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Phosphate Turnover During Anaerobic Digestion of Chicken, Pig and Dairy Manure

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Glossary

AD	Aanaerobic digestion
CAL-P	Calcium acetate lactate soluble phosphate
CEN	Supernatant of centrigufed sample
CMS	Chicken manure mixed with straw
CSTR	Continuous stirred tank reactor
D105	Sample dried at 105 °C
D50	Sample dried at 50 °C
DIN	Deutsches Institut für Normung
DM	Dry matter
DMS	Dairy manure mixed with straw
DTP	Dissolved total phosphorus
EU	European Union
FZD	Freeze dried sample
H_2O-P	Water soluble phosphate
H_2O-Fil	Filtrate after H_2O extraction
HAP	Hydroxyapatite
HCl-P	Hydrochloric acid extractable P
HCl-Fil	Filtrate after HCl extraction
HRT	Hydraulic Retention Time
IP	Inorganic phosphorus

MAP	Magnesium ammonium phosphate
$NaHCO_3$-P	Sodium bicarbonate soluble phosphate
$NaHCO_3$-Fil	Filtrate after $NaHCO_3$ extraction
NaOH-P	Sodium hydroxide extractable phosphate
NaOH-Fil	Filtrate after NaOH extraction
OLR	Organic loading rate
P	Phosphorus or phosphate, defined by chapter
P_O	Organic phosphate
P-SumD105	Sum of Hedley fractions of samples dried at 105°C
P-SumD50	Sum of Hedley fractions of samples dired at 50°C
P_i	Inorganic phosphate
P_{tot}	Total phosphate
PDT	Post digestion traetment
PP	Particulate phosphorus
PSumFZD	Sum of Hedley fractions of freeze dried samples
SEM	Scanning electron microscopy
SMT	Standard measurements and testing program
TK	Total potassium
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
UD	Undried digestate
UD-16h	Undried digestate shaken with extractant for 16 hours

UD-4h	Undried digestate shaken with extractant for 4 hours
UD-2h	Undried digestate shaken with extractant for 2 hours
UD-1h	Undried digestate shaken with extractant for 1 hour
VS	Volatile solids
XRD	X-ray diffraction

1 Introduction

1.1 Nutrients in agriculture

Life on earth especially depends on the three macro nutrients nitrogen, potassium and phosphorus. Nature, being a closed cycle, continuously depletes and replenishes the nutrient pools through sustenance of life. At the same time, nutrients are the limiting factor for growth. Simplified it can be stated that additional nutrients in the cycle lead to increased growth. This correlation led to the extensive use of fertilizer in agricultural systems in order to maximize crop yield. Especially the application of nitrogen fertilizer led to a significant increase in production in the beginning of the 20^{th} century (Cassman & Dobermann, 2022). In traditional agriculture, nutrients would be recirculated to the field in the form of feces and crop residues. However, with growing demand for produce, fertilizer demand rose as well. For nitrogen, this demand could be readily met after the innovation of the Haber Bosch process. In this process nitrogen gas (N_2), which represents over 78% of air (Stevens, 2019), is converted into NH_3 under high pressure and temperature, which can then be processed into the desired final product, e.g. urea (Vojvodic *et al.*, 2014). Phosphorus fertilizer, in the following called phosphate due to its natural occurrence as such, faces an entirely different situation. It cannot be produced from an infinite source like air as nitrogen can. Instead, it is mined as phosphate rock worldwide to cope with the demand. However, world reserves are firstly limited and secondly unevenly distributed over the globe. Of the currently well-known reserves, 71% (50 Gg) are located in Morocco and the Western Sahara (U.S. Geological Survey, 2022). This leads to undesirable geopolitical dependencies. Additionally, the reserves are largely untapped because they contain high amounts of cadmium and other impurities (Niño-

Savala *et al.*, 2019). These need to be extracted before phosphate can be used as a fertilizer, making the process very expensive. However, the recent discovery of huge phosphate reserves in Norway have drawn a lot of attention. The phosphate rich mineral layers are said to be as large as 70 Gg of phosphate rock, thereby doubling the known world reserves. However, the analysis of phosphate content and possible heavy metal impurities is still ongoing (Bushuev, 2021).

Not only is more fertilizer used in today's agriculture but globalization has led to a disruption of nutrient cycles with nutrient consumption being displaced from their point of application (Bouwman *et al.*, 2009). This displacement is especially profound in livestock farming where large amounts of nutrients are imported in the form of feedstuff. Only a small fraction of these are converted into meat and transported off-site as produce. In an ideal scenario the remaining nutrients in form of feces and slaughterhouse waste would be converted to fertilizer and transported back to the point of feedstuff origin. However, this is currently not practiced because it is expensive. Instead, regions with high livestock density face increasing issues of nutrient surplus.

1.2 Nutrient recovery from biogas digestate

Exporting nutrients is expensive because of the high water content in manure. Additionally, open manure storage leads to high greenhouse gas emissions in form of methane (CH_4) and nitrous oxide (N_2O) as well as odor emissions through ammonia (NH_3) emissions. Anaerobic treatment of manure has the target to reduce these emissions and produce a combustible gas mixture consisting of approximately 60% methane (CH_4) and 40% carbon dioxide (CO_2). This gas can be used energetically to produce electricity and heat. The gas production is the result of decomposition of organics by microorganisms, leading to a biological stabilization of manure. Greenhouse gas and odor emissions during storage and application of anaerobically digested manure (digestate) are significantly reduced compared to manure (Clemens *et al.*, 2006). Furthermore, the nutrients of the input substrate of the digestion remain in the digestate, making it a macro and micro nutrient rich fertilizer. Especially nitrogen in digestate shows better plant availability compared to manure because 45% is present as NH_4^+ (Mazzini *et al.*, 2020). Regarding chemical phosphate composition, the digestion process is considered a black box where only inputs and outputs are known (Bachmann *et al.*, 2016; Mazzini *et al.*, 2020).

The high costs for digestate transportation lead to the application on agricultural fields in biogas plant proximity. In regions with high livestock intensity this can lead to nutrient surplus if not monitored properly. Faulty monitoring leads to the addition of more nutrients to the soil than are extracted by harvest. This leads to environmental issues such as eutrophication by run-off and groundwater nitrification by leaching. Especially the issue of nitrification of groundwater through excessive application of nitrogen fertilizer has drawn public attention in the past, leading to the European Nitrate Directive (91/676/EEC) (European Union: Council of the European Union, 1991), whose regulations were implemented in Germany by the Fertilizer Ordinance. This ordinance indicates that the application of nitrogen in form of organic fertilizer (i.e. manure and digestate) on agricultural soil must be limited to 170 kg ha^{-1}.

As a result of the nitrate directive, several state-of-the-art technologies for nitrogen recovery from digestate are available. The most common application is a stripping process, in which nitrogen is stripped from the digestate in form of NH_3 by air insertion into the liquid combined with pH or temperature increase, or a combination of the three. Afterwards NH_3 is scrubbed from the off-gas with H_2SO_4 or water (Latif *et al.*, 2015). The resulting product, ammonium sulfate solution or ammonia solution, is a highly transportable mineral fertilizer. The stripping process normally requires a solid-liquid separation beforehand and only the liquid fraction is treated. In case of Germany this not only has the advantage that it can be transported over long distances but also that it does not fall under the 170 kg ha^{-1} limitation of the Fertilizer Ordinance.

