Dissolution rate and growth performance reveal struvite as a sustainable nutrient source to produce a diverse set of microbial protein

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Abstract

To provide for the globally increasing demand for proteinaceous food, microbial protein (MP) has the potential to become an alternative food or feed source. Phosphorus (P), on the other hand, is a critical raw material whose global reserves are declining. Growing microbial protein on recovered phosphorus, for instance, struvite obtained from wastewater treatment, is a promising MP route production that could supply protein-rich products while handling P scarcity. In this study, effect of MP media was explored with respect to struvite dissolution kinetics, and additionally different MP were characterized with struvite as sole P-source. Different operational parameters, including pH, temperature, contact surface area, and ion concentrations were tested, and struvite dissolution rates were observed between 0.32 and 4.7 g P/L/d and a solubility between 0.23 and 2.22 g P-based struvite/L. Growth rates and protein production of the microalgae *Chlorella vulgaris* and *Limnospira* sp. (previously known as *Arthrospira* sp.), and the purple non-sulfur bacterium *Rhodopseudomonas palustris* on struvite were equal to or higher than growth on conventional potassium phosphate. For aerobic heterotrophic bacteria, two slow-growing communities showed decreased growth on struvite, while the growth was increased for a third fast-growing one. Furthermore, protein content on struvite was always comparable to the obtained when grow on standard media. Together with the low content in metals and micropollutants, these results demonstrate that struvite can be directly applied as an effective nutrient source to produce fast-growing MP, without any previous diluting step. Combining a high purity recovered product with an efficient way of producing protein results in a strong environmental improvement.

Abbreviations

Activity test medium, AT; aerobic heterotrophic bacteria, AHB; bold's basal medium, BBM; chemical oxygen demand, COD; diammonium phosphate, DAP; dry weight, DW; ethylenediaminetetraacetic acid, EDTA; inductively couple plasma-optical emission spectrometry, ICP-OES; inorganic crystal structure database, ICSD; methane oxidizing bacteria, MOB; microbial protein, MP; purple non-sulfur bacteria, PNSB; single cell protein, SCP; particle size distribution, PSD; sludge retention time, SRT; specific surface area, SSA; thermogravimetric analysis, TGA; triple superphosphate, TSD; volatile solids, VS; X-ray powder diffraction, XRD.

Keywords

Spirulina, Single-cell protein, Resource recovery, Magnesium ammonium phosphate, Purple bacteria, Aerobic heterotrophic bacteria

1. Introduction

With the increase in global population and a rise in living standards, it is expected that the demand for protein will increase by approximately 50% by 2050 (FAO 2021). This impressive number is aggravated by the fact that present-day nutrient use efficiencies are extremely low, with crop and livestock production losses of 86% nitrogen (N) and phosphorus (P) in developed countries (Coppens et al., 2016). In addition, P is a nonrenewable resource with rapidly declining reserves. Some researchers estimate that 40-60% of the global P reserves will be consumed by 2100 (van Vuuren et al., 2010). Others do not expect short-term scarcity but, however, point out that environmental impacts, such as radioactive waste and cadmium-rich products (i.e., fertilizers), are increasing as a consequence of the declining P stock quality (Smidt et al. 2011). Therefore, in recent years, the EU has declared P rock as a critical raw material, and also has called for urgent action to replace imported protein crops with sustainable alternatives (Denanot 2018).

One approach that can both relieve pressure on nutrient sources while generating high-value protein products is the recovery of nutrients through the microbial protein (MP) production from wastewater (Verstraete et al., 2016; Vethathirri et al., 2021). MP, also known as single cell protein (SCP), is the high-value protein-rich biomass of microorganisms such as microalgae and bacteria (Ritala et al., 2017). They are of particular interest for recovering essential nutrients, like N and P, from liquid streams, as they have a high nutrient conversion efficiency and a high volumetric biomass/protein productivity rate compared to other protein sources (Pikaar et al., 2017; Verstraete et al., 2016). They also have an exceptionally raised protein content (50-80% dry weight) and feature other functional components such as vitamins, pigments, and antioxidants (Matassa et al., 2016). Elevated nucleic acid content in MP is well tolerated

by most animals when used as feed (Skrede et al., 2009). But when applied as food, due to the lack of urease enzyme in humans, nucleic acids content must be low enough to avoid toxicity (Anupama & Ravindra, 2000; Nasseri et al., 2011). Nonetheless, DNA/RNA content in current commercial microalgae products (1.4-1.6% dry weight) allows a safe consumption for humans up to 425 g/day of *Chlorella* sp. and 275 g/day of *Limnospira platensis* (Muys et al., 2019). Higher nucleic acid content is observed in other MP forms (Nasseri et al., 2011), but several methods are reported to reduce it in SCP to safe levels, like heat treatment over 65ºC for 20 minutes or alkaline hydrolysis (Anupama & Ravindra, 2000; Ritala et al., 2017; Yazdian et al., 2005).

