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Improvement of biogas production and nitrogen recovery in anaerobic digestion of purple phototrophic bacteria by thermal hydrolysis

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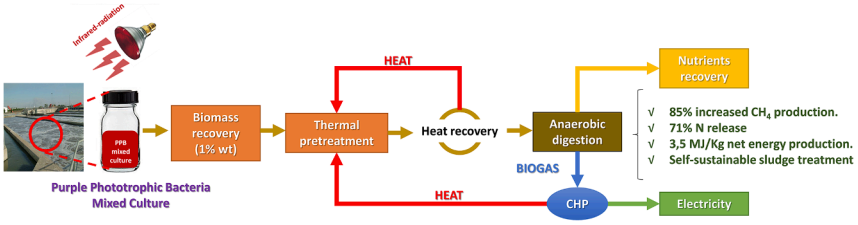
HIGHLIGHTS

- Hydrolysis temperature strongly affects C, N release, and solids destruction.
- Thermal hydrolysis in batch mode is most efficient at 180 °C and 15 min.
- Temperature increases the maximum BMP obtaining up to 380 LCH_4/kgVS.
- 83% and 71 % of solid destruction and N recovery, respectively, are obtained.
- The impact of TH + AD on the release step may be energy positive.

GRAPHICAL ABSTRACT



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ABSTRACT

Purple phototrophic bacteria (PPB) are a novel driver to recover organics and nutrients from wastewater by assimilative growth. Depending on the source, assimilated resources from the PPB biomass can still be recovered after a releasing step. Anaerobic digestion (AD) releases carbon and nutrients, but the release is incomplete. Thermal hydrolysis (TH) as a pretreatment before AD improves the digestibility, release, and subsequent recovery potentials. This work determines the effects of TH in batch and continuous modes regarding methane potential, nutrients' release efficiencies, volatile solids destruction, degradability, and hydrolysis rates. Continuous runs over 165 days (d) confirmed enhanced recovery potentials, achieving up to 380 LCH_4/kgVS (83 % solids destruction) and 73 % N release, respectively. The TH pretreatment is energy-intensive, but with appropriate heat recovery and increased methane production in the AD of the pretreated biomass, a combined configuration is energy positive.

1. Introduction

The Partition-Release-Recovery (PRR) concept for wastewater treatment utilizes fast-growing microbes to concentrate organic matter and nutrients while minimizing chemical dissipation (Batstone et al., 2015). Purple phototrophic bacteria (PPB) are the key driver of the partition stage of PRR. They grow in photo-heterotrophic mode at yields close to unity, enabling one-step organics' and nutrients' recovery within the PPB biomass (Hülsen et al., 2014). However, nutrient removal requires adding organic material (~200–300 mgCOD L^{-1}) and infrared light, enlarging the operational costs of the partition step (Hülsen et al., 2020). Therefore, effective release ("release step") of

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organic material and nutrients in concentrated form has to be achieved to balance those expenses. For the PRR concept, effective anaerobic digestion (AD) during the release step is a critical element. The PRR concept is currently being scaled up to a demonstrative scale by the Spanish company FCC AQUALIA (deep-purple.eu), and the AD of excess PPB biomass is a key step to allow energy and resource profitability and commercialization of the technology in the long-term, which is a critical technological gap.

AD reduces the sludge volume, generates biogas, and up-concentrates nutrients in stabilized digestate (Cao and Pawłowski, 2012). Major limitations are the long retention times (20–30 days) and low solid destruction efficiencies (20–50 %), which are generally associated with limitations on the hydrolysis of solid fractions in the AD (Appels et al., 2008). Improving the hydrolysis by the disintegration of the sludge may overcome these limitations. Several pretreatment techniques have been used for this purpose, including thermal, chemical, mechanical, and combined methods, where the final aims are to enhance biogas production, increase solids destruction and improve dewaterability (Neumann et al., 2016).

Among all the technologies used as pretreatment for AD, thermal hydrolysis (TH) has progressively been recognized as an effective alternative for sludge management during the last years, usually employing temperatures between 100 and 200 °C. The combination of high temperatures and pressures breaks the cellular and molecular structures of the biomass, releasing intracellular content and water, thus making the sludge better hydrolyzable, which improves digestibility and biogas production during AD (Hii et al., 2014). Several authors have reported the potential of TH to improve waste activated sludge (WAS) management (Sapkaite et al., 2017; Carrere et al., 2010; Tyagi and Lo, 2013).

Besides sludge reduction, AD also releases ~ 24–50 % of bound nitrogen as ammonium and 50–70 % of bound phosphorus as phosphate (Tchobanoglous et al., 2003), up-concentrating these nutrients in the digestate and the centrate. TH has the potential to substantially increase the solubilization of bound N and P to ammonia and phosphates via cell walls disruption and cells lysis (Appels et al., 2008), increasing the release, the concentrations, and therefore the potential recovery options (e.g., struvite precipitation, striping, and scrubbing, among others). Recovered products can be used as plant fertilizers (Wong et al., 2006).

However, to maximize nutrient recovery from the wastewater, it is necessary to enhance the accumulation and capture into a solid phase (i.e., biomass), avoiding destructive processes like biological or chemical oxidation that dissipate carbon as CO_2, N as N_2, and P as metal-bound sludge. Physical separation minimizes dissipative removal and maximizes nutrient recovery (Verstraete et al., 2009). However, biological up-concentration might offer a lower energy alternative. In the *PRR concept*, assimilated resources are released back into a liquid stream through the AD, but around a hundred times more concentrated than the raw wastewater (Batstone et al., 2015). Finally, nutrients are recovered by state-of-the-art technologies, whereas carbon is mainly recovered as energy from the methane generated by the AD process. The proposal of the *PRR* concept identified the energy prospect as the bottleneck of the platform. Recently, it has been demonstrated that the AD alone cannot fulfill the energy requirements of the *PRR* concept (methane yields between 190 and 330 LCH_4/kgVS). Also, the low digestibility of PPB biomass (primarily due to low protein degradation) makes the process non-competitive due to excess sludge and insufficient energy recovery (Hülsen et al., 2020). However, that work also identified several potential AD enhancers, including TH as the most promising option, to destroy complex proteins and release nutrients at temperatures above 150 °C (Hülsen et al., 2020). It agrees with results reported for other phototrophic microorganisms, such as microalgae and cyanobacteria, which have a relatively high methane potential, but low hydrolysis rate due to the external cell walls of these microorganisms that limits the overall process rate (Bohutskyi et al., 2014). These low potentials must be raised to secure the energetic feasibility, thus achieving the PRR concept's energy neutrality and optimizing nutrient recovery (Puyol et al., 2017). Therefore, analyzing the TH of PPB biomass is an essential aspect of improving the feasibility of the *PRR* concept.

Hence, this paper aims to evaluate the TH at a temperature range of 80–180 °C as a pretreatment of PPB-based biomass to enhance the methane production and the nutrient recovery potential of the *PRR* concept. For this purpose, the impact of TH on the downstream methane yield, volatile solids (VS) destruction, degradability, and hydrolysis rates, are determined in batch tests and continuous anaerobic digester at hydraulic retention time (HRT) of 10 and 20 days (d). On the other hand, evaluating the energy consumption by TH is critical to establishing the energy self-sufficiency required for successfully integrating the pretreatment in the WWTP. It is a general principle that the amount of additional biogas is insufficient to level the energy balance of the combined TH and AD process due to the high specific energy requirements of the pretreatment (Córdova et al., 2018). However, the recovered heat or electricity from the TH stream can allow an energy-integrated process (Pérez-Elvira et al., 2008). This way, energy balances for the AD of PPB with and without pre-hydrolysis and with or without energy recovering from the TH were performed to evaluate the self-sufficiency and net energy export to other parts of the wastewater treatment plant. Additionally, the nitrogen and phosphorous released in all the cases were evaluated.