The most commonly used equipment for solid-liquid separation is a screw press. In a screw press, roughly 40% of the total dry matter (DM) ends up in the filter cake and can be transported economically over longer distances. The solids contain about 40% of total P, 20-25% of total nitrogen and 8% of potassium from the fresh digestate (Bachmann *et al.*, 2016; Mazzini *et al.*, 2020). The liquid fraction has a DM content of about 5% and is rich in NH_4^+ nitrogen as well as some phosphate and potassium. It can either be stripped or needs to be applied in biogas plant proximity due to the high water content.

Other technologies such as thickening and drying aim to concentrate nutrients through thermal treatment of digestate. However, because of their high energy demand and necessary secondary process steps such as treatment of the off-air from driers with a nitrogen scrubber (Maurer & Müller, 2012), they have not gained large popularity.

1.3 Framework for phosphate in agriculture

Until the renewal of the Fertilizer Ordinance in 2017 (publication language German: Bundesministerium der Justiz (2017)) phosphate was of lesser concern for biogas plants because only nitrogen application was limited. Additionally, phosphate is not as mobile in soil as nitrogen and, therefore, the risk of groundwater contamination by leaching is not as high. However, issues with run-off and soil erosion led to high inputs of phosphate into surface water bodies as well as groundwater at narrow depth. Phosphate is thought to be the driver of eutrophication because it is the limiting nutrient for algal growth in natural waters. Additionally, with increasing global warming eutrophication will increase as well, making it even more important to reduce anthropogenic nutrient input into water bodies (Nazari-Sharabian *et al.*, 2018; Rodgers, 2021). As a result, the Fertilizer Ordinance of 2017 includes application limits for phosphate, which depend on the crop rotation, harvest and soil class. The soil class is based on the amount of calcium acetate lactate soluble phosphate (CAL-P). Based on this level German soils are divided into the classes A to E with A being phosphate depleted and E oversaturated soil. The VDLUFA has lowered the threshold values for these classes in 2018, resulting in an indirect further restriction of phosphate application to agricultural fields by digestate application (Wiesler *et al.*, 2018).

Meanwhile, the European Union (EU) placed phosphate on the list of critical raw materials in 2014 (European Union: European Commission, 2014) and it has not been removed since (European Union: European Commission, 2020). This is owed to its geographical distribution with no major resources located in the EU (potentially the discovery of phosphate reserves and start of mining activity in Norway resolves this issue). Manure and sewage sludge are considered to be huge phosphate reserves in the EU. According to Roskosch and Heidecke (2019), 444 Tg of phosphorus from manure and 125 Tg of phosphorus from digestate as well as 50 Tg from sewage sludge and 50 Tg from sewage sludge ash can theoretically be recovered annually in Germany alone. To tap into this potential, Germany has implemented a renewed sewage sludge ordinance, which forces sewage sludge treatment plant operators to recover phosphorus from sludge with a phosphorus content above 20 g $\mathrm{kg}_{\mathrm{DM}}^{-1}$ (AbfKlärV (Bundesministerium für Umwelt Naturschutz und Reaktorsicherheit, 2017)).

The regulatory pressure has led to a number of innovative processes for phosphate recovery, of which TetraPhos and Phos4Green have reached industrial scale in Germany. The TetraPhos process aims to produce pure phosphoric acid from sewage sludge ash, which can afterwards be used in fertilizer and food production or industrial applications. Several side products arise during recovery such as gypsum, salt-solution and non usable landfill waste. A large scale plant has been commissioned in 2021 in Hamburg (Bertau *et al.*, 2018; Remondis SE & Co. KG, n.d.). The Phos4Green process aims to produce a fertilizer granulate from sewage sludge ash with adjustable phosphate availability and without producing any side products (Glatt, n.d.). Like most approaches for phosphate recovery, these two processes first acidify the sewage sludge ash to mobilize phosphate. Afterwards, the liquid is either purified to remove salt ions and produce pure phosphoric acid (TetraPhos) or the liquid is dried in a spray drying system to produce fertilizer granules from the remaining solids in the fluid (Phos4Green). The advantage in treating sewage sludge ash is that the ash is a highly concentrated, phosphate rich substrate and it is produced in large amounts centrally. When treating biogas digestate the volume is firstly smaller and decentralized and secondly the phosphate concentration is lower. However, the acceptance among farmers for digestate derived phosphate fertilizer is much greater than derived from sewage sludge. Additionally, the carbon in digestate is a valuable humus builder in agricultural soil (Golkowska *et al.*, 2014), whereas organics and germs in sewage sludge are usually considered a health risk. Therefore, thermal conversion of carbon before phosphate recovery from digestate in not desired. The so called NuTriSep process, developed by Geltz Umwelt-Technologie considers this and aims to recover phosphate and nitrogen fertilizer directly from biogas digestate as ammonium sulfate solution and phosphate salt granules. The process is currently being tested in southern Germany (Wraneschitz, 2020). Furthermore, not only high-tech solutions with a strict separation of nutrients from other components but also low-tech solutions such as simple solid-liquid separation, as described, should be considered for nutrient concentration in specific digestate fractions.

1.4 Plant availability of phosphate

Phosphate availability to plants is a much-debated topic in plant and soil science. As a result, many different methods for the analysis of plant availability exist today. The Mehlich-3-P, CAL-P, Olsen-P and Citric Acid-P method are among the most popular. They are all wet extraction methods that intent to extract only the phosphate fraction from the sample that the plant can acquire from the soil. However, the analyzed value commonly deviates from the real value measured in pot trials because plant availability depends on many factors such as the chemical and morphological soil composition and soil pH as well as the crop itself as some cultivars can mobilize phosphate in soil (Kamh *et al.*, 2002; Lambers *et al.*, 2006; Wuenscher *et al.*, 2016).