However, the production of SCP from waste effluents is challenging, mainly due to the presence of micropollutants like heavy metals, pharmaceuticals, or polycyclic aromatic hydrocarbons. These contaminants may accumulate in the biomass or inhibit the growth of the MP themselves. Some other waste effluents, like brewery wastewater, are more suitable for SCP production due to their very low contaminants content (Lee et al., 2015). Nonetheless, these effluents do not possess enough nutrients like P, which is crucial for the microorganism growth and SCP formation (Muys et al., 2020), and supplementation with traditional P source is still needed (Lee et al., 2015). Also, the high turbidity of some kinds of wastewater makes phototrophic MP production less efficient (e.g., microalgae), as they require excellent light penetration for optimal growth (Pacheco et al., 2015).

The recovery of nutrients from wastewater, to provide them afterwards for SCP production, can be a sustainable alternative that overcome wastewater disadvantages. Magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$), also known as struvite, is one of such recovered substances rich in nutrients. Struvite precipitation has already evolved into a mature technology with many full-scale applications in municipal wastewater

112 treatment (e.g., Aquafin, Berliner Wasserbetriebe, and Waternet) and the food industry
113 (e.g., Clarebout potatoes and Agristo). The struvite has also showed to meet all legal
114 requirements for its use as fertilizer, due to its low content in heavy metals and
115 micropollutants (Muys et al., 2021; Tong et al., 2017), and to be a good slow-release P
116 source, especially in acidic soils (Hertzberger et al., 2020). Its market price of 0 - 0.8
117 €/kg P is lower than traditional fertilizers like 1 €/kg P for triple superphosphate (Muys
118 et al., 2021).

119 As stated above, both price and sustainable origin makes struvite a potential P source
120 for SCP production. However, considering the known slow-release nature of struvite in
121 soils (Hertzberger et al., 2020; Muys et al., 2021), reliable struvite dissolution data in an
122 aqueous medium are essential to design strategies for bioreactor dosage. Struvite
123 dissolution rates should at least match P uptake rates of MP to guarantee optimal
124 growth. Only a few studies report struvite dissolution rates, with values between 8 and
125 60 mg P/L/d (Bhuiyan et al., 2007). This dissolution rate range is acceptable for
126 relatively slow growing SCP; however, fast-growing microbes used for SCP production,
127 such as purple non-sulfur bacteria (PNSB) (Alloul et al., 2021; Wada et al., 2022) and
128 aerobic heterotrophic bacteria (AHB) (Muys et al., 2020; Papini et al., 2022), would
129 require higher dissolution rates to avoid growth limitations. Indeed, successful growth
130 on struvite was only reported for the relatively slow-growing eukaryotic microalgae
131 *Chlorella vulgaris*, *Scenedesmus* sp., *Nannochloropsis salina* and *Phaeodactylum*
132 *tricornutum* (Barbera et al., 2017; Chaoyu et al., 2017; Davis et al., 2015; Moed et al.,
133 2015), and slow-growing prokaryotic *Limnospira platensis* (previously classified in
134 *Arthrospira* genera) generally known as Spirulina (Markou et al., 2019; Nowicka-
135 Krawczyk et al., 2019). Recently, struvite was applied as P source to fast-growing

methane oxidizing bacteria (MOB), but struvite was dissolved in acids previously to the addition to the cultures (Goonesekera et al., 2022).

Consequently, the production of fast-growing microorganism, like PNSB or AHB, has not yet been examined on struvite without any previous dissolution step. Additionally, struvite dissolution kinetics under MP production bioreactors have not been addressed (i.e., stable pH), even though it is known that is influenced by several process parameters such as temperature, contact surface and ion concentrations (medium components)(Ariyanto et al., 2014; Bhuiyan et al., 2007; Roncal-Herrero & Oelkers, 2011). Bioinformatic models are available to determine the solubility of struvite, but conditions like the presence of EDTA, often used in SCP production (Barbera et al., 2017; Markou et al., 2019), are not available.