2. Materials and methods

2.1. Purple phototrophic bacteria biomass production

PPB biomass was grown on domestic wastewater from the Móstoles campus at Rey Juan Carlos University (Madrid, Spain) and cultivated in 10 L enrichment bottles using a synthetic media optimized for PPB growth, as described elsewhere (Hülsen et al., 2014). Cultivating a poorly known AD substrate as PPB biomass in a synthetic media ensures the homogeneity of the novel feedstock. Furthermore, it focuses on the biomass itself rather than the potential variability of the domestic wastewater due to successive feeds that add non-desirable uncertainty to the goals of this study. Acetate, propionate, butyrate, and ethanol (1:1:1:1 as chemical oxygen demand, COD) were used as organic substrates at 4 g COD/L to ensure fast growth. The culture was enriched in 10 L ISO glass bottles during 7 d in anaerobic conditions, initial pH 6.5 and at room temperature, illuminated with infrared lamps (Philips BR125 IR 150 W), and covered with VIS/UV filters (ND 1.2 299, Transformation Tubes, Banstead, UK), providing 70 W/m^2 of near-infrared (NIR) irradiance. The PPB culture was refreshed weekly using 10 % of the previous culture volume. The biomass used for the hydrolysis experiments and for feeding the batch and continuous anaerobic digestion experiments was harvested via centrifugation and then resuspended to a final concentration of 1 % as total solids (TS).

2.2. Thermal hydrolysis and biochemical methane potential tests

Hydrolysis of PPB biomass was tested in a 100 mL autoclave (Selecta Presoclave-II, Barcelona, Spain) at autogenous pressure. Temperature (80, 130, 180 °C) and time (15, 30, 60 min) were selected as optimization parameters in a 3^2-factorial design of experiments. First, 50 mL of PPB biomass per batch was hydrolyzed at selected conditions in an N_2 atmosphere. The experiments' severity was based on the Severity Factor (SF), which the following equation can calculate:

$$SF = log\left[t \bullet e^{\left(\frac{T-100}{14.75}\right)}\right] \quad (1)$$

where T is the reaction temperature (°C), and t is the reaction time (min). Therefore, the SF varies between 0.58 and 4.13. Higher SF values are not recommended due to the formation of recalcitrant Maillard compounds and furans (Steinbach et al., 2019). The relationship

between T, t, and the SF can be accessed in Supplementary Information.

The biomass was then used as a feedstock for standard biochemical methane potential (BMP) tests. These tests were performed at 37 ± 1 °C in static mode in 160 mL serum bottles inoculated with methanogenic sludge extracted from a full-scale anaerobic reactor at the wastewater treatment plant (WWTP) of Móstoles (Madrid, Spain). The flocculent sludge had a biomass concentration of 9.5 ± 0.2 g VS/L, a volatile content of 64 ± 4 %, and specific methanogenic activity (on acetate) of 0.11 ± 0.01 gCOD-CH_4/(gVS·d). The sludge has carbonate alkalinity of 0.78 ± 0.09 gCO_3^{2-}/L and bicarbonate alkalinity of 3.21 ± 0.09 g HCO_3/L (pH = 7.82), giving a high buffer capacity to counteract the effect of acidification due to anaerobic batch tests. These characteristics agree with those proposed by Angelidaki et al. (2009).

The experiments were performed in triplicate, and the bottles were swirled once a day, always before taking a gas sample. An inoculum-to-substrate ratio of 2:1 as vS was used in all cases, and a control test with no substrate was included to subtract the methane produced by the endogenous digestion of the inoculum, as detailed previously (Segura et al., 2016), which is based on the standard protocol established by Angelidaki et al. (2009). The BMP tests served to determine the best conditions to maximize the biogas production and release of N and P.

2.3. Continuous experiments

The best TH conditions (temperature and time), determined during BMP experiments, were selected for the pretreatment of PPB biomass as a feedstock for continuous experiments. Completely mixed anaerobic digesters of 2L were continuously operated for 165 d at 37 ± 1 °C. The reactors were inoculated with 1 % VDS of the same anaerobic sludge described in the BMPs tests. One (test reactor) was fed with hydrolyzed PPB biomass, whereas the other (control reactor) was fed with fresh PPB biomass (non-hydrolyzed). The reactors start-up phase was 40 d until achieving a steady state. Then, two hydraulic retention times (HRTs, 20 and 10 d) were consecutively tested. The reactors were sampled at least twice per week (usually once per day during the workdays), including the feedstock, digestate, and biogas.

2.4. Analytical methods

Samples from the raw PPB biomass, after the TH process, before and after the BMP tests, as well as inlet and outlet samples from the anaerobic digesters, were analyzed for ammonium, phosphate, total Kjeldahl Nitrogen (TKN), total phosphorus (TP), total and soluble COD (TCOD, SCOD), total solids (TS), volatile solids (VS), total and volatile suspended solids (TSS, VSS) by using standard methods (APHA, 2017). Biogas production was quantified by a Boyle-Mariotte apparatus (measuring pressure) and a Milligascounter (Ritter, Colonia, Germany) (measuring gas flow) in BMP tests and the continuous AD systems, respectively. The biogas composition (H_2, CO_2, and CH_4) was analyzed by using a 7820A GC system equipped with a 3Ft 1/8 2 mm Poropak Q 80/100 SS column, a 6Ft 1/8 2 mm Poropak Q 80/100 SS column and a 6Ft 1/8 2 mm MolSieve 5A 60/80 SS column, a fitting external Luer lock and a thermal conductivity detector (TCD) (Agilent Technologies, Santa Clara, CA, USA). The mobile phase was Argon at a flow rate of 5 mL/min. The temperature of the oven and the detector were 45 °C and 220 °C, respectively.

2.5. Data management

Solubilization equations: The solubilization factor (SB; Eq. (1)) was calculated based on the particulate fraction of the chemical oxygen demand (TCOD-SCOD) (Sapkaite et al., 2017). In this expression, the particulate matter is the potentially hydrolyzable fraction during the pretreatment. Subindexes $_{TH}$, $_C$, and $_O$ indicate "after TH", "in control", and "in the untreated sludge" samples, respectively. Nitrogen and phosphate solubilizations were calculated in the same way.

$$\%SB_{COD} = \frac{\left(\frac{SCOD}{TCOD}\right)_{TH} - \left(\frac{SCOD}{TCOD}\right)_C}{\left(\frac{TCOD-SCOD}{TCOD}\right)_0} x100 \quad (2)$$

According to Salsabil et al. (2010), suspended or volatile solids solubilization (SS or SVS) can also be calculated as shown in equations (2) and (3), respectively:

$$\%SS = \frac{TS_0 - TS_{TH}}{TS_0} X100 \quad (3)$$

$$\%SVS = \frac{VS_0 - VS_{TH}}{VS_0} X100 \quad (4)$$

Statistical analysis: Experimental data are presented as arithmetic means. Error bars in experimental data represent 95 % confidence intervals in mean values based on a two-tailed *t*-test (5 % significance threshold).

Kinetic analysis of BMP: Kinetic parameters of methane production in BMP assays were obtained by fitting first-order models to the data as per Segura et al. (2016), thereby calculating the kinetic constant, k_H (in 1/d) and BMP (F_0, in LCH_4/kgVS). Parameter uncertainty was determined using two-tailed t-tests calculated from the standard error in the parameter value obtained from the Fisher information matrix. Where parameter optimization problems involve multiple parameters (k_H, F_0), parameter uncertainty surface ($J = J_{crit}$, 5 % significance threshold) has also been assessed, as described in Batstone et al. (2003). Confidence intervals (at 95 %) were calculated based on two-tailed t-tests from the parameter's standard error, as above, and used for statistical representative comparisons. All the statistical analyses of BMP assays were performed by using Aquasim 2.1d.

2.6. Energy evaluation

The analysis was restricted to the release step of the *PRR* concept, where the inlet of the excess sludge from the photobioreactor of the partition step flows through a proposed TH treatment and a subsequent AD process and includes the biogas, heat, electricity, and digestate. The main objectives for this evaluation are i) to establish if TH coupled with AD of the PPB biomass is energetically self-sustainable; ii) to evaluate the excess of energy production upon satisfying the plant's energetic needs; and iii) to evaluate and compare the solid destruction and release of nutrients (N and P) with and without the TH step, to get a holistic perspective of each system within the release step of the *PRR* concept. A critical challenge in the release stage to make the *PRR* practically feasible is to get at least 85 % of solids destruction (Batstone et al., 2015). The wastewater mainline and processes to recover nutrients from the digestate (e.g., centrifugation or crystallization) are out of the scope of this balance, as they remain invariable irrespective of the presence or absence of the TH system.

