in mixed cultures and on wastewater sources.

Production of biomass was related to PPB growth as well as input solids. Biomass fractionation (X_{PB}, X_S and X_I) along the simulation period is depicted in Figure 6. When acetate was not added, PPB biomass was produced at 44% of the total biomass in the outlet (sludge line). Adding acetate increased this value up to 53% of total biomass. Accumulation of X_S within the reactor is a direct consequence of slow hydrolysis due to low HRT. Additional substrate increased biomass concentration due to assimilation of remnants of N and P. This also boosted the SRT and decay was more evident, increasing XS concentrations up to values above 1000 mgCOD L-1 (see stage (II) in Figure 6). Inerts fraction, however, was always below 23% of the total particulates concentration, probably due to the slow hydrolysis rate. ~~Increasing substrate addition not only caused a net increase of PPB concentration inside the reactor due to assimilative growth on the remnant N and P, , but also caused an increase of SRT and decay was gaining importance. This was traduced in increasing X_S concentrations until achieving values above 1000 mg COD L^{-1} (see stage (II) in Figure 6). However, inerts fractionation was always below 23% of the total biomass exiting the reactor, which is explained by the slow hydrolysis rate.~~ These results have an important effect on energy distribution in the PRR platform since all energy balances are directly related with the biomass management through anaerobic digestion, and the relative amount of PPB will influence potential anaerobic degradability and biomass consistency. An important aspect identified by this continuous analysis is that the biomass fraction X_{PB} is always relatively small, even when applying a settler (compared with activated sludge streams predicted by the ASM1). This is because the hydrolysis coefficient is very low (<0.1 d^{-1}) compared with the levels of >2 d^{-1} typically applied in the ASM1-2d (Henze et al. 2006). This means that while growth rates are comparable to activated sludge, hydrolysis rates are far lower, and hence metabolic activity is dominated by available soluble substrate (and possibly N and P) rather than electron acceptor availability. In any case, there will always be a large proportion of undegraded particulates, due to the slow hydrolysis coefficient, and in a stable, solids dominated system, PPB sludge

should be more analogous to primary sludge rather than activated sludge, with both negative and positive consequences.

Simulation of biomass behaviour has implications on biomass production upon main line biological treatment. There is a net increment of biomass production yield compared to typical activated sludge processes. This could have an impact in energy recovery (through biogas) but also in sludge waste disposal expenses, which can be partially counteracted by downstream production of high value-added bioresources as proteins, prebiotics and probiotics (Matassa et al. 2015) or bioplastics (Padovani et al. 2016), as well as energetic resources as third generation liquid biofuels (Castro et al. 2016).

5 Conclusions

Anaerobic phototrophic growth in domestic wastewater treatment is fast, comparable to activated sludge (in k_M values) with very low K_S values, indicating that purple phototrophic bacteria behave as K strategist. However, hydrolysis is relatively slow (~0.1 d^{-1}), which means that particulate substrates will not be degraded at short HRTs. The predominant mechanism is photoheterotrophy, with autotrophy and chemotrophy generally slow. The decay rate is relatively high, comparable to activated sludge under aerobic conditions. The dynamics under continuous conditions indicate that biological processes are adaptable to normal flow variations such that performance at a given mode is stable.

The model has the following limitations:

(i) The model is only valid for anaerobic conditions, and hydrogen production for redox balancing is assumed to be inhibited, so this model cannot be implemented for hydrogen production systems as it is.

(ii) poly-P and other polymers accumulation is not included due to a lack of foundational research. Also, nitrogen fixation is not included since it is assumed to be inhibited by ammonium.

(iii) Detailed pH simulation is needed, particularly for batch processes, where pH is likely to vary due to removal of acids.

A key priority should be inclusion of poly-P and PHA accumulation as well as N_2 fixation and side H_2 production, as these processes (poly-P without carbon, PHA

without oxidation or organics, and N_2/H_2 production) are unique to photoanaerobic organisms.

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784

785TABLES

786**Table 1.** Petersen matrix of the PAM-1 model for domestic wastewater 787treatment by PPB.