1.5 Sequential extraction of phosphate

The essential parameter for plant nutrition is the plant availability of phosphate, which can be estimated by the mentioned methods. However, methods using sequential chemical extraction have been developed to quantify the entire spectrum of phosphate salts in soil. In these methods different solvents are used sequentially with the same sample to extract different phosphate types successively. Among others, the Standard Measurements and Testing protocol (SMT) and the Hedley fractionation have gained popularity. The SMT is used for the analysis of freshwater sediments and separates phosphates into total phosphate (P_{tot}), inorganic phosphate (P_{i}), organic phosphate (P_{O}), non-apatite inorganic phosphate and apatite phosphate (European Commission *et al.*, 2001; Pardo *et al.*, 2004). The Hedley fractionation developed by Hedley *et al.* in 1982 differentiates between resin extractable phosphate (Resin-P), sodium bicarbonate extractable phosphate ($NaHCO_3$-P), phosphate release by fumigation with $CHCl_3$ and extraction with $NaHCO_3$, sodium hydroxide extractable (NaOH-P), sonicated NaOH extractable phosphate (sonicate/NaOH-P), hydrogen chloride extractable phosphate (HCl-P) and phosphate extractable by boiling with H_2SO_4 (Residual-P). Resin-P represents the amount of phosphate in soil solution and, therefore, represents readily available phosphate. In the following fractions, the used solvents permit conclusions on the associated metal ions. $NaHCO_3$-P is phosphate loosely adsorbed to particle surfaces and easily released into solution. NaOH-P is associated with Fe^{2+} and Al^{3+} while HCl-P is associated with Ca^{2+} and Mg^{2+}. The difference of sonicated/NaOH-P and NaOH-P represents the amount of P_O. The difference of $NaHCO_3$-P and $CHCl_3/NaHCO_3$, on the other hand, represents microbial phosphate. In conclusion these methods give a quantitative overview of the phosphate groups in soil but do not identify individual phosphate salts. For a general overview of phosphate fixation by salt formation and determination of predominant salts this is not necessary. Furthermore, it can be seen by the fractions that the resolution of inorganic phosphate analysis is higher in the Hedley fractionation compared to SMT. Additionally, the first two fractions of the Hedley fractionation (H_2O-P and $NaHCO_3$-P) are considered to represent most of the plant available phosphate as they resemble Olsen-P. This information is not available from the SMT method.

1.6 Phosphate turnover during anaerobic digestion

Literature on phosphate fractionation in biogas digestate is scarce. Dou *et al.* (2000) have described their adjusted Hedley fractionation for the analysis of animal manure. In this method samples were freeze-dried and afterwards extracted with H_2O, $NaHCO_3$, NaOH, HCl and H_2SO_4. The extracts were than analyzed by ICP-OES for P_{tot} and photometrically for

P_i. Their results suggest that 53 - 72% of total phosphates in dairy and poultry manure are H_2O and $NaHCO_3$ soluble and 14 to 20% of phosphates are organic. Other authors used this method for the analysis of digestate from two different biogas plants. Their results showed that roughly 70% to 90% of phosphates in digestate are extractable with H_2O and $NHCO_3$ and organic phosphates represent only 5 to 18% of total phosphate in digestate (Bachmann *et al.*, 2016). These results are in line with Mazzini *et al.* (2020) who investigated the effect of anaerobic digestion (AD) on the Hedley fractions (based on the adapted Hedley fractions according to (Turner & Leytem, 2004)), comparing input and output of biogas plants. However, their results were non-conclusive due to a high dependence on plant operation and feedstock. In four out of six investigated plants they found that AD led to a decrease in $NaHCO_3$-P and increase in Fe/Al associated P, meaning NaOH-P, especially with high amounts of manure as input material (Mazzini *et al.*, 2020). This is in line with Möller and Müller (2012), who concluded that AD leads to a decrease in plant available phosphate. However, in one biogas plant Mazzini *et al.* (2020) found that AD has the opposite effect of an increase in $NaHCO_3$-P and in another they found no significant difference between in- and output of the plant. They concluded that the increase of $NaHCO_3$-P in one plant was connected to a high amount of recirculated digestate in the input. From this it could be concluded that an increase in hydraulic retention time (HRT) would lead to an increase in readily available phosphate (H_2O-P and $NaHCO_3$-P) due to a higher degradation of organics leading to a higher release of mineralized phosphate. However, since process conditions, especially above pH 7 during AD favor the formation of insoluble calcium and magnesium phosphates it seems unlikely that an extended reaction time will increase available phosphate.

Regarding phosphate turnover, AD remains a black box. While some evidence suggests a mineralization of phosphates due to decomposition of organic matter the kinetics of mineralization and the effects of process parameters on the formation chemistry remain unknown. However, with this information biogas plants could enhance their contribution to closed nutrient cycles in agriculture and a recovery of phosphate could be facilitated because the biogas plants could produce targeted phosphate compositions in the digestate. This way the use of conventional phosphate fertilizer could be reduced and, in case of phosphate recovery, the chemical input for pH adjustment could be optimized. Therefore, the objective of this work is to elucidate the changes of phosphate salt distribution during AD and their dependency on process parameters.

References

Bachmann, S., Uptmoor, R., & Eichler-Löbermann, B. (2016). Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Scientia Agricola, 73(1), 9–17. https://doi.org/10.1590/0103-9016-2015-0069

Bertau, M., Fröhlich, P., & Eschment, J. (2018). Phosphatrecycling-Königsweg zu einer nachhaltigen Phosphorversorgung Phosphorrückgewinnung Phosphatreycling-Königsweg zu einer nachhaltigen Phosphorversorgung. In O. Holm, E. Thomé-Kozmiensky, P. Quicker, & S. Kopp-Assenmacher (Eds.), Verwertung von klärschlamm (pp. 519–533). Thomé-Kozmiensky Verlag GmbH.

Bouwman, A. F., Beusen, A. H. W., & Billen, G. (2009). Human alteration of the global nitrogen and phosphorus soil balances for the period 1970-2050. Global Biogeochemical Cycles, 23(4), n/a–n/a. https://doi.org/10.1029/2009GB003576

Bundesministerium der Justiz. (2017). Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen (Düngeverordnung - DüV). https://www.gesetze-im-internet.de/d_v_2017/BJNR130510017.html

Bundesministerium für Umwelt Naturschutz und Reaktorsicherheit. (2017). Verordnung zur Neuordnung der Klärschlammverwertung - AbfKlärV. http://www.bgbl.de/xaver/bgbl/start.xav?startbk=Bundesanzeiger%7B%5C_%7DBGBl%7B%5C&%7DjumpTo=bgbl117s3465.pdf

Bushuev, M. (2021). Rohstoff-Fund in Norwegen lässt EU hoffen. Deutsche Welle. https://p.dw.com/p/3mmpR

Cassman, K. G., & Dobermann, A. (2022). Nitrogen and the future of agriculture: 20 years on. Ambio, 51(1), 17–24. https://doi.org/10.1007/s13280-021-01526-w

Clemens, J., Trimborn, M., Weiland, P., & Amon, B. (2006). Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. Agriculture, Ecosystems & Environment, 112(2-3), 171–177. https://doi.org/10.1016/j.agee.2005.08.016

Dou, Z., Toth, J. D., Galligan, D. T., Ramberg, C. F., & Ferguson, J. D. (2000). Laboratory procedures for characterizing manure phosphorus. Journal of Environmental Quality, 29(2), 508–514. https://doi.org/10.2134/jeq2000.00472425002900020019x

European Commission, Innovation, Directorate-General for Research, Ruban, V., Rauret, G., López-Sánchez, J., Muntau, H., & Quevauviller, P. (2001). The certification of the extractable contents (mass fractions) of phosphorus in freshwater sediment following a five-step extraction procedure : BCR 684. (Tech. rep.). Publications Office.

European Union: Council of the European Union. (1991). Council Directive 91/676/EEC of 12 December 1991 concerning the protection of water against pollution caused by nitrates from agricultural sources. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:31991L0676

European Union: European Commission. (2014). Communication from the commission of the European parliament, the council, the European economic and social committee and the committee of the regions On the review of the list of critical raw materials for the EU and the implementation of the Raw Materia. COM/2014/0297 final; European Union: European Comission.