In this study, struvite valorization route was investigated as a sustainable P source for MP cultivation without previous dilution step. Specifically, this study explores for the first time fast-growing microbes used for SCP on struvite, using as model organisms the PNSB *Rhodopseudomonas palustris* and 3 different AHB consortia from food processing industries. Additionally, provides a complete description of growth rates, biomass, and protein content of commonly slow-growing microbes used for SCP (i.e., *Chlorella vulgaris* and *Limnospira platensis*) using struvite as P source. On the other hand, the influence of relevant physicochemical parameters on struvite dissolution kinetics was assessed with batch dissolution experiments at constant pH.

2. Materials and Methods

2.1. Struvite characterization

Struvite granules were obtained from a full-scale NuReSys installation that treats anaerobically digested potato processing water (Clarebout Potatoes, Nieuwkerke,

Belgium). Prior to analysis, struvite granules were solubilized in 10 mL of 65% HNO_3 and diluted with milli-Q water up to 50 mL. Total phosphorus was determined following the method of (Scheel, 1936). Ammonium was measured by colorimetric analysis with a continuous flow AA3-Autoanalyzer (BranLuebbe) according to DIN and ISO standard methods. Magnesium, potassium, calcium, and sodium were analyzed by ICP-OES (Varian Vista-MPX) after sample filtration (0.45 µm). Zn, Cu, Pb, Ni, Cd, Cr, Mn, Hg, As, Mo and Fe were analyzed by MP-AES (Agilent). Organic crystal intrusions were quantified by thermogravimetric analysis (TGA) (Mettler Toledo TGA/SDTA851). In brief, 25 ± 5 mg of struvite was heated to 900°C at a rate of 1°C/min and the organic matter was quantified as the difference in weight loss under oxygen and nitrogen atmospheres. Free water (non-crystal water) content was determined by drying 10 g of struvite to a constant weight at 35°C. To determine the bulk mineralogical composition around 3 g of sample was dried at 40°C and ground in a wet milling device in ethanol and then spray-dried. Next, the samples were measured by X-ray diffraction (XRD Siemens D5000; using CuKα radiation). Corundum (Al_2O_3) was used as internal standard. The subsequent quantification was performed based on the Rietveld method, in which an XRD pattern is calculated based on the structure models of the minerals present as they are found in literature or in databases (ICSD). The pattern is then fitted to the measured pattern by refining the structure parameters. Impurities in the tested struvite accounted for only 3.59% w/w; containing low amounts of organic matter (0.73 wt.%), potassium (1.73 wt.%), calcium (0.07 wt.%), sodium (0.02 wt.%), free water (1.01 wt.%) and metals (0.036 wt.%), of which manganese (0.009 wt.%) and iron (0.025 wt.%) were the most abundant (SM Figure S1 and S2). Furthermore, XRD spectrometry analysis revealed that a large sample fraction was

184 composed of struvite (98.4%), indicating specific precipitation, while a smaller part
185 (1.6%) was identified as quartz (SiO_2) (SM Figure S3).

186 The particle size distribution (PSD) was determined by laser diffraction (Mastersizer
187 3000, Malvern Instruments), after it was visually confirmed that no particles were
188 present larger than 3.5 mm (using a sieve with mesh size 2 mm; SM Figure S4),
189 whereby data analysis was performed using the Mie theory. The specific surface area
190 (SSA) of the particles was calculated using the Sauter mean diameter.

191 **2.2. Physicochemical parameters impacting struvite dissolution kinetics**

192 Prior to the MP growth experiments, the influence of single relevant physical and
193 chemical parameters on struvite dissolution rates was assessed at constant pH. (SM
194 Table S1). All dissolution experiments were performed in batch in closed one-liter
195 Erlenmeyer flasks and stirred at 300 rpm (Thermo Scientific Variomag Poly 15) at 20 ±
196 1°C. The parameters assessed were particle size (74-771 cm^2/L), struvite dosage (1-4
197 g/L), pH (7-9), temperature (20-28ºC), and concentration of several components, named
198 Mg^{2+} (2.4-9.6 mM), NaCl (17-510 mM), Ca^{2+} (1-5 mM), CO_2/HCO_3^- (100-800 mM),
199 and EDTA (0.25-4 mM). The pH was buffered at 7 (or at 9 for the pH experiment) with
200 HEPES (50 mM). For all experiments, 2 g of struvite was dissolved in 1 L of medium,
201 except for the struvite dosage experiment.