Component (C) →	i	1	2	3	4	5	6	7	8	9	10
j	Process↓	S_S	S_{ac}	S_{IC}	S_{h2}	S_{IN}	S_{IP}	S_I	X_{PB}	X_S	X_I
1	Hydrolysis/ fermentation	$f_{ss,xs}$	$f_{Sac,xs}$	$f_{IC,xs}$	$f_{h2,xs}$	$f_{IN,xs}$	$f_{IP,xs}$	$f_{si,xs}$	0	-1	$f_{xi,xS}$
2	Acetate uptake	0	-1	$f_{IC,ph,ac}$	0	$-f_{N,B}Y_{PB,ph}$	$-f_{P,B}Y_{PB,ph}$	0	$Y_{PB,ph}$	0	0
3	Photoheterotrophic uptake	-1	0	$-f_{IC,ph,Ss}$	0	$-f_{N,B}Y_{PB,ph}$	$-f_{P,B}Y_{PB,ph}$	0	$Y_{PB,ph}$	0	0
4	Chemoheterotrophic uptake	-1	$(1-Y_{PB,ch})f_{ac,ch}$	0	$(1-Y_{PB,ch})f_{h2,ch}$	$-f_{N,B}Y_{PB,ch}$	$-f_{P,B}Y_{PB,ch}$	0	$Y_{PB,ch}$	0	0
5	Autotrophic uptake	0	0	$-f_{IC,a}$	$-f_{h2,a}$	$-f_{N,B}Y_{PB,a}$	$-f_{P,B}Y_{PB,a}$	0	$Y_{PB,a}$	0	0
6	Decay of XPB	0	0	$-\sum_{i=8-9}^{\square} Ci$	0	$-\sum_{i=8-9}^{\square} Ci$	$-\sum_{i=8-9}^{\square} Ci$	0	-1	1	0

		Soluble substrate (mg COD L^{-1})	Acetate (mg COD L^{-1})	Inorganic carbon (mol C_HCO_3 L^{-1})	H_2 (mg COD L^{-1})	Inorganic nitrogen (mg N_NH_4 L^{-1})	Inorganic phosphorous (mg P_PO_4 L^{-1})	Soluble inert (mg COD L^{-1})	Phototrophic biomass (mg COD L^{-1})	Biodegradable particulate (mg L^{-1})	Particulate inert (mg COD L^{-1})

788

789**Table 2:** Batch conditions of the different metabolic tests.

Mechanism	Medium	Buffer system*	COD/N/P (C/N/P)***	C source (mgCOD L^{-1})	Electron donor (mg L^{-1})	Electron acceptor	Positive control	Negative control
Photoheterotrophy	Ormerod	HEPES	100/10/2	Acetate (130), propionate, butyrate, ethanol (100)	Organic	CO_2	Adding 1 g $NaHCO_3$	-
Nitrogen limitation	Ormerod	HEPES	100/1.4/2	Acetate (130)	Organic	CO_2	No N limitation	-
Phosphorus limitation	Ormerod	HEPES	100/10/0.15	Acetate (130)	Organic	CO_2	No P limitation	-
Photoautotrophy	Ormerod	Phosphate	(100/20/∞)	$NaHCO_3$ (0.012)**	Na_2S (300)	CO_2	-	No Na_2S
Chemoheterotrophy (dark)	Ormerod	HEPES	100/10/2	Ethanol (60), Acetate (130)	Organic	Acetate	With light	-
Inhibition of H_2 production	DWW	-	100/12/4	DWW (278)	Organic	CO_2	-	Acetate (600)
	Ormerod	Phosphate	100/15/∞	Acetate (600)	Organic	CO_2	-	N limitation (1/10)

790* Buffer systems: HEPES (5.9 g L^{-1}), Phosphate (0.9 g K_2HPO_4 + 0.66 g KH_2PO_4). ** mol C L^{-1} *** ∞ means in high
791excess due to buffering

792

Table 3: Comparison of estimated parameters with those reported in the literature.