European Union: European Commission. (2020). Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions Critical Raw Materials Resilience: Charting a Path towards greater Security and Sustainability. COM/2020/474 final; European Union: European Commission.

Glatt. (n.d.). Technology - Glatt Phos4green. Retrieved June 1, 2021, from https://phos4green.glatt.com/technology/

Golkowska, K., Vázquez-Rowe, I., Lebuf, V., Accoe, F., & Koster, D. (2014). Assessing the treatment costs and the fertilizing value of the output products in digestate treatment systems. Water Science and Technology, 69(3), 656–662. https://doi.org/10.2166/wst.2013.742

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal, 46(5), 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Kamh, M., Abdou, M., Chude, V., Wiesler, F., & Horst, W. J. (2002). Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. Journal of Plant Nutrition and Soil Science, 165(5), 566–

572. https://doi.org/10.1002/1522-2624(200210)165:5<566::AID-JPLN566>3.0.CO;2-O

Lambers, H., Michael W., S., Michael D., C., Stuart J., P., & Erik J., V. (2006). Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Annals of Botany, 98(4), 693–713. https://doi.org/10.1093/aob/mcl114

Latif, M. A., Mehta, C. M., & Batstone, D. J. (2015). Low pH anaerobic digestion of waste activated sludge for enhanced phosphorous release. Water Research, 81, 288–293. https://doi.org/10.1016/j.watres.2015.05.062

Maurer, C., & Müller, J. (2012). Ammonia (NH_3) emissions during drying of untreated and dewatered biogas digestate in a hybrid waste-heat/solar dryer. Engineering in Life Sciences, 12(3), 321–326. https://doi.org/10.1002/elsc.201100113

Mazzini, S., Borgonovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., & Adani, F. (2020). Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. Science of The Total Environment, 734, 139284. https://doi.org/10.1016/j.scitotenv.2020.139284

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242–257. https://doi.org/10.1002/elsc.201100085

Nazari-Sharabian, M., Ahmad, S., & Moses, K. (2018). Climate Change and Eutrophication: A Short Review. Engineering, Technology & Applied Science Research, 8(6), 3668–3672. https://doi.org/10.5281/zenodo.2532694

Niño-Savala, A. G., Zhuang, Z., Ma, X., Fangmeier, A., Li, H., Tang, A., & Liu, X. (2019). Cadmium pollution from phosphate fertilizers in arable soils and crops: An overview. Frontiers of Agricultural Science and Engineering, 6(4), 419–430. https://doi.org/10.15302/J-FASE-2019273

Pardo, P., Rauret, G., & López-Sánchez, J. F. (2004). Shortened screening method for phosphorus fractionation in sediments. Analytica Chimica Acta, 508(2), 201–206. https://doi.org/10.1016/j.aca.2003.11.005

Remondis SE & Co. KG. (n.d.). Phosphorus recovery // REMONDIS Sustainability. Retrieved May 3, 2022, from https://www.remondis-sustainability.com/en/acting/phosphorus-recovery/

Rodgers, E. M. (2021). Adding climate change to the mix: responses of aquatic ectotherms to the combined effects of eutrophication and warming. Biology Letters, 17(10). https://doi.org/10.1098/rsbl.2021.0442

Roskosch, A., & Heidecke, P. (2019). Sewage Sludge Disposal in the Federal Republic of Germany. Umweltbundesamt (German Environment Agency).

Stevens, C. J. (2019). Nitrogen in the environment. Science, 363(6427), 578–580. https://doi.org/10.1126/science.aav8215

Turner, B. L., & Leytem, A. B. (2004). Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure. Environmental Science and Technology, 38(22), 6101–6108. https://doi.org/10.1021/es0493042

U.S. Geological Survey. (2022). Mineral commodity summaries 2022. https://doi.org/10.3133/mcs2022

Vojvodic, A., Medford, A. J., Studt, F., Abild-Pedersen, F., Khan, T. S., Bligaard, T., & Nørskov, J. (2014). Exploring the limits: A low-pressure, low-temperature Haber–Bosch process. Chemical Physics Letters, 598, 108–112. https://doi.org/10.1016/j.cplett.2014.03.003

Wiesler, F., Appel, T., Dittert, K., Ebertseder, T., Müller, T., Nätscher, L., Olfs, H.-W., Rex, M., Schweitzer, K., Steffens, D., Taube, F., & Zorn, W. (2018). Standpunkt - Phosphordüngung nach Bodenuntersuchung und Pflanzenbedarf (tech. rep.). Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). Speyer. https://vdlufa2.kdprojekte.de/wp-content/uploads/2021/05/2018_Standpunkt_P-Duengung.pdf

Wraneschitz, H. (2020). NuTriSep: Nährstoffextraktion und Torfersatz aus Gärprodukten. Biogas Journal, (5).

Wuenscher, R., Unterfrauner, H., Peticzka, R., & Zehetner, F. (2016). A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. Plant, Soil and Environment, 61(No. 2), 86–96. https://doi.org/10.17221/932/2014-PSE

2 Objective of the thesis

Kinetics phosphate turnover during anaerobic digestion

The change of composition and turnover rate of phosphate (P) are important attributes for targeted P salt production by AD. A mineralization of P during AD would reduce immediate plant availability but could enhance the mid- to long-term fertilization effect depending on the formed P salts. Therefore, the objective of this work was to analyze the effect of digestion time on the salt formation by quantifying and categorizing formed P salts in a batch digestion over 30 days.

Phosphate analysis in biogas digestate extracts

During the sample extraction for the analysis of kinetics by Hedley fractionation, it was noted that according to the method samples were dried before extraction. Furthermore, the wavelength used for spectral analysis of the samples was not clearly stated in literature. This work had the objective to firstly evaluate the ideal wavelength for analysis between 600 nm and 1000 nm and secondly the effect of sample drying on Hedley fractions. Additionally, the extraction time of the samples was varied to evaluate the ideal exposure time to the solvents. The ultimate objective was to conclude an adapted Hedley fractionation for the analysis of undried digestate based on the results of the forgoing objectives.

Effects of process parameters on phosphate turnover

While the effect of hydraulic retention time (HRT) was analyzed in the first part of this work, the AD process remains a black box as to the effect of other process parameters on P salt formation. Therefore, chicken manure mixed with straw (CMS) and Dairy manure mixed with draw (DMS) were digested in continuous stirred tank reactors and process parameters were varied. The objective was to analyze the effect of the parameters organic loading rate (OLR), temperature and substrate composition on P fractions analyzed by the adapted Hedley fractionation during AD.