202 Next to the effect of single physicochemical parameters, struvite dissolution was
203 assessed in a model fresh and saltwater algal growth medium. The freshwater medium,
204 adapted from WC Medium (Guillard and Lorenzen 1972), contained 0.25 mM $CaCl_2$,
205 0.15 mM Mg_2SO_4, 0.15 mM $NaHCO_3$ and 0.05 mM K_2HPO_4 as relevant ions. The
206 marine medium, adapted from f/2 Medium (Guillard & Ryther, 1962), contained 217

mM NaCl, 0.94 mM $NaHCO_3$, 12.98 mM Mg_2SO_4, 12.19 mM $MgCl_2$, 4.58 mM $CaCl_2$, 0.58 mM KH_2PO_4 and 0.047 mM EDTA as relevant ions.

Samples were regularly analyzed for ammonium and ortho-phosphate (San++ Automated Continuous Flow Analyzer). Maximum struvite dissolution rates (i.e., P and N release rates) were determined according to the ammonium and phosphate concentrations after 2 hours (linear behavior with $R^2 > 0.95$; SM Figure S5). Struvite solubility was calculated based on the equilibrium phosphorus concentration after 30 hours (P content were stable; SM Figure S5). Struvite dissolution in deionized water at pH 7, 300 rpm and 20 ± 1°C served as control.

A comparison of the data from the dissolution experiments was made with thermodynamic simulations using PHREEQC 3.0 speciation software (not for EDTA and particle size as the software did not allow simulation of different particle sizes and EDTA could not be implemented). The PHREEQC 3.0 software package, used for the simulation, was obtained from the USGS website (Parkhurst & Appelo, 2013). The standard Amm.dat database was expanded with enthalpy data and solubility products of different phosphate, ammonium, sulphate, and carbonate salts, as calculated from the Gibbs free energies collected from literature (la Iglesia, 2009; Masterton et al., 1983; Oelkers et al., 1995; Ronteltap et al., 2007). When the required enthalpy data was absent in literature, the simulation used solubility products at standard conditions (SM Table S2).

2.3. Struvite as a recovered nutrient source for MP production

The three classes of investigated MP included (i) microalgae in photoautotrophic growth conditions with pure cultures of the eukaryotic *Chlorella vulgaris* (211-11b; SAG, Göttingen University, Germany) and prokaryotic *Limnospira* sp. PCC 8005

231 (SCK-CEN, Belgium), (ii) purple non-sulfur bacteria (PNSB) in photoheterotrophic
232 growth conditions with a pure culture of *Rhodopseudomonas palustris* (BCCM,
233 Belgium), and (iii) three consortia of aerobic heterotrophic bacteria (AHB). Two
234 consortia of AHB originated from full-scale activated sludge plants treating wastewater
235 in the potato processing industry with high sludge retention time (SRT); one originated
236 from Remofrit (Belgium) and was pre-grown at 28°C at a SRT of 20 days and one
237 originated from Agristo (Belgium) and was pre-grown at 20°C at an SRT of 30 days.
238 These communities will be referred as low-rate growth communities, named 'potato 1'
239 and 'potato 2' respectively. The third consortium was acquired from a pilot-scale MP
240 reactor (Avecom, Belgium) and was pre-grown under high-rate growth conditions on a
241 wheat flour-based medium mimicking starch-rich wastewater at 25°C with an SRT
242 between 1.5 and 3 days.

243 Within the growth experiments, struvite provided 100% of the phosphorus and 5.9-8.3%
244 of the nitrogen. Nutrient limitation was avoided by supplying a sufficient amount of
245 nutrients to reach a theoretical biomass concentration of 2 g/L of *C. vulgaris* and PNSB,
246 4 g/L of *Limnospira* sp. and 5 g/L of AHB. Hereby, an initial nutrient ratio was set
247 according to Redfield for the microalgae and PNSB and according to the theoretical
248 biomass composition of $C_{60}H_{87}O_{23}N_{12}P$ for the AHB.