Parameter	Units	Estimated values	Literature values	Refs.
$k_{M,ac}$	mg COD mg^{-1} COD d^{-1}	2.4	1.5 (0.5), n=2	1
$k_{M,ph}$	mg COD mg^{-1} COD d^{-1}	1.4	11 (13), n=12	2
$k_{M,ch}$	mg COD mg^{-1} COD d^{-1}	0.074	5 (4), n=8	3
$k_{M,ic}$	mol IC g^{-1} COD d^{-1}	3.4 10^{-6}	2.5 10^{-5} (1.7 10^{-5}), n= 9	4
$K_{S,s}$	mg COD L^{-1}	0.5	4,333 (6,036), n=2	5
$Y_{PB,ph}$	mg COD mg^{-1} COD	1.1	0.78 (0.37), n=17	6
$Y_{PB,ch}$	mg COD mg^{-1} COD	0.5	0.23 (0.12), n= 8	7
$Y_{PB,a}$	mg COD mg^{-1} C	36,100	132,000 (84,000), n=4	8
k_{hyd}	d^{-1}	0.07	0.27 (0.06), n=2	9
k_{dec}	d^{-1}	0.09	0.2 (0.02), n=2	10

	~~$k_{M,ac}$~~	~~$k_{M,ph}$~~	~~$k_{M,ch}$~~	~~$k_{M,ic}$~~	~~$K_{S,s}$~~	~~$Y_{PB,ph}$~~	~~$Y_{PB,ch}$~~	~~$Y_{PB,a}$~~	~~k_{hyd}~~	~~k_{dec}~~
	~~mg COD mg^{-1} COD d^{-1}~~	~~mg COD mg^{-1} COD d^{-1}~~		~~mol IC g^{-1} COD d^{-1}~~	~~mg COD L^{-1}~~	~~mg COD mg^{-1} COD~~	~~mg COD mg^{-1} COD~~	~~mg COD mg^{-1} C~~	~~d^{-1}~~	~~d^{-1}~~
~~Estimated~~	~~2.4~~	~~1.4~~	~~0.074~~	~~3.4 10^{-6}~~	~~0.5~~	~~1.1~~	~~0.5~~	~~36100~~	~~0.07~~	~~0.09~~
~~Literature average~~	~~1.5~~	~~11~~	~~5~~	~~2.5 10^{-5}~~	~~4333~~	~~0.78~~	~~0.23~~	~~132000~~	~~0.27~~	~~0.20~~
~~Standard deviation~~	~~0.5~~	~~13~~	~~4~~	~~1.7 10^{-5}~~	~~6036~~	~~0.37~~	~~0.12~~	~~84000~~	~~0.06~~	~~0.02~~
~~*n (observations)*~~	~~2~~	~~12~~	~~8~~	~~9~~		~~17~~	~~8~~	~~4~~	~~2~~	
~~References~~	~~1~~	~~2~~	~~3~~	~~4~~	~~5~~	~~6~~	~~7~~	~~8~~	~~9~~	~~10~~

[1] (Golomysova et al. 2010, McKinlay and Harwood 2011), [2] (Gadhamshetty et al. 2008, Golomysova et al. 2010, Klein et al. 1991, McKinlay and Harwood 2011, Obeid et al. 2009), [3] (Madigan and Gest 1978, Schultz and Weaver 1982), [4] (Sarles and Tabita 1983, Wang et al. 1993), [5] (Gadhamshetty et al. 2008, Obeid et al. 2009), [6] (Gadhamshetty et al. 2008, Klamt et al. 2002, Klein et al. 1991, McKinlay and Harwood 2011, Obeid et al. 2009, Schultz and Weaver 1982), [7] (Madigan and Gest 1978, Schultz and Weaver 1982), [8] (Wang et al. 1993), [9] (Huang et al. 1999, Huang et al. 2001), [10] (Huang et al. 1999, Huang et al. 2001)

FIGURE CAPTIONS

Figure 1: Schematic summary of PPB metabolism under domestic wastewater treatment. Key: N2ase: Nitrogenase complex. TCA-c: Tri-carboxylic acid cycle. DF: Dark fermentation. VFA: volatile fatty acids. e^-: electrons. Dash: electron cycles. Dot: proton pumps. *: Model components.

Figure 2: Experimental (symbols) and modelled (lines) time curse of substrates uptake (left) and parameters determination including 95% confidence intervals and confidence regions (right) of PPB metabolism in photoheterotrophy (a), chemoheterotrophy (b) and photoautotrophy (c) growth modes.

