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Goonesekera, Estelle Maria; Tsapekos, Panagiotis; Angelidaki, Irini; Valverde Pérez, Borja

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Use of struvite and PCP as phosphorus sources for microbial protein production

*E. M. Goonesekera, P. Tsapekos, I. Angelidaki, B. Valverde-Pérez**
Technical University of Denmark, Department of Environmental Engineering, Building 113, Bygningstorvet, DK-2800 Kgs. Lyngby, Denmark (Corresponding author: bvape@env.dtu.dk)*

Introduction: Microbial protein (MP) has regained popularity as an alternative to traditional protein, being methane oxidising bacteria (MOB) one option for its production. Current production processes rely on fossil fuels, although there is an increasing trend of using residual streams to obtain a more circular production model. Up to now, most research on nutrient recovery via MOB has focused on the nitrogen and methane sources, while little attention has been payed to phosphorus, also crucial for protein production. This study aims to understand the phosphorus requirements of methane oxidising bacteria, thus allowing an optimised use of phosphorus recovered at WWTP.

Methods and data: Batch experiments were run with different residual phosphorus sources and testing ammonia and nitrate as nitrogen sources in gas tight serum bottles. Inoculum was obtained from fermenters growing MOB enrichments. Optical density, pH and concentration of soluble NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}, COD, TN and TP were monitored in the liquid phase, while O_2, CH_4 and CO_2 content was tracked in the headspace. Each experimental condition was run in duplicate. At least three consecutive runs for each condition were carried out. Microbial protein and amino acid profile were analysed at the end of the batches. Struvite was collected from Helsingør Forsyning A/S (Helsingør, Denmark) and PCP was provided by EasyMining Sweden AB. Both were analysed for heavy metal content. Both struvite and PCP cannot be dissolved in water with characteristics that promote MOB growth. Thus, a preliminary screening of dissolution methods was performed, including different temperatures (25 and 37°C), pH levels (2 and 5) and acids (H_2SO_4 and HCl).

Results and Discussion: pH has a larger effect than temperature on phosphate dissolution. PCP dissolved best at pH 2 and 25°C, whilst struvite did at 37°C. Recommended dissolution times are 4 h for struvite and 30 min for PCP. Using H_2SO_4 had a detrimental effect on MOB due to the resulting high ionic strength. Thus, HCl was preferred and used for the preparation of the cultivation media with PCP and struvite. Although lower, growth rates were not statistically different when supplying phosphorus from PCP or struvite (Table 1). Lowest yield was for PCP with NH_3, although differences were not significant. It should be noted that the appearance of this batch was cloudy due to precipitation of NH_4^+ and PO_4^{3-}. This could have generated cellular stress, which could lead to lower biomass yields. Indeed, for this batch the highest concentrations on soluble COD, TN and TP were detected by the end of the experiment, suggesting incomplete methane oxidation to different organic by-products, like external polymeric substances or low molecular weight organic acids (28.2 ± 5.4 % of the fed methane was converted into soluble organics). When using PCP as phosphorus source, the protein content was lower than in the controls. The batch where struvite was used as phosphorus and nitrogen source showed the highest protein content. Protein content higher than 70% of CDW is desirable for using microbes as feed ingredient. However, the use of residual resources from anthropogenic origin (i.e., sewage) for producing feeds and foods is currently banned. Indeed, biomass harvested from the control with NO_3^- and NH_3 with struvite exceeded the limit on lead content for feeds. Biomass from NH_3 with struvite also exceeded the limit for cadmium. Thus, another promising application is the hydrolysis of the protein content to produce amino acid hydroxylates, which can be used as plat bio-stimulant with a market value higher than mineral fertilizers.

Take-home messages: Key findings of our work are: 1) both PCP and struvite are good phosphorus sources for production of microbial protein via methanotrophs cultivation; 2) struvite yields highest protein content, although it has high content of cadmium and lead; 3) PCP induces microbial stress, which results in lower biomass yields and high conversion of methane into dissolved organic carbon, which needs to be treated before effluent discharge.

Table 1. Growth rates, biomass yields on methane, nitrogen and phosphorus and protein content.

Condition	Growth rate (d^{-1})	$Y_{CDW/CH4}$	$Y_{CDW/N}$	$Y_{CDW/P}$	Protein content (% CDW)
Control NH_3	1.11 ± 0.00	0.24 ± 0.03	14.7 ± 1.1	33.0	52.7 ± 1.6
Control NO_3^-	1.06 ± 0.07	0.26 ± 0.02	22.5 ± 1.2	93.5 ± 3.0	62.0
PCP NO_3^-	0.88 ± 0.05	0.29 ± 0.03	24.3 ± 1.1	67.3 ± 20.3	48.9 ± 1.5
Struvite NH_3	0.72 ± 0.17	0.26 ± 0.06	16.2 ± 3.1	26.8 ± 1.3	80.4 ± 4.7
PCP NH_3	-	0.21 ± 0.06	16.18 ± 0.57	22.0 ± 0.6	12.1 ± 3.2

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