3 Influence of anaerobic digestion on the labile phosphorus in pig, chicken, and dairy manure

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Abstract

Phosphorus (P) loss from livestock and poultry industry causes serious threat to agro-ecological environments. Anaerobic digestion (AD), through recycling of P-containing resources and biogas production, prevails as a promising solution to the resource, energy, and environment trilemma. In this study, the dynamic transformation of P in batch AD processes fed with chicken, pig and dairy manures was investigated. Results showed that the Labile-P of total phosphorus (TP) in pig, chicken and dairy manure digestates decreased from 37.35% to 23.79%, 36.79% to 17.29%, and 60.47% to 20.39%, respectively, and was associated with an increase of NaOH-P during the AD process. However, the Labile-P in raw manures ranging from 64.67% to 81.10%, indicated that AD could reduce the pollution risk caused by the overuse of high Labile-P animal manure as fertilizer. Metal ions had a significant influence on P transformation because of their ability to combine with PO_4^{3-}/HPO_4^{2-}. During AD, the species of phosphates increased: $AlPO_4$, $FePO_4$, $Mg_3(PO_4)_2$, $CaHPO_4$, $Mg(NH_4)PO_46H_2O$ and $Ca_{10}(PO_4)_6(OH)_2$ were the main phosphates qualified by X-ray diffraction (XRD). AD produced a satisfactory fertilizer for plants that were able to activate the precipitated P, which could provide readily available N and slow-release P. This study provides a meaningful theoretical guide for recycling P from animal manure resources.

Keywords: P; animal manure management; AD; Transformation mechanism; fertilizer

3.1 Introduction

Phosphorus, essential for meeting the growth requirements of humans, animals and plants, plays an important role in almost all the biochemical reactions (Leytem *et al.*, 2006; Li *et al.*, 1998; Rehim *et al.*, 2012). However, it is also one of the most limited resources due to its uneven spatial distribution (Pradel & Aissani, 2019; U.S. Geological Survey, 2019; Ye *et al.*, 2017). The widely accepted P cycle is typically characterized as an open mode where natural P is supplied from phosphate containing rock through mining, utilized by all living beings and disposed into the environment (Chowdhury *et al.*, 2017). This means that current P sources face two challenges including resource shortage and environmental pollution.

Generally, livestock manure is considered as a nutrient resource bank because it contains a large amount of organic matter, being a source of nitrogen (N), phosphorus (P), and potassium (K) (Tittonell *et al.*, 2010). However, the TP losses in China increased from 0.2 million tons in 1950 to 3.1 million tons in 2010 (Bai *et al.*, 2016a), most of which were lost due to the direct release of animal manure from livestock and poultry farms into water bodies (Bai *et al.*, 2016b; Ma *et al.*, 2010; Meals *et al.*, 2008). The leached and accumulated P would eventually trigger eutrophication and hypoxia (Dalu *et al.*, 2019). Therefore, it is necessary to reduce P losses by means of efficient P recycling from animal manure based on advanced collection, storage, and treatment technologies.

Anaerobic digestion (AD) of animal manure is one of the technologies considered to be an effective solution for nutrient recycling, pollution reduction, and energy production (Arthurson, 2009; Gurmessa *et al.*, 2020; Sobhi *et al.*, 2019). Compared to readily available N and K, P transformation in the AD system shows complicated precipitation mechanisms with different metal ions or struvite formation which depend on the characteristics of digested wastes and the AD process (Cerrillo *et al.*, 2015; Hao *et al.*, 2018; Marti *et al.*, 2008; Möller & Müller, 2012). Möller and Müller (2012) indicated that the positive ions and pH in the AD system strongly influence the turnover and solubility of P, because the fraction of dissolved P mineralizes during anaerobic digestion by reacting with metal ions or binding to particulate solids (Güngör & Karthikeyan, 2008). The high pH of digestate supports the salt formation because the chemical equilibrium of phosphoric acid shifts towards reactive phosphate (Möller & Müller, 2012). Meanwhile, different substrates also result in different changes in P species and their distribution during AD, which would further influence the effective application of solid digestate as organic P fertilizer. So far, few studies have determined the detailed effect of AD on P transformations. Some results showed no effects (Bachmann *et al.*, 2014; Möller & Stinner, 2010), while others indicated AD could reduce P availability for plants (Möller & Müller, 2012). Generally, plant-soil interaction processes can be influenced by different P fractions in the fertilizer, among which, Labile-P is the fraction that can be directly absorbed by most plants to increase their growth in the early stage. Metal-bound P can be used by some special plants such as white lupine and pigeon pea, because the roots of these plants can secrete organic acids to activate the metal-bound phosphate by chelating Fe or Al, or by competing for adsorption sites with phosphate radicals (Ae *et al.*, 1990; Chen *et al.*, 2008b; Gahoonia *et al.*, 2000; Ström *et al.*, 2002). Therefore, it is necessary to understand P distribution and transformation in the AD process and compare the difference in P characteristics between different manure digestates. With this knowledge, it is possible to optimize the plant-soil interaction process by applying the digestate as the organic fertilizer.

In this study, transformation of P in the AD process by treating three different types of animal manures was investigated. Phosphate fractions in different AD phases were classified by the sequential chemical extraction method. The detailed phosphates characteristics were explored by X-ray diffraction (XRD) and scanning electron microscopy (SEM). From the perspective of fertilizer, this study contributes to the improvement of the AD process to produce the satisfactory fertilizer.

3.2 Materials and methods

3.2.1 Substrates and inoculum

Chicken, dairy and pig manure were obtained from the animal farms Liuminying, Doudian and Donghuashan, respectively, located in Beijing, China. Inoculated sludge was obtained from the anaerobic digester treating sewage sludge from a wastewater treatment plant in Beijing. Table 3.1 shows the main characteristics of the three animal manures and inoculated sludge used in this study.

Table 3.1: Typical characteristics of three animal manures and incoculum

Characteristics	Pig manure	Chicken manure	Dairy manure	Inoculated sludge
TS (%)	23.96 ± 0.12^{a}	24.26 ± 1.28^{a}	17.91 ± 0.79^{b}	5.03 ± 0.27^{c}
VS (%)	19.09 ± 0.18^{a}	14.75 ± 0.25^{b}	12.76 ± 0.47^{b}	2.87 ± 0.43^{c}
TP (mg/g TS)	15.37 ± 0.55^{a}	12.34 ± 0.39^{b}	3.44 ± 0.18^{c}	2.03 ± 0.35^{c}
TN (mg/g TS)	30.07 ± 0.86^{a}	49.95 ± 1.01^{b}	20.21 ± 0.56^{c}	5.42 ± 0.54^{c}
TK (mg/g TS)	13.89 ± 0.32^{b}	18.12 ± 0.43^{a}	6.21 ± 0.62^{c}	5.85 ± 0.62^{c}

Note: Means of the same row with different lowercase (a, b, c) are significantly different ($p < 0.05$) using Tukey-test

3.2.2 Experimental design

Brown glass bottles with a working volume of 120 mL were used as batch reactors (i.e. digesters). Pig, chicken and dairy manure were mixed with inoculum in a dry matter ratio of 1:1. Afterwards distilled water was added to adjust the dry matter content to 5%. Additionally, a control group containing solely inoculum was used. After inoculation, all digesters were flushed with standard nitrogen (99.999%) to ensure an anaerobic environment and immediately covered by butyl rubber sealant and closed with aluminum caps. AD temperature was set to 37 ± 2 °C (i.e. under mesophilic conditions) by a temperature controlled biochemical incubator (LRH-70, Shanghai Yiheng Scientific Instrument Co., Ltd., China). The digestate was sampled and analyzed on day 0, 1, 2, 3, 5, 7, 10, 15, 20, 25 and 30 of the experiment. Aliquots of each sample were stored for further analysis, and the remaining samples were dried at 105 °C, crushed with a mortar, sieved in a 0.425 mm sieve and stored at 4 °C in a refrigerator for further sequential chemical extraction, X-ray diffraction, and scanning electron microscopy.