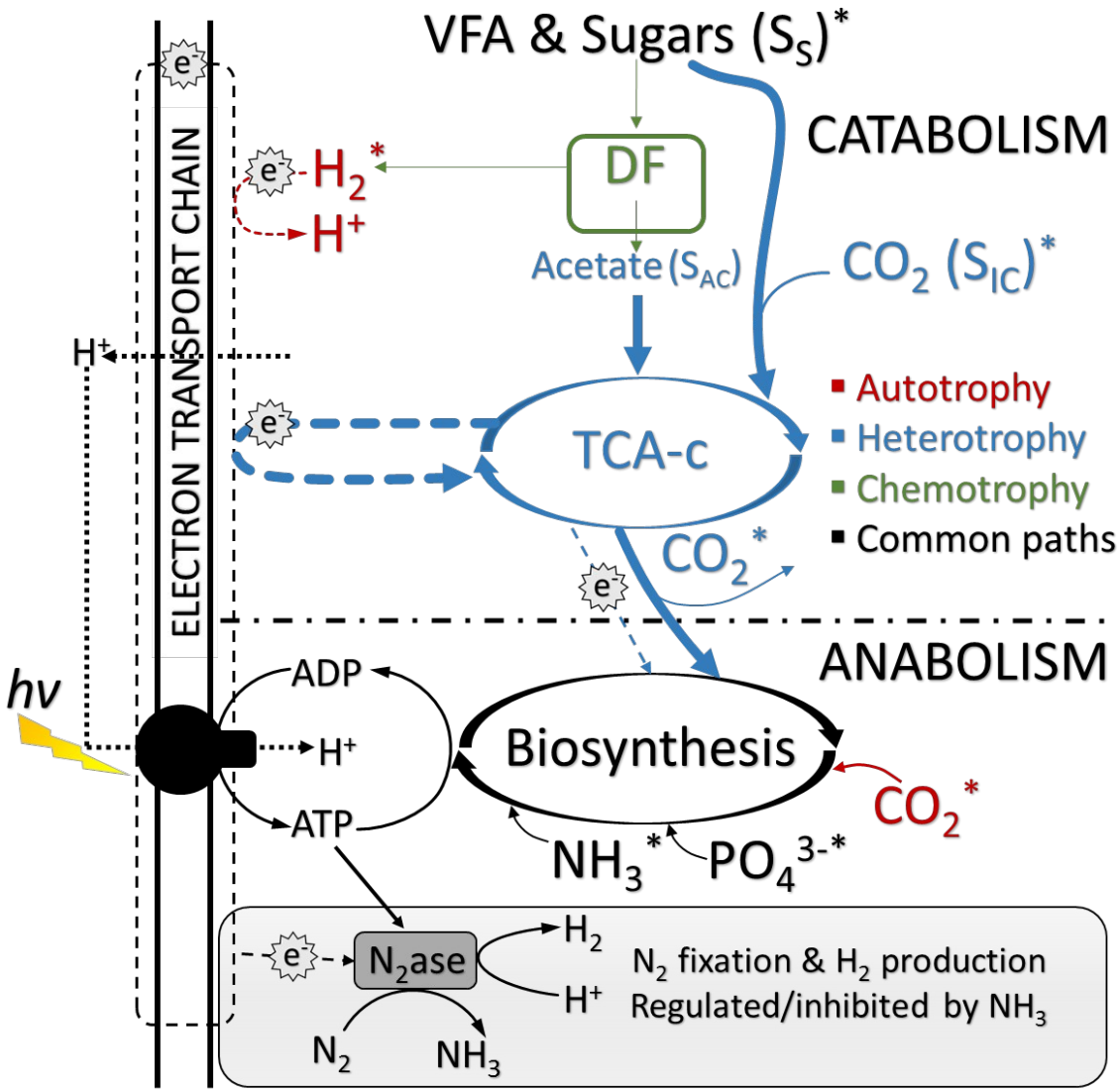
Figure 3: Mechanism of decay rate. Time course of specific phototrophic activity of PPB subjected to starvation under full illumination.

Figure 4: Time course of released products upon starvation in dark conditions demonstrating hydrolysis: soluble organic compounds but acetate (squares), acetate (diamonds), hydrogen (triangles), TIC (pluses), NH_4^+-N (circles) and PO_4^{3-}-P (crosses).