249 Growth experiments were performed in triplicate in one-liter Erlenmeyer flasks stirred
250 at 300 rpm with the same reactor configuration that was used for the dissolution
251 experiments (SM Table S3 and S4). The conventional control medium for *C. vulgaris*
252 was adapted Bold Basal Medium (BBM) (Bischoff and Bold, 1963) and adapted
253 Zarrouk medium was used for the cultivation of *Limnospira* sp. (Zarrouk et al., 1966).
254 The amount nitrogen provided by the struvite, in the form of NH_4^+, was compensated in
255 all MP with a reduction of the nitrogen provided by other medium elements, therefore

maintaining the same amount of N between the control medium and struvite medium (SM Table S3). Since BBM medium contains NO_3^- while struvite contains NH_4^+, the effect of the nitrogen source on *C. vulgaris* growth was also researched.

For *R. palustris*, adapted AT medium was used as the control (Madigan et al., 2015). Erlenmeyer flasks were made anoxic by flushing with nitrogen gas and closed with rubber stoppers. Microalgae and PNSB in late exponential phase were inoculated at an optical density (OD) of 0.1. Flasks were randomized daily to minimize influences of site-bound light variations. All AHB were cultivated in adapted *E. coli* medium (Madigan et al., 2015) and inoculated at an OD of 1.0. Dissolved oxygen levels were measured regularly (Hach HQ40d).

OD was measured daily at 680 nm for the microalgae and at 660 nm for PNSB and AHB (UV - 2501 PC; SHIMADZU). Maximum specific growth rates were obtained from a regression analysis on the exponential growth data. The pH was measured daily and corrected if necessary. Protein content was analyzed according to (Markwell et al., 1978), and dry weight were measured during exponential and stationary growth. Microalgal protein was extracted following (Slocombe et al., 2013).

Since AHB are the fastest growing MP and *Limnospira* sp. are cultivated at pH 9, limiting struvite dissolution rates were expected. For this reason, PO_4^{3-} and NH_4^+ levels were measured during the growth of two types of AHB and during the first day of *Limnospira* sp. growth. The results of experiments performed in triplicate are presented as the means ± standard deviations. An independent sample t-test in SPSS statistics 24 was used to compare data at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Parameters impacting struvite dissolution kinetics at constant pH

In this study, struvite is considered mainly as a P source for MP production, as it can deliver all the P necessary to support microbial growth but only a portion of the N required. Indeed, with N/P ratios of 5.4/1 (w/w) for typical microorganisms ($C_{60}H_{87}O_{23}N_{12}P$), the lower ratio in struvite (0.4/1) (w/w) supplies only 7.4% of the required N. Therefore, the focus is set on the maximum P dissolution rate.

Observed P dissolution rates between 0.16 and 2.4 g P/g struvite/d (0.32 and 4.7 g P/L/d) and values for solubility between 0.23 and 2.22 g struvite/L confirms the significant influence of physical and chemical parameters on struvite dissolution kinetics (Figure 1; SM Table S5). Literature values for struvite solubility in distilled water are significantly lower, between 0.15 g/L at 20°C and 0.21 g/L at 35°C (Bhuiyan et al., 2007), mainly because the pH was not controlled in these studies, resulting in a pH increase.

The obtained results indicate that among physical parameters, contact surface area is an effective parameter to increase when higher struvite dissolution rates are needed (Figure 1 A). Depending on the type of MP produced and power consumption considerations, also elevated temperatures can be applied, however, with a more limited gain in struvite dissolution rate (Figure 1 B). Among the tested chemical parameters, carbonate (Figure 1 D), EDTA (Figure 1 E) and elevated salinity (Figure 1 F; SM Figure S6 A) concentrations can increase the struvite dissolution rate sensitively. Carbonate and EDTA are both common elements applied in the medium of some photosynthetic microorganism (Barbera et al., 2017; Markou et al., 2019; Moed et al., 2015), therefore the presence of this elements assure rapid dissolution rates and enough phosphate supply. PNSB and AHB media, on the other hand, normally do not contain those

303 components but their use has been considered occasionally (Cerruti et al., 2020). In
304 contrast, when dealing with elevated medium concentrations of calcium, care should be
305 taken to ensure a sufficiently high dissolution rate to meet MP demand (Figure 1 C).
306 Elevated Mg^{2+} ion concentrations (up to 10 mM; SM Figure S6 B) presented a less
307 severe influence on dissolution rate (More detailed description available in SM
308 Appendix A).