3.2.3 Analytical methods

Total solids (TS) and volatile solids (VS) of manure, inoculated sludge, and digestate in different AD phases were measured according to the standard method (American Public Health Association, 2012). Total nitrogen (TN), total potassium (TK), inorganic phosphorus (IP) and total phosphorus (TP) were determined according to Chinese standard water and wastewater monitoring and analysis methods (State Environmental Protection Administration (SEPA), 2002). The pH value was measured using a desk type pH meter (FiveEasy Plus FE28, Mettler Toledo Co. Ltd., China). Gas was collected in graduated glass syringes. The gas volume was concluded from the position of the piston. Gas was standardized by measuring a standardized gas volume and calculating the error arising from ambient conditions. This error was then used to correct the measured gas volume of the reactors. Methane content was analyzed by gas chromatography (GC-2010, Shimadzu International Trading Co., Ltd., China) equipped with a CP-Porabond Q column and a thermal conductivity detector.

3.2.4 Sequential chemical extraction

In this study, the P characteristic of dried manure and the solid phase of digestates were detected by using the sequential chemical extraction method based on the fractionation principle by Hedley *et al.* (1982). This method has been widely used for characterizing P in soils and organic waste (Ajiboye *et al.*, 2004; Toor *et al.*, 2005; Turner & Leytem, 2004). Accordingly, five types of phosphate fractions were extracted sequentially from dried digestate. First, deionized water (H_2O) and sodium bicarbonate ($NaHCO_3$ 0.5M) were used to extract water-soluble phosphorus and exchangeable phosphorus, respectively. These two fractions combined are considered Labile-P in the following. These extractions are followed by NaOH (0.1 M), HCl (1.0 M), and ultimately nitration acid (H_2SO_4 and HNO_3) extraction to separate alkali-soluble phosphorus (NaOH-P, mainly combined with iron and aluminum), acid-soluble phosphorus (HCl-P, mainly combined with calcium and magnesium) and residual phosphorus (Residual-P, chemically stable and relatively insoluble phosphates), respectively (Yin *et al.*, 2013). The ratio of solution to sample was 100:1 as used by Dou *et al.* (2000) for manure. 30mL of extractant were consecutively added to 0.3g of dried sample. After each addition, the sample was shaken on a vertical shaker at 150 $r \cdot min^{-1}$ for 16 h and filtered with phosphorus-free filter paper (MN616G; MACHEREY-NAGEL GmbH & Co. KG., Germany). The filtrate was stored for later analysis and residues on the filter papers were mixed with the subsequent extractant. After completing the final shaking and filtering processes with HCl, the residues were transferred into digestion tubes with deionized water, H_2SO_4 and HNO_3 for digestion. Digestion took place at 350 °C for 75 min. Afterwards, the samples were filtered and the liquid sample was stored for later analysis.

3.2.5 X-ray diffraction (XRD)

X-ray diffraction was used to measure the main phosphate species in manure and digestate samples. The diffraction patterns of samples in different AD phases were determined by using an X-ray diffractometer (XD-2, Beijing Purkinje General Instrument Co., Ltd., China) with a Cu kα radiation at a voltage of 36 kV and current of 20 mA over a scanning angular range from 5°to 80°at a rate of 4°/min. The chemical compositions were analyzed by the phase retrieval function of MDI Jade 5.0 software (Materials Data, Inc., Livermore, CA).

3.2.6 Scanning electron microscopy (SEM)

The scanning electron microscopy images were taken to the show the surface and shape of phosphates in different manure digestates. The morphology of samples was observed by using SEM (JSM-56700F, JEOL, Japan). First, the sample was coated with a thin layer of gold before viewing to avoid charging effects and then examined at 1000 and 5000 times magnifications by using an acceleration voltage of 10 kV.

3.2.7 Statistical analysis

All data are presented as the mean ± standard deviation. Significance analysis were conducted with a one-way analysis of variance followed by Tukey test performed by SPSS 13.0 statistical software.

3.3 Results and discussion

3.3.1 Performance of batch AD system

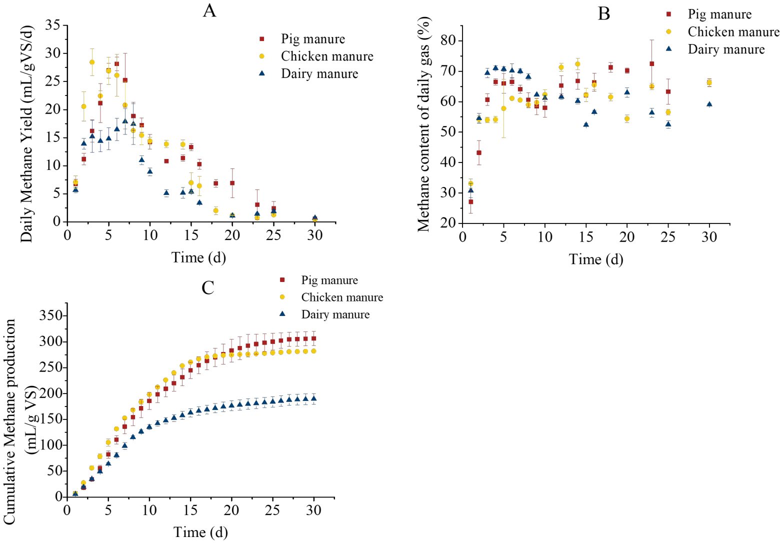

Figure 3.1: Gas production during anaerobic digestion process: (A) Daily methane yield; (B) Methane content of daily gas; (C) Cumulative methane production

The cumulative methane production (mL/g VS), daily methane yield (mL/g VS) and daily gas quality (methane content in %) in the batch reactors are presented in Figure 3.1. AD systems worked successfully and biogas production started immediately on the first day in all digesters. The daily methane yield began to decrease after day 7 of fermentation in each group. (Figure 3.1). After 30-day fermentation, the cumulative methane production or specific methane yield was 318.71 ± 13.68 mL/g VS, 294.35 ± 2.12 mL/g VS and 202.05 ± 10.43 mL/g VS for pig manure, chicken manure and dairy manure, respectively (Figure 3.1). The cumulative methane production of dairy manure was the lowest and the difference was significant ($p < 0.05$). The Figure 3.2 shows the changes of pH and VFAs during the AD process. Results indicated that the VFAs increased rapidly within two or three days then decreased gradually due to the conversion by methanogen and the change of pH was closely related to the change of VFAs (Figure 3.2). The lowest pH in pig and dairy manure digestates were reached after two days with 6.91 and 7.12 and 6.94 in chicken manure digestates after three days. Afterwards, pH gradually increased and stabilized at the end of fermentation at 7.62 in chicken manure digestate, 7.50 in pig manure digestate and 7.35 in dairy manure digestate due to VFA decomposition and the rising buffer capacity in the reactor (Zhou *et al.*, 2020). In fact, the batch process would experience phases of hydrolysis, acidification, acetogenesis and methanogenesis because of the different generation time of microorganisms, which would cause the accumulation of VFAs and the decrease of pH at the beginning of fermentation (Bianco *et al.*, 2020).