Figure 5: Influent (continuous line) and effluent concentrations (dash line) over time for PAnMBR simulation for SCOD (a), ammonium (b) and phosphate (c) upon primary settling. Different operational periods are indicated as vertical shades separators.

Figure 6: Biomass fractionation including active phototrophic bacteria (dash line), biodegradable particulate biomass (continuous line) and inert particulate (dot lines) over time for the PAnMBR continuous simulation. Different operational periods are indicated as vertical shades separators.

830**FIGURE 1**



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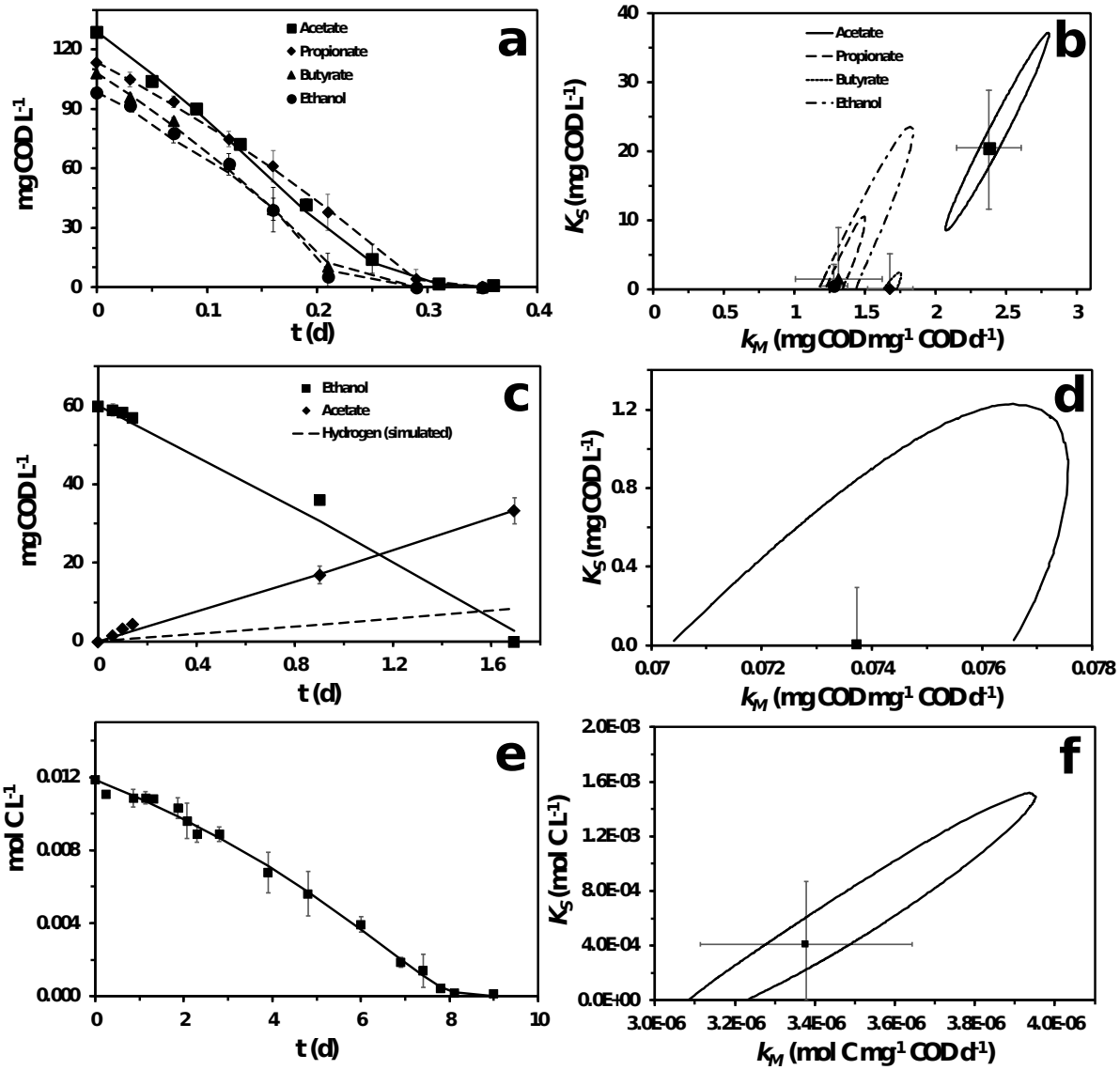
833**FIGURE 2**