309 PHREEQC simulations of struvite solubility in function of tested physicochemical
310 parameters (including temperature, pH, concentration of Mg^{2+}, NaCl, Ca^{2+} and
311 inorganic carbon (CO_2/HCO_3^-)) approached the experimental data well for most
312 parameters (SM Figure S7). Struvite solubility simulations in function of Mg^{2+}
313 concentrations were systematically too low, while at carbonate concentrations higher
314 than 200 mM, the software simulations overestimates struvite solubility, compared to
315 the experimental data. This could be explained by the complexity of struvite dissolution
316 chemistry, which is only approached in modeling software (Derese, 2018). Furthermore,
317 for several compounds, enthalpy data is missing, which may affect the accurate
318 prediction of the solubility products at reaction temperatures different from 25°C.
319 Finally, several kinetic models are described in literature that allow simulation of
320 dissolution rates. (Lasaga & Lüttge, 2003) introduced the dissolution step-wave model
321 (DSM) which includes effects of crystal defects. Follow-up research is necessary to
322 understand struvite surface topography during dissolution and to validate existing
323 kinetic models for struvite in different physicochemical conditions.

324 **3.2. Struvite as a recovered nutrient source for MP production**

325 The feasibility of growing three metabolic MP types was addressed using struvite as the
326 only P source and a partial N source. With the exception of two of the three AHB
327 communities, growth using struvite was equal to or better than the standard media. This

328 indicates that elevated Mg^{2+} concentrations, originating from dissolved struvite, had no
329 adverse effects on MP growth. In addition, the protein content of the MP produced on
330 struvite was at least as high as that produced on standard media.

331 The growth rate of *C. vulgaris* on struvite was on average 20% higher compared to the
332 control BBM medium, regardless of the N source (NH_4^+ or NO_3^-) (Figure 2 A). More
333 specifically, the maximum specific growth rate on 'struvite+NO_3^-' was 23% higher
334 compared to the NO_3^--based control; however, the difference was not significant ($p >$
335 0.05). Furthermore, the final biomass concentration (1.22 ± 0.11 g DW/L) was
336 comparable to the control (1.23 ± 0.03 g DW/L), as shown in Table 1. For the
337 'struvite+NH_4^+' condition, the maximum specific growth rate was 17% higher ($p <$
338 0.05) compared to the NH_4^+-based control, with a 33% higher final biomass
339 concentration (1.22 ± 0.33 g DW/L) compared to the control (0.83 ± 0.19 g DW/L).
340 This observation could indicate an additional growth-promoting struvite component.
341 However, this effect should be nuanced since not all the repeats in the 'control+NH_4^+'
342 triplicate followed the same behaviour, reflected in the large standard deviation. For the
343 potentially positive effect of struvite, following reasons could be hypothesized. Firstly,
344 additional magnesium in struvite could be a growth promoting factor. However, (Ayed
345 et al., 2016) did not see a difference in the growth rate with increasing Mg^{2+}
346 concentrations. In addition, trace elements in struvite (e.g., iron) could have a positive
347 influence (Wan et al., 2014), while struvite organic matter (up to 1.69% w/w) makes
348 heterotrophic growth possible. The latter is however unlikely, since the dosed struvite
349 contained a maximum of 3.6 mg VS/L (5 mg COD/L), yielding only 2.5 mg/L of
350 heterotrophic biomass, assuming hydrolysis of all organics.

351 With respect to the effect of different nitrogen species, neither of the two nitrogen
352 sources (NH_4^+ or NO_3^-) were more beneficial than the other in terms of growth rate,

while the final biomass concentration was 33% lower on the NH_4^+-based control, but not in the struvite NH_4^+-based experiment (Figure 2 A). Literature confirms this, although the preferred nitrogen source has been reported to be ammonium since its uptake and assimilation demands less energy (Markou et al., 2014). The highest protein content (44.0 ± 4.9% DW) was noted during stationary growth on 'struvite+NH_4^+' and was 31% higher compared to the protein content during stationary growth on the NH_4^+-based control. This resulted in a protein productivity of 0.020 g/L/d (Table 1). These findings suggest that ammonium, including the derived from struvite, is equally accessible for *Chlorella* growth, with limited effect in growth rate or protein content.