The excess biomass generated in the partition step of a PPB-based photobioreactor like that proposed in the PPR concept is the basis for the energy/nutrient balance (Batstone et al., 2015). Thus, the wastewater treatment plant is designed based on a nominal 50 000 EP (10 ML average dry weather flow) design, with influent COD, total Kjeldahl nitrogen (TKN), and total phosphorous (TP) of 500 mgCOD/L, 50 mgN/L, and 10 mgP/L, respectively. The processes aim at effluent limits of 5 mgN/L, 1 mgP/L, and 20 mgCOD/L. The photobioreactor generates a PPB biomass, with the following characteristics: flow: 380 m^3/d, COD flow: 4800 kgCOD/d, N flow: 488 kgN/d, P flow: 90 kgP/d, solids concentration: 1 %, TS flow: 3780 kgTS/d, vS flow: 3380 kgVS/d. The biomass flow is thickened in a gravity filter bed before introduction to the anaerobic digester (scenario B) or the TH reactor (scenario A). The anaerobic digester is dimensioned for an HRT of 10 d, with a height-to-diameter ratio of 0.5. A hemispherical dome (0.5 m) was designed as the upper cap for the digester, whereas the bottom was assumed to be partially buried in the ground (0.5 m). Ambient and ground

temperatures of 20 and 10 °C, respectively, were fixed, and losses from the vessels to the surroundings and the ground were considered. The construction is made in concrete covered by a glass fiber insulation film, establishing values for heat transfer global coefficients between walls and air and between bottom and ground, as shown in Supporting Information. The thermal hydrolyzer was dimensioned using 15 min HRT and a diameter/height ratio of 1. Due to its small size, it was supported directly on the ground. As for the anaerobic digester, the reactor is made of concrete covered by a glass fiber insulation film and provided with a mechanical stirring system. The energy balance for heat exchangers assumes a heat exchange efficiency of 90 % for heating and cooling operations. The heater units are modeled assuming an efficiency of 90 % and a factored capacity of 20 %. Finally, the energy consumption for gravity filter thickeners is fixed at 0.04 kWh/kgTS. All the design data, final dimensions, and main energy costs (pumping and mixing) are summarized in Supporting Information, and full calculations are provided in a Microsoft Excel spreadsheet (see Supporting Information).

3. Results and discussion

3.1. Effect of thermal hydrolysis on the solubilization of C, N, and P in the purple phototrophic bacteria sludge

The characteristics of the PPB biomass (control) after centrifugation are as follows (average values with standard deviations, number of replications: 3): (in mg/L) TCOD: 14700 ± 400, SCOD: 140 ± 10, NH_4-N: 20 ± 3, PO_4-P: 5.6 ± 0.1, TP: 310 ± 20, TKN: 730 ± 30, (in g/L) TS: 10.4 ± 0.2, VS: 9.3 ± 0.2, TSS: 10.2 ± 0.1, VSS: 9.2 ± 0.1. The data show high COD/VSS ((TCOD-SCOD)/VSS = 1.58), organic N/VSS (0.076), and organic P/VSS (0.033) ratios revealing the high potential for energy and nutrient recovery. The high COD/VSS ratio implies a maximum theoretical methane potential of around 550 LCH_4/gVS (based on a theoretical COD to CH_4 ratio of 350 $mLCH_4$/gCOD at Standard Conditions), likely due to high protein and lipid content. An average crude protein content (around 48 %) can be inferred based on nitrogen balance using TKN and estimating organic nitrogen (Hülsen et al., 2020), which is in the mid to upper part of values reported for microalgae and WAS (17.1–74.7 and 36.4–69.1 %, respectively) (Leow et al., 2015; Bougrier et al., 2008). PPB can also accumulate a high amount of lipids even during growth, with values up to 35 % of the total cellular content (Assawamongkholsiri et al., 2016). In addition, the absorbance peaks at 805 and 865 nm due to the presence of bacteriochlorophyll *a*, combined with the purple color (data not shown), indicating an enriched PPB culture (Hülsen et al., 2014).

Thermal hydrolysis at 80, 130, and 180 °C for 15, 30, and 60 min was carried out with the PPB sludge. Fig. 1 shows the solubilization (%) of VS COD, NH_4-N, and PO_4-P after TH pretreatments. In general, the hydrolysis batch tests indicated that temperature (p-value < 0.05 in all cases) and not reaction time (p-value > 0.05 in all cases) had a strong effect on C (soluble COD) and nutrients (soluble N, P) release and on the destruction of solids (where destruction indicates the conversion of vS into soluble components of their transformation into biogas).

A high solids destruction efficiency of 40 % was observed at 180 °C and 15 min of the hydrolysis reaction due to the higher cell destruction and lysis and probably reduced particle size. At 15 min, the vS solubilization increases 3-fold when increasing temperature from 80 to 130 °C (6.2 to 17.9 %, respectively) and doubles for temperature increases from 130 to 180 °C (17.9 to 40.4 %, respectively). The hydrolysis time was not as relevant as temperature, obtaining similar solubilization efficiencies at intervals of 15, 30, and 60 min at 180 °C (40.4 to 49.9 %). At 180 °C (30 and 60 min), almost 50 % of solids are destroyed (49.9 % after both reaction times). An effective thermal pretreatment can degrade not only extracellular substances (e.g., polysaccharides, proteins, lipids) and eventually release them into the soluble phase but also intracellular organics (especially proteins) that can be solubilized by breaking the chemical bonds in the cell walls (Tyagi et al., 2011). It might destroy the microbial cell wall, making proteins (carbohydrates and lipids in the sludge are generally easily degradable compared to proteins) available for biological degradation (Muller et al., 2001; Kor-Bicakcia et al., 2019).

Regarding organics and nutrient solubilization, SCOD, N, and P releases increased from 7.6 to 33.4 %, 0.1 to 35.2 %, and 1.0 to 7.1 %, respectively, upon temperature rises from 80 to 180 °C at 15 min. Longer reaction times (30 and 60 min) had an insignificant effect on the soluble COD, N, and P releases. The results clearly show that *N*-solubility is mainly affected by increased temperatures (Fig. 1c). Solubilities increase

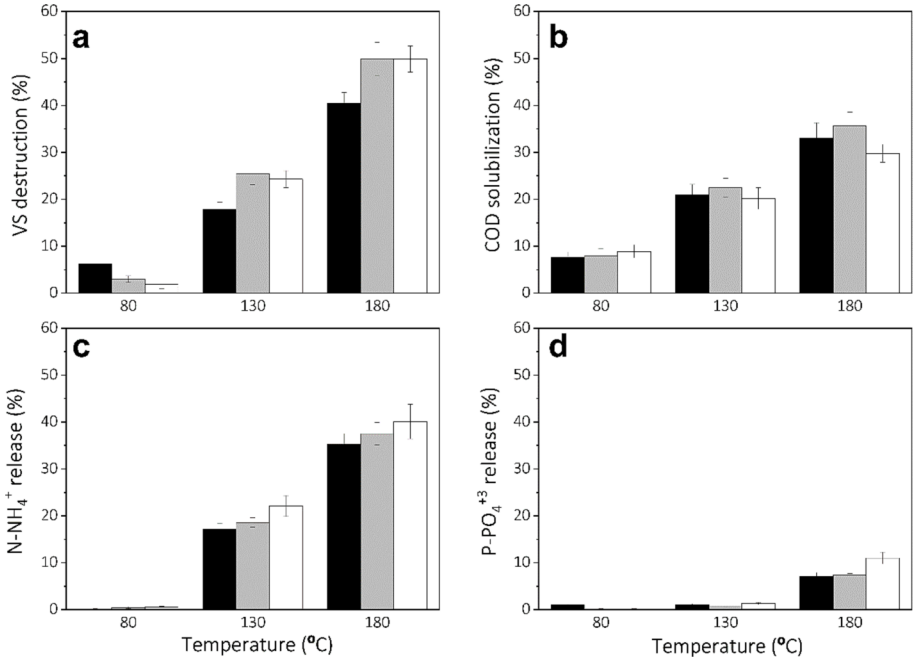

Fig. 1. Solubilization (%) of VS (a), COD (b), NH_4-N (c), and PO_4-P (d) after thermal hydrolysis of the PPB biomass at temperatures of 80, 130, and 180 °C and reaction times of 15 (black columns), 30 (grey columns), and 60 (white columns) min.