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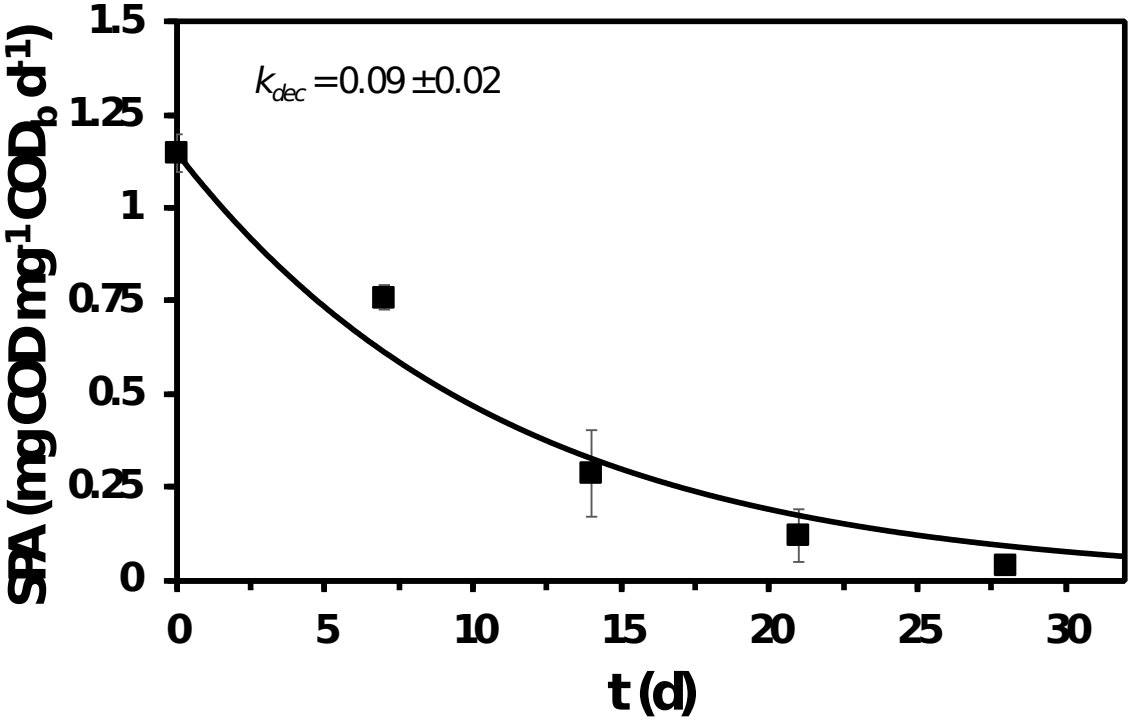
836**FIGURE 3**



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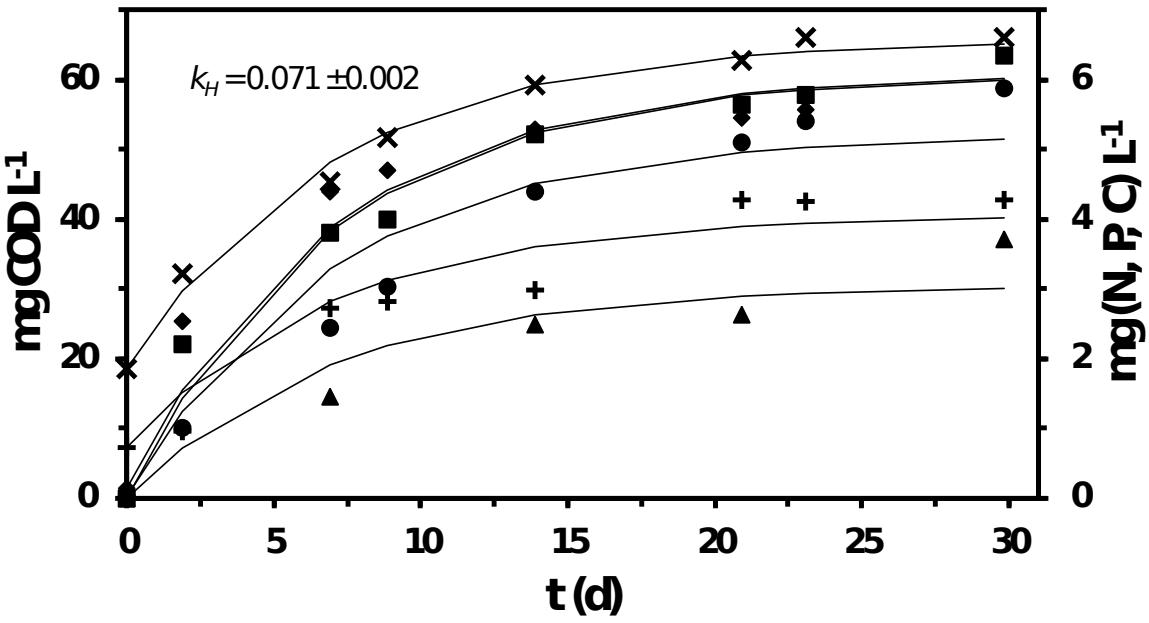
839**FIGURE 4**