For the prokaryotic cyanobacterium *Limnospira* sp., the maximum specific growth rate on struvite was equivalent to the control (Figure 2 B) and the final biomass concentration (1.76 ± 0.17 g DW/L) was not significantly different from the control (1.95 ± 0.06 g DW/L) ($p > 0.05$). Indeed, the dosed struvite contained a maximum of 9 mg VS/L (13 mg COD/L), potentially yielding only 6.5 mg/L of heterotrophic biomass. Ammonium derived from struvite was depleted after 2 days, after which nitrate was also consumed. Furthermore, the amount of dissolved phosphorus reached 17.9 ± 0.56 mg P/L after 2 hours, indicating rapid phosphate availability (SM Figure S8). Growth on struvite did not result in a significant difference in protein content. The highest protein content (49.0 ± 0.8% DW) was observed during linear growth on struvite (Table 1), which was 24% higher compared to the protein content during stationary growth ($p < 0.05$). The highest protein productivity was observed during linear growth in the control treatment (0.064 g/L/d). Both growth rates and protein content were slightly higher than results obtained when pH was not controlled (Markou et al., 2019), which could indicate that a pH control system would be favorable for spirulina MP production on struvite.

The second metabolic type, photoheterotrophic PNSB *R. palustris*, did not present a significant difference in growth rate on struvite ($p > 0.05$), although it was 43 ± 19% higher compared to the control (Figure 2 C). Since an increase in the PNSB growth rate has been observed previously with increasing Mg^{2+} concentrations (Wu et al., 2015), the dose of 25 mg Mg^{2+}/L supplemented with struvite could be the reason for this increase. In contrast, the final biomass concentration on struvite (1.42 ± 0.04 g DW/L) was 10% lower compared to the control (1.57 ± 0.03 g DW/L). The highest protein content (43.3 ± 0.7% DW) observed during stationary growth on struvite was 34% higher than during linear growth and 11% higher than the control during stationary growth ($p < 0.05$). The highest protein productivity was obtained during linear growth on struvite (0.027 g/L/d; Table 1).

Within the last metabolic growth type, the maximum specific growth rates on struvite of the two low-rate AHB communities originating from wastewater treatment at 28°C (potato 1) and at 20°C (potato 2) were 36% and 28% lower compared to the control ($p < 0.05$), respectively (Figure 2 D and E). Final biomass concentrations were 64% and 42% lower compared to the control for potato 1 (3.85 ± 0.10 g DW/L) and potato 2 (2.54 ± 0.60 g DW/L), respectively. This could not be attributed to a lack of phosphorus since 13 ± 3.4 mg P/L was available for the AHB at 28°C after 1 hour (Figure 3 A). The earlier discussed struvite dissolution tests demonstrate that at 20°C, P release rates are only 37% lower, which still results in 8 mg P/L after 1 hour. Furthermore, free ammonia only reached 4.3 and 2.4 mg NH_3-N/L at 28°C and 20°C, respectively, which is too little to result in adverse effects on growth. Finally, since (Lock et al., 2014) observed a toxic effect of magnesium on *E. coli*, magnesium toxicity was tested up to 7.6 mM Mg^{2+} (195 mg Mg^{2+}/L), which did not show any adverse effects (SM Figure S9).

The average protein content during stationary growth was higher compared to linear growth in all treatments, but a significant difference (+44%) was only observed for the potato 2 AHB on struvite ($p < 0.05$). The highest protein productivity was observed for the potato 1 AHB control during linear growth (4.4 g/L/d; Table 1).

In contrast to the low-rate AHB, the high-rate AHB pre-grown on wheat flour presented a 10% higher maximum specific growth rate on struvite compared to the control, although this was not significant ($p > 0.05$) (Figure 2 F). The final biomass concentration for struvite (1.97 ± 0.26 g DW/L) was not significantly different from the control (2.21 ± 0.52 g DW/L). After 1 hour, the P concentration reached 29 ± 8.3 mg P/L, indicating a sufficient rate of nutrient release from struvite (Figure 3 B). Similar to the low-rate consortia, a 43% and 23% higher protein content was observed during stationary growth compared to linear growth for the control and struvite treatments, respectively ($p < 0.05$). Moreover, a 36% higher protein content was observed during linear growth on struvite compared to the control ($p <0.05$), resulting in a protein productivity of 1.9 g/L/d (Table 1).