150-fold for a temperature increase from 80 to 130 °C during 15 min (0.1 to 17.1 %, respectively) and double again from 130 to 180 °C (17.1 to 35.2 %). Protein disruption increases at 180 °C, where the registered maximum NH_4-N solubilization is obtained (42 % at 60 min). The COD and N release results confirm that temperatures higher than 120 °C are necessary to optimize COD and N release, as we suggested in a previous paper (Hülsen et al., 2020). However, the PO_4-P solubilization remained constant at reaction times of 15 and 30 min at 180 °C and only increased by 3.6 % between 30 and 60 min. While, at 180 °C, 11-fold more phosphorous is released compared to 130 °C (at 60 min), though the release is still below 10 % of the TP content. The release of phosphate might be due to phosphorous accumulation or nucleotide breakage. The TH accelerates the release of intracellular P, but high temperature also induces the immobilization of P with Mg, Fe, Ca, and Al, so phosphorus recovery potential is reduced in thermal treatments (Han et al., 2019).

3.2. *Effect of thermal hydrolysis of the purple phototrophic bacteria sludge on the biochemical methane potential*

The solubilized PPB biomass, post TH pretreatment, was used for additional BMP tests to determine the effect of TH conditions (temperature and time) on methane production. Fig. 2a shows the methane potential (as LCH_4 per kg of added VS) of the pre-hydrolyzed PPB biomass compared with raw PPB biomass (control) over time. The methane production of the untreated biomass showed a two-step trend, usually associated with a first degradation of the particulate substrate to soluble monomers, followed by the subsequent methane production (Yap et al., 2017), yielding a BMP of around 335 LCH_4/kgVS. The hydrolysis temperature enhanced the BMP only at 180 °C, whereas it was maintained at 130 °C and considerably decreased at 80 °C. Thereby, it seems clear that 180 °C is the only tested temperature that allows increasing the BMP of PPB biomass. The hydrolysis time barely affected the BMP (between 15 and 60 min at 180 °C, only 8 % was enhanced), indicating that 15 min seems the most favorable hydrolysis time of those explored in the present paper since it allows a smaller hydrolysis reactor, which decreases the capital expenditure when scaling-up.

Interestingly, the gas production of the control and pretreatment at 80 °C exhibited a similar gas production until day 18 (142 LCH_4/kgVS). Then, a sharp increase to 130 °C pretreatment BMP levels was observed (301 LCH_4/kg vS after day 30). Previous studies reported the survival of PPB microorganisms in AD systems, even under dark conditions (Hülsen et al., 2020). The AD of PPB appears to be delayed where a fraction is not

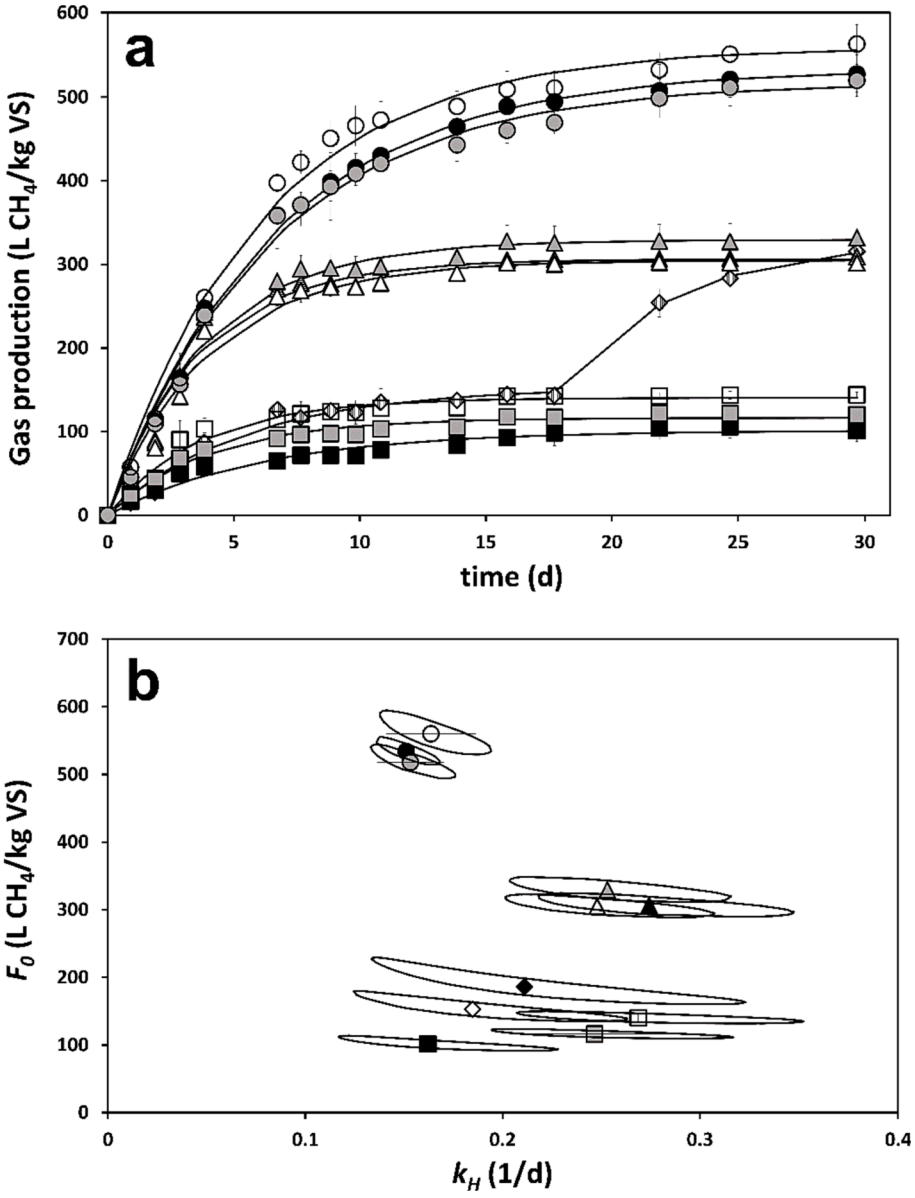

Fig. 2. Results of the BMP tests (a) and first-order hydrolysis constants (k_H; d^{-1}) and biochemical methane potential (F_0; LCH_4 $kgVS^{-1}$) (b) after TH of samples at different temperatures (80 (squares), 130 (triangles) and 180 (circles) °C) and times (15 (black symbols), 30 (grey symbols) and 60 (open symbols) min). Control raw biomass without TH in dashed diamonds (in a) and open diamonds (up to day 18), and full diamonds (from day 18 onwards) (in b). Lines in panel a represent fitting to the first-order model. Oval areas in panel b represent the confidence regions (at 95%) for the confluence of the values of k_H and F_0. Vertical and horizontal error bars indicate confidence intervals (at 95%).

degraded in the first 17 d (potentially still alive) but becomes bioavailable afterward, which explains the increased methane production in the control experiment. This lag phase does not occur at 130 °C, as this temperature is sufficient to cause thermal deactivation. However, the temperature of 80 °C might not deactivate the PPB but could instead promote sporulation that considerably improves the resistance to chemical attacks caused by hydrolytic bacteria (Berleman and Bauer, 2004), causing a decrease of BMP upon TH at 80 °C. This suggestion aligns well with previous studies using other photosynthetic microorganisms. For example, in the case of microalgae, TH as a pretreatment releases the intracellular algogenic organic matter (AOM) in the media due to cell wall break, enhancing biogas yields by 60–100 %, depending on the type of algae. Maximum methane improvement occurred at 165 °C for 30 min with *Scenedesmus obliquus*, yielding 268 ± 2 LCH_4/kgVS, which enhanced the methane potential by 208 % compared to non-pre-treated biomass (Ometto et al., 2014).

The effect of the hydrothermal pretreatments on the PPB biomass was also analyzed by comparing the kinetic parameters. Fig. 2b shows the values of the hydrolysis constant (k_H) and the biochemical methane potential (F_0) of the BMPs at temperatures of 80, 130, and 180 °C and times of 15, 30, and 60 min and the control (raw PPB biomass). For the control experiment, a two first-order piecewise-defined model was assumed (Yap et al., 2017; Velázquez-Martí et al., 2018), where the discontinuity between the sub-functions occurred at 18 d. This discontinuity defines a change in k_H and F_0 values. Therefore, the total BMP is calculated as the sum of the two F_0 values. For the rest of the cases, a single first-order function was used.