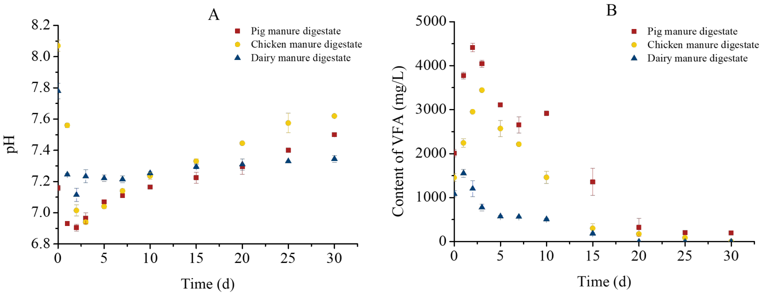

Figure 3.2: Changes of (A) pH and (B) VFAs during anaerobic digestion process

3.3.2 Effect of AD on P fraction and distribution

The types and distribution of phosphates in three animal manures were determined by sequential chemical extraction method in Figure 3.3. The results of sequential fractionation of phosphates indicated that H_2O-P of 8.06, 5.16 and 1.87 mg/g DM (orthophosphate and micromolecule organophosphorus), as well as $NaHCO_3$-P of 2.06, 2.82 and 0.96 mg/g DM were extracted from pig, chicken and dairy manures. $NaHCO_3$-P is an exchangeable phosphate, easily desorbed from particle surfaces and transformed into H_2O-P by dissolution/desorption processes. Because of their easy transformation, the two types are considered Labile-P and are similar to Olsen-P, which could be directly used by plants, and consequently, considered plant-available. Therefore, Labile-P is the most important fraction for fertilization. Results showed the content of Labile-P in dairy manure (2.83 mg/g DM) was much lower than pig (10.12 mg/g DM) and chicken manures (7.98 mg/g DM), but the ratio of Labile-P to TP in dairy manure (81.10%) was higher than pig manure (65.84%) and chicken manure (64.67%). The content of NaOH-P is generally low in animal manures. Figure 3.3 shows that there are only 1.54, 0.46 and 0.12 mg NaOH-P/g DM in pig, chicken and dairy manures. As for the HCl-P, there are 3.53, 3.69 and 0.46 mg/g DM in pig, chicken and dairy manures.

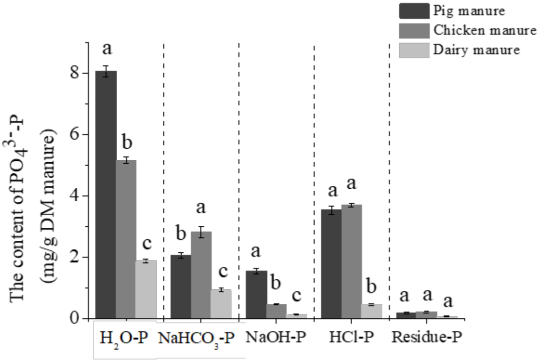

Figure 3.3: P types and distribution in three animal manures. (Means of the same type of P with different lowercase letters (a,b,c) are significantly different ($p < 0.05$) using Tukey-test)

It is worth noting that dissolved total phosphorus (DTP, mainly H_2O-P) and particulate phosphorus (PP, mainly $NaHCO_3$-P) are considered to be the main contributors to eutrophication (Akselsson *et al.*, 2008; Selig *et al.*, 2002). Therefore, excessive amounts of Labile-P can pollute water bodies by soil erosion, leaching and surface runoff. This means that direct application of animal manures as fertilizer in agricultural soil can not only supply P nutrients but increase the risk of water pollution if it is overused (McDowell, 2012; Zheng *et al.*, 2014). In fact, both NaOH-P and HCl-P are considered as slow-release fertilizer, which could be activated by organic acid and contribute to plant-available phosphorus in soil in an environmental-friendly way. Moreover, NaOH-P is a desirable P fertilizer, because it is fixated to particles and not prone to run-off, and consequentially, plant roots and microorganisms can decompose and metabolize it. Based on previous studies and our results, the application of animal manure should follow appropriate techniques, such as AD and compost, to ensure nutrients recycling and environmental protection.

As a useful treatment of organic waste, AD can not only produce renewable energy (i.e. biogas), but also recycle P resources (Arthurson, 2009; Khalid *et al.*, 2011). The digestates of AD systems fed with animal manure are generally rich in P and should be used as a valuable supplement or even replacement of chemical fertilizer (Arthurson, 2009; Khalid *et al.*, 2011). After AD of three types of manure used in this study, the solid–phase of the digestates contained more than 95% of total P, while the liquid-phase was characterized by low DM and P contents. These findings coincide with findings by Möller *et al.*, who concluded that the N:P and K:P ratios were higher in liquid-phase digestate than in solid-phase digestate. As shown in Table 3.2, the inorganic phosphorus (IP) in animal manure ranged from 63.21% to 84.64% of TP. Through anaerobic digestion, this fraction increased in pig and dairy manure but remained constant in chicken manure. The increase of IP is connected with the decrease of organic matter due to degradation processes in AD.

Table 3.2: Percentage of IP of the total phosphorus of animal manures and digestates.

Characteristics	**Pig manure (%)**	**Chicken manure (%)**	**Dairy manure (%)**
Manure	67.68-80.43	72.71-84.64	63.21-71.52
Digestate	80.56-92.24	74.88-84.26	72,61-77.52

The types and distribution of P in the three animal manure digestates during different AD phases are presented in Figure 3.4. After mixing with inoculum, H_2O-P in animal manures rapidly combined with metal ion, such as Fe^{2+}, Al^{3+}, Mg^{3+}, Ca^{2+}, Cr^{3+} and Mn^{3+} (Figure 3.4). Stable forms of P, including NaOH-P, HCl-P and residue-P, dominated the phosphate types in the digestates of pig, chicken and dairy manures. The content of H_2O-P and $NaHCO_3$-P in three manure digestates decreased significantly during AD ($p < 0.05$). In addition, the content of Labile-P in pig manure digestate changed from 5.88 mg/g DM (0 day) to 3.66 mg/g DM (30 days), showing a significant reduction by AD ($p < 0.05$). Similar trends were found in chicken and dairy manure digestates where the content of Labile-P decreased from 4.52 mg/g DM (0 day) to 2.14 mg/g DM (30 days) and from 2.09 mg/g DM (0 day) to 0.69 mg/g DM (30 days) respectively. Meanwhile, the content of stable phosphates in digestates gradually increased because some Labile-P were transformed to stable ones during AD. Compared to pig and dairy manure digestates, the effect of AD on the NaOH-P increase was more profound in chicken manure digestate. Coinciding with TP in the manures, the content of each P fraction in dairy manure digestate was lower than in pig and chicken manure digestates. In comparison to animal manure, there was less Labile-P but more NaOH-P, HCl-P and residue-P in digestates.