3.3. Towards large-scale MP production on struvite

Since P release rates from struvite were sufficiently high to support the rapid growth of a diverse set of metabolic MP types, a predissolution tank is redundant, which enables the cheaper strategy of dosing struvite directly into the bioreactor. Only an elevated Ca^{2+} levels could limit the P dissolution rate and restrict P uptake rate of the AHB consortia. In case higher struvite dissolution rates are required, struvite milling, EDTA, or inorganic carbon addition could still be performed. However, these steps may increase production costs.

On a larger scale, residual nutrients in the cultivation liquid can be reused after biomass harvesting along with fresh nutrient dosage. However, repeated addition of struvite to the (partially) reused cultivation medium implies the accumulation of unconsumed magnesium. Considering a medium recuperation efficiency of 90%, an amount of 0.14, 0.38, 0.25 and 100 mg Mg^{2+}/L would accumulate daily for *C. vulgaris*, *Limnospira* sp., *R. palustris* and the AHB, respectively (Calculations S1). Even though no toxic effects were observed for AHB up to 7.6 mM Mg^{2+} (195 mg Mg^{2+}/L; SM Figure S9), further research should investigate the potential of magnesium-related growth inhibition. Current struvite market prices of 0-100 €/ton or 0-0.8 €/kg of P (Muys et al., 2021) are lower than the price of conventional P fertilizers, such as KH_2PO_4, diammonium phosphate (DAP) and triple superphosphate (TSP). In the end, the P, N, Mg and K related medium costs to produce 1 ton DW of MP biomass can be lowered by 34-65%, compared to the cost of conventional nutrients, depending on the applied conventional P source (KH_2PO_4, TSP or DAP). Hereby, 100% of the P and Mg^{2+} and part of the N is replaced with struvite, at current price ranges for chemicals and struvite. This makes struvite a sustainable alternative for MP cultivation with no extra cost for commercial phosphorus and nitrogen fertilizers.

Along with low content in heavy metals, microbiological assessments performed in struvite from food and beverage wastewater show that pathogen levels are generally lower than legal requirements (Muys et al., 2021). Moreover, simple procedures like struvite storage, washing, or drying at temperatures above 35 ºC are effective reducing pathogens in struvite (Decrey et al., 2011; Muys et al., 2021). However, micropollutants and other pathogens originated from other types of wastewaters could end up in the recovered struvite. In essence, the safety of the final product (microbial protein) should be closely examined in the future to sufficiently minimize risks.

Several microbial protein products from AHB communities are being already commercialized (i.e., ValProMic, from Avecom; HiTechPro, from iCell), and could benefit from the use of struvite as sole P source and partial N source. It was stated in Muys et al., 2020 that the quality of the MP coming from AHB cultures may be more linked to the culture operational parameters rather than microbial community composition. These results are supported by recent studies on microbial protein from AHB grown in brewery wastewater (Papini et al., 2022). Nonetheless, further research in the microbial composition of the community would be necessary to understand the relation with the quality and the influence of the use of struvite in it.

Finally, besides microalgae, PNSB, and AHB, other microorganisms like methane oxidizing bacteria (MOB) and hydrogen oxidizing bacteria (HOB) have been shown to be suitable to produce SCP (Kerckhof et al., 2021; Matassa et al., 2015). Even though the growth rates of MOB ($0.04h^{-1}$) and HOB ($0.4h^{-1}$) are much lower than the obtained in this study when using struvite as P/N source ($6\text{-}8h^{-1}$ for AHB communities), the effect of their specific media in struvite dissolution and growth rates should be investigated.

4. Conclusions

The low content in metals and pathogens, together with high dissolution rates determined in this study, establish struvite as a promising P source for MP production. Therefore, it can contribute towards the upgrade of wastewater to MP, by providing P when it is deficient in the reactor influent. Struvite performs good as a sole P and partial N source for the three classes of MP tested, including microalgae, purple non-sulfur bacteria and fast-growing aerobic heterotrophic bacteria, promoting similar biomass and

476 protein production compared to conventional P sources. Struvite contact surface area,
477 salinity, inorganic carbon and EDTA concentrations are the most effective parameters to
478 increase struvite dissolution rates, and therefore their use can be considered when high
479 dissolution is needed in MP reactors.

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