The hydrolysis rate was optimal at 130 °C, irrespective of the reaction time, yielding values around 0.22–0.27 1/d. Values at 80 and 180 °C were lower (ca. 0.15–0.2 1/d). However, the biodegradability followed the same trend as the BMP, increasing while the temperature increased from 80 to 180 °C (Fig. 3). The biodegradability was lower at 80 °C (~120 LCH_4 /kgVS), followed by the control and the 130 °C experiment (~335 and 340 LCH_4/kgVS, respectively). Finally, the 180 °C experiment got the highest values (~560 LCH_4/kgVS). The highest biodegradability obtained at 180 °C is probably due to the increased hydrolysis at high temperatures (Li et al., 2019). It also agrees with previous work, where temperatures higher than 120 °C were necessary to optimize COD and N release (Hülsen et al., 2020).

Fig. 3 shows the solubilization parameters after the BMPs. The *N*-ammonium solubilization (Fig. 3a) increased with temperature, obtaining around 24.2 %, 49.6 %, and 64.4 % at 80, 130, and 180 °C after 15 min of TH, respectively. It constitutes a 3-fold increase from 80 to 180 °C, underlining the improved degradation of proteins and nucleic acids pretreated at higher temperatures. Relative to the control, the N solubility increased by 26 and 36 % at 130 and 180 °C (15 min). Comparing the release in the control experiment with the release at 80 °C reinforces the potential of bacterial sporulation at this temperature, where the proteins may be protected from enzymatic hydrolysis caused by the AD process.

The PO_4 release (Fig. 3b) followed the same trend. The results show an increase of 0.7, 2.5, and 5.3 %, for temperatures of 80, 130, and 180 °C, respectively, after 15 min of TH. In this case, the phosphate release is tripled when the temperature increases from 80 to 180 °C, but still low, as reported for other pretreatments (Hülsen et al., 2020). While cell destruction after pretreatment favors the digestibility of cellular compounds such as nucleotides to release phosphate-containing molecules, the release appears incomplete. As previously mentioned, methane production doubled from 130 to 180 °C. It is directly related to the increase of solids destruction efficiency, as shown in Fig. 3c, following the same trend of methanization. However, as observed with the N release, the temperature of 80 °C did not improve the release of organics, and both methanization and solids destruction values were slightly lower than those obtained for the control experiment (using raw PPB-based biomass). Again, this reinforces the idea of the sporulation of PPB as a heat resistance mechanism. In any case, these data all indicate

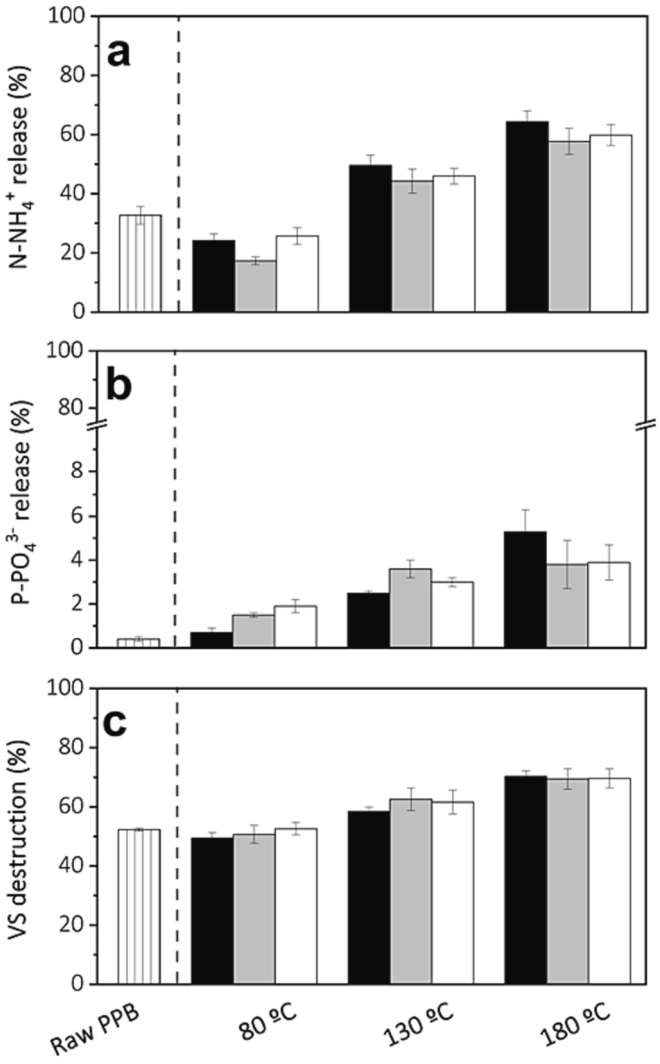

Fig. 3. Solubilization of NH_4-N (a), PO_4-P (b), and VS destruction (c) after BMP tests of pretreated PPB biomass at 80, 130, and 180 °C and 15 (black columns), 30 (grey columns) and 60 (white columns) minutes, compared with the control experiment where raw PPB biomass was used as substrate (dashed columns).

the decisive temperature effect in the increase of methane production, as reported in the case of microalgae biomass, where neither time nor pressure had a relevant influence on the TH, unlike the temperature (Córdova et al., 2018).

3.3. Continuous anaerobic digestion of pre-hydrolyzed purple phototrophic bacteria

Batch results have indicated that the optimum conditions for improving the BMP were attained at 180 °C and 15 min. These values correspond to an SF of 3.53 min, and as this value falls under the experimental intervals for the SF, the results can be considered validated. Therefore, those conditions were selected for the pretreatment of the PPB biomass before continuous experiments. Furthermore, to evaluate the thermal pretreatment's efficiency at longer and shorter times of reaction during continuous runs, HRTs of 20 and 10 d were evaluated.

Fig. 4a shows the time course of the methane potential of the control and the test reactors, whereas Table 1 shows a summary of the reactors' performance. In both cases, the production of hydrogen was negligible, and the pH in the outlet was always in values between 6.9 and 8.5, being the means values and confidence intervals (at 95 %): 7.2 ± 0.2 and 7.1 ± 0.1 for the control reactor, and 7.4 ± 0.2 and 7.3 ± 0.1 for test reactor,

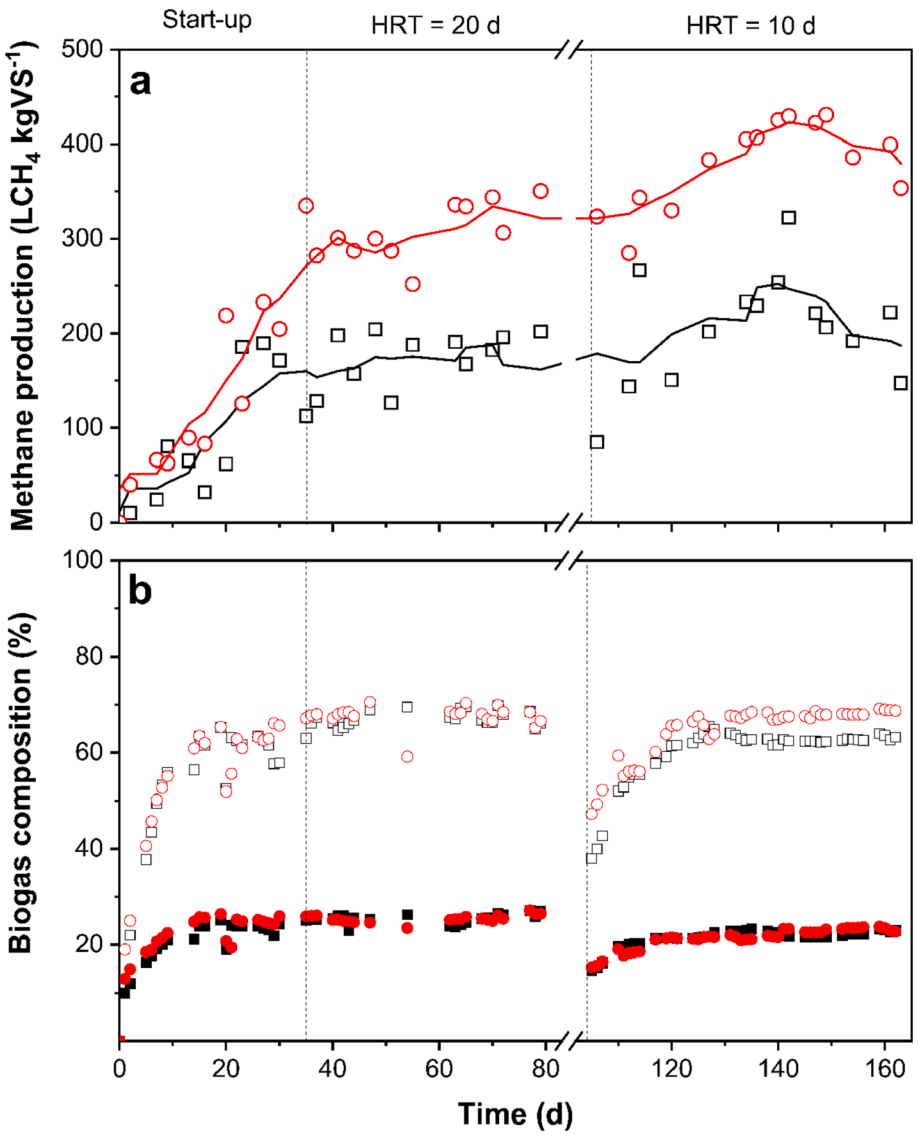

Fig. 4. Methane production (a) and biogas composition (b) in control (raw biomass, black squares) and test (hydrolyzed at 180 °C for 15 min, red circles) reactors at 20 and 10 d HRT. Continuous lines in panel a represent 5-d moving average values. Panel b shows methane (open symbols) and carbon dioxide (closed symbols). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for HRT values of 20 and 10 d, respectively. This indicates a pretty stable performance of both reactors, where no critical inhibitory episodes were observed during the whole experimental time.