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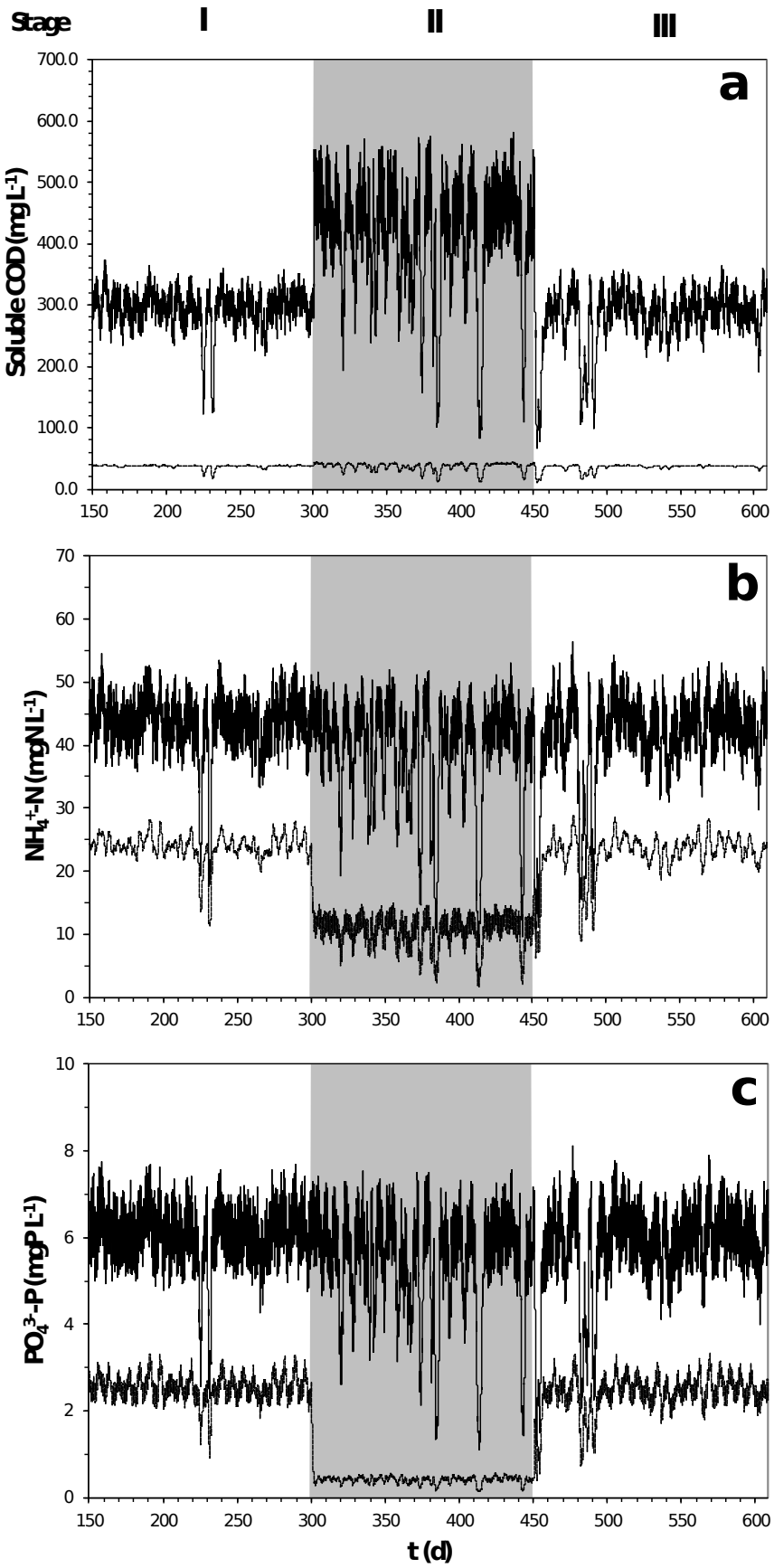
842**FIGURE 5**