The methane production was higher in the test than in the control reactor under both HRTs. During the first 40 d of stabilization (HRT of 20 d), the control production was 68 % lower than the test reactor. At an HRT of 20 d, the test reactor achieved a methane potential of 307 ± 19, much higher than the control (177 ± 17 LCH_4/kgVS). This increase was even more evident at HRT of 10 d, where the methane production in the test reactor was 85 % higher than that obtained in the control reactor (380 ± 27 vs 205 ± 35 LCH_4/kgVS, respectively). These results confirm the impact of thermal pretreatment on digestibility. The increase of the methane production in both reactors at lower HRTs of 10 d may indicate an adaptation of the anaerobic communities over time and a positive impact of the increased loading rate. It is likely due to the under-loading of the digesters at HRT of 20 d (OLR around 0.5 gVS/(L·d), potentially causing a degree of starvation of the methanogenic community (de Jonge et al., 2017; Magdalena et al., 2019).

The analysis of the biogas over time showed that methane composition was 65.9 and 62.7 % for the control reactor and 67.1 and 67.9 % for the test reactor at HRT of 20 and 10 d, respectively (Fig. 4b). This exceptionally high CH_4 composition may be due to high lipids content of the PPB biomass, as has been recently demonstrated (Assawamongkholsiri et al., 2016). Furthermore, the stability of the biogas composition (after subtracting the N_2 content used for attaining anaerobic conditions) at 20 d of HRT is achieved at around 5 d and maintained. These results indicate that TH pretreatment increases the COD, N, and P release; therefore, the digester volume can be halved while maintaining a high methane production, underlining findings from Hülsen et al. (2020).

The nutrient recovery potential was analyzed by calculating the percentage of N and P mobilized in the AD process compared to the original biomass feedstock (thereby accounting for the release caused by hydrolysis) (Table 1). The nitrogen release (as ammonium) is enhanced in the case of the hydrolyzed biomass, achieving N recovery potential of 47.3 and 71.4 % at HRT of 20 and 10 d, respectively, which compared positively to the obtained in the control digester (31.8 and 28.5 % at 20 and 10 d HRT, respectively). As commented previously, the protein solubilization may explain this increase. These results are promising for N recovery, as the low protein digestibility has been suggested to be a

Table 1
Average values (with 95 % confidence intervals) of process performance parameters from control (raw PPB biomass) and test (PPB biomass pretreated at 180 °C and 15 min) AD reactors at start-up, and HRT of 20 and 10 d.

	TH	Start-up		HRT = 20 d		HRT = 10 d	
		Control	Test*	Control	Test*	Control	Test*
Solubilization factor							
$\%SB_N$	21 ± 8	25 ± 3	47 ± 5	30 ± 6	47 ± 4	35 ± 8	71 ± 12
$\%SB_P$	10 ± 3	11 ± 2	12 ± 1	10 ± 2	11 ± 2	3 ± 2	5 ± 3
Loading rate ($gVS\ L^{-1}\ d^{-1}$)	–	0.52 ± 0.02	0.57 ± 0.02	0.4 ± 0.02	0.59 ± 0.04	0.87 ± 0.04	0.81 ± 0.10
Methane production ($LCH_4\ kgVS^{-1}$)	–	85 ± 43	125 ± 68	177 ± 17	307 ± 19	205 ± 35	380 ± 27
VS destruction (%)	40	19 ± 10	54 ± 8	34 ± 4	75 ± 2	33 ± 6	83 ± 3

*Calculated as the sum of TH + AD.

limiting factor for the economic feasibility of the *PRR* concept (Hülsen et al., 2020).

However, phosphorus release was not significantly affected by TH sludge pretreatment. The P release (as phosphate) in the test reactor was 10.9 and 5.0 % for 20 and 10 d HRT, respectively. The control reactor showed an even lower P solubilization potential, only 10.0 and 3.4 % for 20 and 10 d HRT, respectively. The results are close to those obtained by authors in other experimental studies (Hülsen et al., 2020). They indicate general low solubilization of P after AD. As explained above, P might be precipitated during TH and upon AD, likely due to high pH and the strong presence of cations like Mg^{2+} and Ca^{2+} on the synthetic growth media that may affect the P solubility. Therefore, a possible strategy to improve the P recovery would be to recover the P before submitting the biomass to AD through, e.g., acidic treatment. Another possibility impacting both the N and P recovery potential would be to use the PPB-based digestate directly as an N—P organic fertilizer, which requires the pre-conditioning of the biomass with operations like acidic hydrolysis to remove metals (in case needed) (Sakarika et al., 2019; Zarezadeh et al., 2019).

Regarding the vS destruction, similar values were obtained in the control and the test reactor during the AD. Thus, the pre-hydrolyzed PPB sludge is not more biodegradable than the raw sludge. However, accounting for the TH process (yielding 40.4 % vS destruction), the overall vS destruction was 2.2–2.5-fold higher in the test reactor than in the Control reactor, achieving vS destruction values of 83 % for an HRT of 10 d. Therefore, the sludge's digestibility increased due to the pretreatment. These data sets were used for a comparative energy balance to analyze the implications of the experimental results on improving the *PRR* platform.

3.4. Energy balance

As it has been explained, TH of PPB-based biomass at 180 °C for 15 min considerably enhanced the methane and N recovery in the continuous AD process, and it could be suitable to improve the overall feasibility of the *PRR* concept. However, an essential requirement for the TH pretreatment is to minimize the energy input needed to reach reaction temperature (180 °C) in the combined process (TH + AD) and then drop the temperature at the entrance of the anaerobic digester (37 °C). Consequently, both the energy requirements of the TH + AD system and the overall N and P released from PPB should be evaluated together. Thereby, the combined TH + AD (scenario A) was compared to a single AD of the PPB biomass (scenario B). The optimal conditions reported in this work for TH (T = 180 °C, HRT = 15 min) and anaerobic digester (HRT 10 d and 37 °C) as well as a heat exchange system to use the heat released when cooling down the effluent of the TH reactor (from 180 to 37 °C) for preheating the influent to TH reactor was used in scenario A. In the case of scenario B, only the anaerobic digester in the optimal conditions was employed (HRT 10 d and 37 °C). In both scenarios, a gravity belt thickening increased the solid concentration from 1 to 5 % by dewatering the sludge from the photobioreactor. Also, the biogas produced in the anaerobic digester is fed to a combined heat-and-power-gas-engine (CHP) for cogeneration of heat and power, where both energy flows are available to be reused in the sludge treatment system itself or any other part of the wastewater treatment plant. The energy balance of both scenarios was performed assuming the excess of biological sludge reported in a PPB-based bioreactor in the framework of the partition-release recover (PPR) concept with a sludge flow of 378 m^3/d and a solid concentration of 5 % (Batstone et al., 2015). The Supplementary Material summarizes the main characterization data of inlet and outlet streams of PPB-based bioreactor and the further assumptions and design parameters (see Supplementary Material). It includes the standard energy cost of pumping and mixing, electrical and heat yields of cogeneration of heat and power with biogas, heat exchange and heater efficiencies, and essential energy sinks and sources. In addition, supplementary material also provides calculations and results in an Excel spreadsheet. Fig. 5 illustrates the process flowcharts and schematic process energy balances of both scenarios.

As expected, the anaerobic digester (scenario B) is an energy-positive system in which 48 % and 11 % of total heat and electricity produced from the biogas in the CHP (4890 kJ/kgTS and 2450 kJ/kgTS, respectively) can be used for self-supply, being the rest available for different uses in the plant (Fig. 5, scenario B). These results, besides the NH_4-N released (45 gN/kgTS; 34.8 % of the initial N content in the PPB-sludge coming from the photobioreactor, Fig. 5), are close to those obtained by authors in other experimental studies with continuous mesophilic digester in literature (Hülsen et al., 2020). However, they are far from the estimated data reported in a previous paper (85 % solids destruction and 90 % nitrogen recovery) to balance the energy costs of the partition stage in the PRR concept (Batstone et al., 2015).

The combination of TH and AD (scenario A) enhances the methanogenic potential of the sludge in the anaerobic digester (from 205 to 380 LCH_4/kgVS), obtaining a solids destruction and N release of 83 % and 71 %, respectively (close to optima values of 85 % and 90 % previously mentioned). Furthermore, although TH is highly energy-demandant, the heat recovery from the TH outlet stream allows preheating of the inlet sludge flow up to 148 °C, reducing the net heat required to reach 180 °C. Considering this, the energy balance in Fig. 5., scenario A, reveals that the combined system is energy-self-sufficient, as the heat and electricity produced in the CHP are enough to cover the energy requirements of the TH + AD system and produce an excess of energy of 3701 kJ/kgTS, only 19 % lower than that obtained in scenario B. However, in this case, it is accompanied by a considerable increase in the destruction of solids (up to 83 %) and the release of nitrogen to values close to optima suggested in the description of the *PRR* to balance the energy costs of the partition stage (Batstone et al., 2015). Therefore, the high amount of solids produced in the PRR concept due to the assimilative growth of PPB, which is its main bottleneck, can be diminished by the hydrolysis stage. Additionally, the nitrogen released after the TH + AD reached a value up to 92 gN/kg TS (Fig. 5), an increase of 104 % compared to that obtained in the AD system.

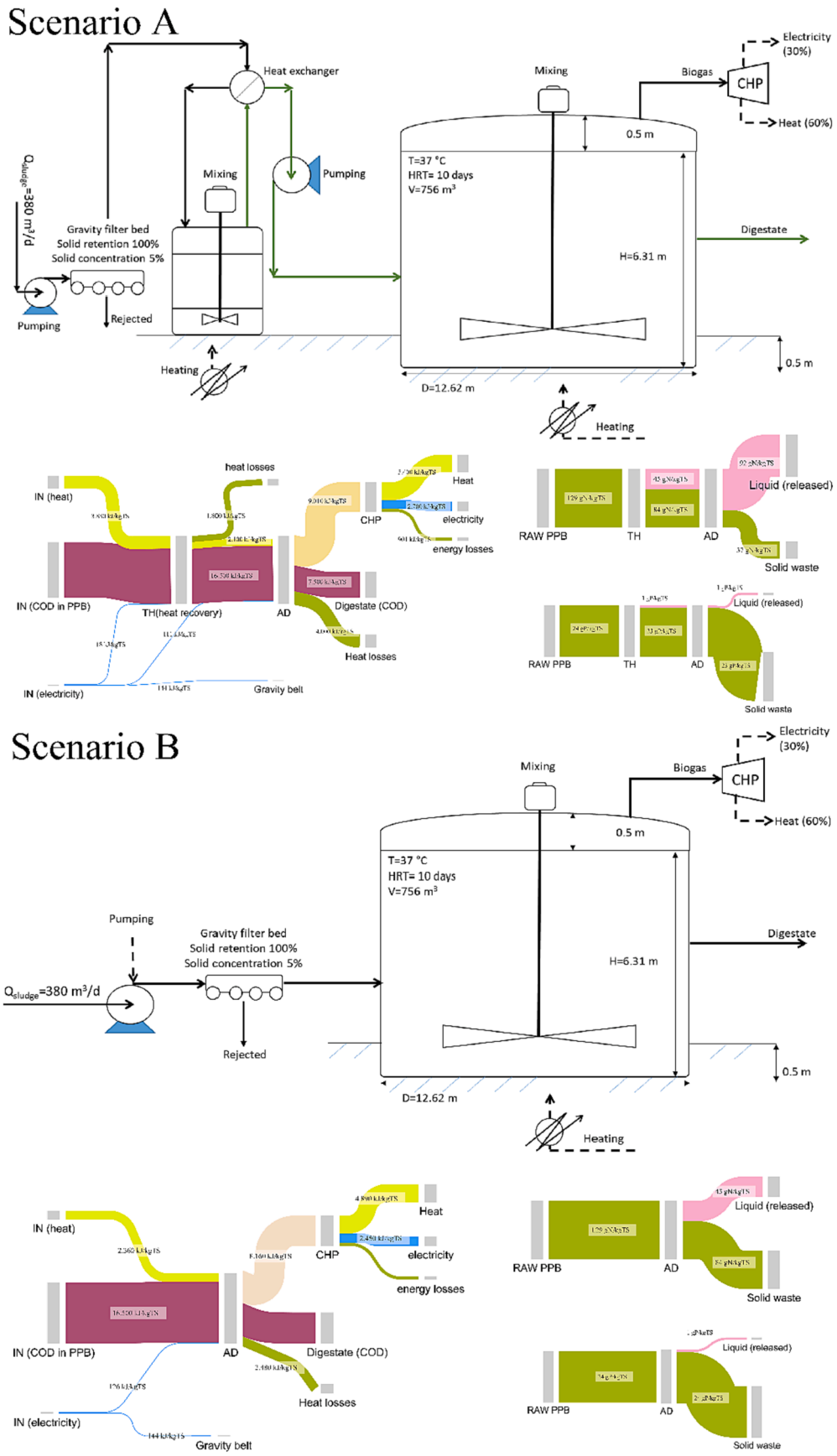

Fig. 5. Process and energy, nitrogen, and phosphorus flow charts for scenarios A (TH with heat recovery and AD) and B (only AD) within the release stage of the *PRR* concept.

3.5. Implications of energy-integrated thermal hydrolysis with anaerobic digestion

A previous study reported overall solid destruction of ca. 59 % and release of nitrogen in the range of 25 to 55.7 gN/kg in a combined system involving TH (T = 120 °C, 60 min) as pretreatment of AD of PPB sludge under mesophilic conditions (T = 37 °C) (Hülsen et al., 2020). The results obtained in this work demonstrated that the TH could be performed at a higher temperature (180 °C) with a lower reaction time (15 min) in continuous mode, increasing the solids' destruction (ca. 83 %) and nitrogen released to the nutrients' recovery stage (92.3 gN/kg). In this case, the combined system (TH + AD) in the release step is energy positive, producing an excess of energy that could contribute to the energy requirements in other parts of the wastewater treatment plant. Nevertheless, it should be remarked that the concentration of digestate before that recovery step, nutrients' recovery, or disposal of final sludge activities should be considered for a complete evaluation of the system.

4. Conclusions

This work demonstrates the feasibility of TH for improving PPB biomass digestibility. The combination of TH (including heat recovery) and AD results in an energy-positive release step within the *PRR* concept, only 19 % less favorable than the AD process alone. Temperature, not reaction time, strongly affects the solids' destruction and carbon and nutrients releases. In the continuous experiments, the hydrolysis at 180 °C and 15 min enhanced the N recovery (71 %) and the methane potential at HRT of 10 d so that the energy balance of the release step is improved, and the anaerobic digester size can be halved.

CRediT authorship contribution statement

Y. Segura: Conceptualization, Methodology, Writing – original draft, Supervision, Writing – review & editing. **R. Molina:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **I. Rodríguez:** Investigation, Methodology. **T. Hülsen:** Conceptualization, Writing – review & editing. **D. Batstone:** Conceptualization, Writing – review & editing. **V. Monsalvo:** Conceptualization, Writing – review & editing, Project administration. **F. Martínez:** Conceptualization, Supervision, Writing – review & editing. **J.A. Melero:** Conceptualization, Supervision, Writing – review & editing. **D. Puyol:** Conceptualization, Methodology, Supervision, Funding acquisition, Validation, Project administration, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.128250.

References

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jemicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water. Sci. Technol. 59 (5), 927–934.

APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association (APHA), Washington, DC, USA, 2017.