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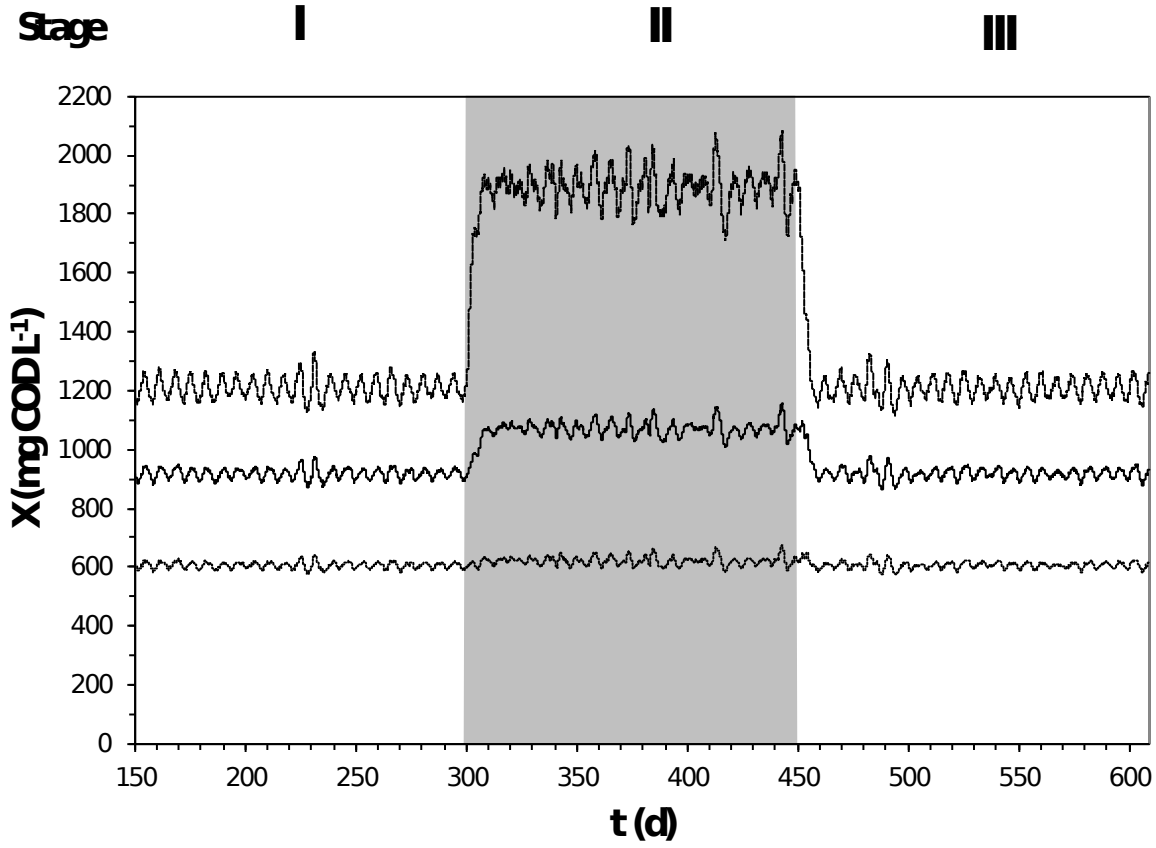
844**FIGURE 6**



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