Appels, L., Baeyens, J., Degreve J., Dewil R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy. Combust. Sci., 34, 755-781.

Assawamongkholsiri, T., Plangklang, P., Reungsang, A., 2016. Photofermentaion and lipid accumulation by Rhodobacter sp. KKU-PS1 using malic acid as a substrate. Int. J. Hydrog. Energy 41 (15), 6259–6270.

Batstone, D.J., Pind, P.F., Angelidaki, I., 2003. Kinetics of thermophilic, anaerobic oxidation of straight and branched chain butyrate and valerate. Biotechnol. Bioeng. 84, 195–204.

Batstone, D.J., Hülsen, T., Mehta, C.M., Keller, J., 2015. Platforms for energy and nutrient recovery from domestic wastewater: A review. Chemosphere 140, 2–11.

Berleman, J.E., Bauer, C.E., 2004. Characterization of cyst cell formation in the purple photosynthetic bacterium Rhodospirillum centenum. Microbiology 150 (2), 383–390.

Bohutskyi, P., Betenvaugh, M.J., Bouwer, E., 2014. The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains. Bioresour. Technol. 155, 366–372.

Bougrier, C., Delgenès, J.P., Carrère, H., 2008. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties, and anaerobic digestion. Chem. Eng. J. 139 (2), 236–244.

Cao, Y., Pawłowski, A., 2012. Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: brief overview and energy efficiency assessment. Renew. Sustain. Energy Rev. 16, 1657–1665.

Carrere, H., Dumas, C., Battimelli, A., Batstone, D., Delgenes, J., Steyer, J., et al., 2010. Pretreatment methods to improve sludge anaerobic degradability: a review. J. Haz. Mater. 183 (1–5), 1–15.

Córdova, O., Santis, J., Ruiz-Filipi, G., Zuñiga, M.E., Fermoso, F.G., Chamy, R, et al., 2018. Microalgae digestive pretreatment for increasing biogas production. Renewable and Sustainable Energy Reviews 82, 2806–2813.

de Jonge, N., Moset, V., Møller, H.B., Nielsen, J.L., 2017. Microbial population dynamics in continuous anaerobic digester systems during start up, stable conditions and recovery after starvation. Bioresour. Technol. 232, 313–320.

Han, X., Wang, F., Zhou, B., Chen, H., Yuan, R., Liu, S., Zhang, R., 2019. Phosphorus complexation of sewage sludge during thermal hydrolysis with different reaction temperature and reaction time by P K-edge XANES and 31P NMR. Sci. Total Environ. 688, 1–9.

Hii, K., Baroutian, S., Parthasarathy, R., Gapes, D.J., Eshtiaghi, N., 2014. A review of wet air oxidation and Thermal Hydrolysis technologies in sludge treatment. Bioresour. Technol. 155, 289–299.

Hülsen, T., Batstone, D.J., Keller, J., 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. Water Res. 50, 18–26.

Hülsen, T., Lu, Y., Rodríguez, I., Segura, Y., Martínez, F., Puyol, D., Batstones, D.J., 2020. Anaerobic digestion of purple phototrophic bacteria – The release step of the partition-release-recover concept. Bioresour. Technol. 306, 123125.

Kor-Bicakcia, G., Eskicioglua, C., 2019. Recent developments on thermal municipal sludge pretreatment technologies for enhanced anaerobic digestion. Renew. Sustain. Energy Rev. 110, 423–443.

Leow, S., Witter, J.R., Vardon, D.R., Sharma, B.K., Guest, J.S., Strathmann, T.J., 2015. Prediction of microalgae hydrothermal liquefaction products from feedstock biochemical composition. Green Chem. 17 (6), 3584–3599.

Li, Y., Chen, Y., Wu, J., 2019. Enhancement of methane production in anaerobic digestion process: A review. Appl. Energy 240 (15), 120–137.

Magdalena, J.A., Tomás-Pejó, E., González-Fernández, C., 2019. Volatile fatty acids production from microalgae biomass: anaerobic digester performance and population dynamics during stable conditions, starvation, and process recovery. Molecules 24 (24), 4544.

Muller, J.A., 2001. Prospects and problems of sludge pretreatment processes. Water Sci. Technol. 44, 121–128.

Neumann, P., Pesante, S., Venegas, M., Vidal, G., 2016. Developments in pretreatment methods to improve anaerobic digestion of sewage sludge. Rev. Environ. Sci. Biotechnol. 15, 173–211.

Ometto, F., Quiroga, G., Psenicka, P., Whitton, R., Jefferson, B., Villa, R., 2014. Impacts of microalgae pretreatments for improved anaerobic digestion: Thermal treatment, thermal hydrolysis, ultrasound, and enzymatic hydrolysis. Water Res. 65, 350–361.

Pérez-Elvira, S.I., Fernandez-Polanco, F., Fernandez-Polanco, M., Rodriguez, P., Rouge, P., 2008. Hydrothermal multivariable approach. Full-scale feasibility study Electron. J. Biotechnol. 11 (4).

Puyol, D., Batstone, D.J., Hülsen, T., Astals, S., Peces, M., Krömer, J.O., 2017. Resource Recovery from Wastewater by Biological Technologies: Opportunities, Challenges, and Prospects. Front. Microbiol. 7, 2106.

Sakarika, M., Spanoghe, J., Sui, Y., Wambacq, E., Grunert, O., Haesaert, G., Vlaeminck, S. E., 2019. Purple non-sulphur bacteria and plant production: benefits for fertilization, stress resistance and the environment. Microb. Biotechnol. 13 (5).

Salsabil, M.R. Laurent J., Casellas M., Dagot C., 2010, Techno-economic evaluation of thermal treatment, ozonation and sonication for the reduction of wastewater biomass volume before aerobic or anaerobic digestion. J. Hazard. Mater., 174, 323-333.

Sapkaite, I., Barrado, B., Fernández-Polanco, F., Pérez-Elvira, S.I., 2017. Optimization of a thermal hydrolysis process for sludge pretreatment. J. Environ. Manage. 192, 25–30.
Segura, Y., Puyol, D., Ballesteros, L., Martínez, F., Melero, J.A., 2016. Wastewater sludges pretreated by different oxidation systems at mild conditions to promote the biogas formation in anaerobic processes. Environ. Sci. Pollut. Res. 23, 24393–24401.
Steinbach, D., Wüst, D., Zielonka, S., Krümpel, J., Munder, S., Pagel, M., Kruse, A., 2019. Steam explosion conditions highly influence the biogas yield of rice straw. Molecules 24 (19), 3492.
Tchobanoglous, G., Barton, F., Stensel, H., 2003. Wastewater Engineering Treatment and Reuse, fourth ed. Metcalfe and Eddy, New York.
Tyagi, V.K., Lo, S.-L., 2011. Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review. Rev. Environ. Sci. Biotechnol. 10, 215–242.
Tyagi, V.K., Lo, S.L., 2013. Sludge: a waste or renewable source for energy and resources recovery? Renew. Sustain. Energy. Rev. 25, 708–728.
Velázquez-Martí, B., Meneses-Quelal, O.W., Gaibor-Chavez, J., Niño-Ruiz, Z., 2018. Review of Mathematical Models for the Anaerobic Digestion Process. Open Access Peer-Reviewed Chapter. Available from: https://www.intechopen.com/books/anaerobic-digestion/review-of-mathematical-models-for-the-anaerobic-digestion-process.
Verstraete, W., Van de Caveye, P., Diamantis, V., 2009. Maximum use of resources present in domestic "used water". Bioresour. Technol. 100 (23), 5537–5545.
Wong, W.T., Chan W.I., Liao, P.H., Lo, K, V., Mavinic, D.S., 2006. Exploring the role of hydrogen peroxide in the microwave advanced oxidation process: solubilization of ammonia and phosphates. J. Environ. Eng. Sci., 5, 459-465.
Yap, S.D., Astals, S., Jensen, D.D., Batstone, D.J., Tait, S., 2017. Indigenous microbial capability in solid manure residues to start-up solid-phase anaerobic digesters. Waste Manage. 64 (2017), 79–87.
Zarezadeh, S., Moheimani, N., Jenkins, S., Hülsen, T., Riahi, H., Mickan, B., 2019. Microalgae and phototrophic purple bacteria for nutrient recovery from agri-industrial effluents: Influences on plant growth, rhizosphere bacteria and putative carbon and nitrogen cycling genes. Front. Plant Sci. 10, 1193.