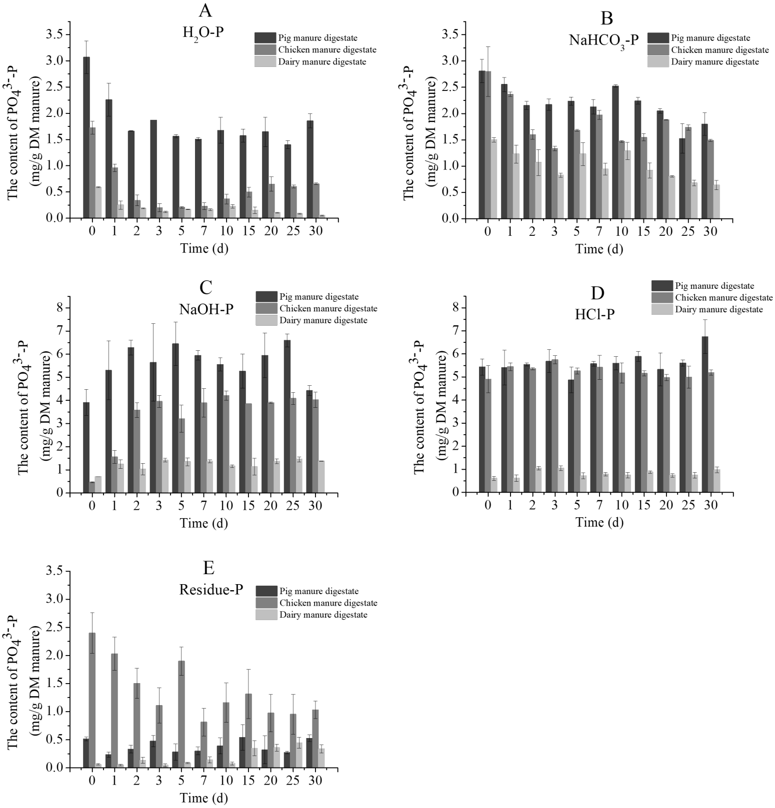

Figure 3.4: Changes in five fractions of phosphorus in pig manure, chicken manure and dairy manure during anaerobic digestion (A) H_2O-P; (B) $NaHCO_3$-P; (C) NaOH-P; (D) HCl-P; (E) Residue-P

Although AD reduces the Labile-P from manure in soil, it is still a more ideal fertilizer than manure for two following reasons. On one hand, digestate fertilizer is more suitable for legumes or grass family plants who can release organic acids through the plant rhizosphere to activate the strongly adsorbed P fractions by solubilizing NaOH P including Fe- and

Al-phosphates (Bhattacharyya *et al.*, 2013; Gahoonia *et al.*, 2000). In fact, NaOH-P is an important contributor to plant-available phosphates. On the other hand, from the perspective of P fertilizer, digestate application can reduce the risk of P loss to the environment in form of Labile-P and provide readily available N and biologically stabilized organic matters for plants compared to direct application of animal manure on farmland, therefore reducing greenhouse gas emissions. However, risk of nitrate leaching and nitrous oxide emissions still need to be contained when using digestate (Möller & Stinner, 2009; Nabel *et al.*, 2016).

3.3.3 P transformation in different AD phases

The phosphate compositions of pig, chicken and dairy manure digestates in different AD phases are shown in Fig 5 as XRD images. The XRD patterns of the three animal manure digestates were similar to the typical amorphous nature of digestate reported by previous research (Ge *et al.*, 2014a), however, there were also some phosphate crystals detected in this study due to the differences in feedstock of the AD system. As reported by Ryden *et al.* (1977) and McLaughlin *et al.* (1977), NaOH-P is generally combined with iron and aluminum components by chemisorption, and the other stable phosphates include calcium-phosphate (Ca-P) and magnesium-phosphate (Mg-P). In this study, $AlPO_4$, $FePO_4$, $Mg_3(PO_4)_2$ and $CaHPO_4$ were the main phosphates found in pig and chicken manures, while $Mg_3(PO_4)_2$ and $CaHPO_4$ were dominant in dairy manure. Additionally, the transformation of crystal phosphate crystals during different AD phases was investigated through the XRD results. After inoculation with activated sludge, the main phosphates gradually changed to $AlPO_4$, $FePO_4$ and $CaHPO_4$ in pig manure digestate (Figure 3.5A), to $AlPO_4$, $Mg_3(PO_4)_2$ and $CaHPO_4$ in chicken manure digestate (Figure 3.5B) and to $AlPO_4$ and $CaHPO_4$ in dairy manure digestate (Figure 3.5C). These changes were significantly affected by different AD phases. In pig manure, $Ca_{10}(PO_4)_6(OH)_2$ was

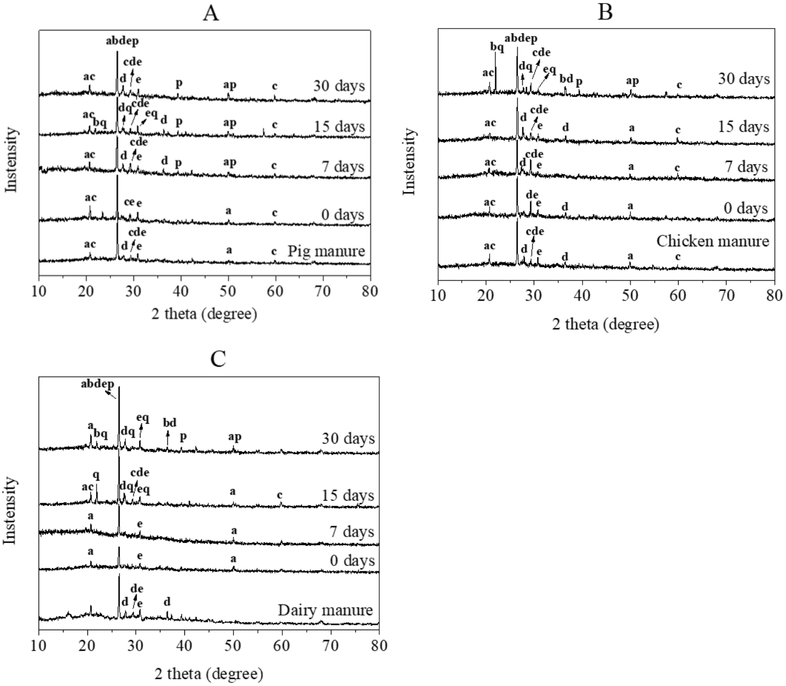

Figure 3.5: XRD patterns of (A) pig manure and digestates, (B) chicken manure and digestates, (C) dairy manure and digestates. a-$AlPO_4$; b-$Fe_3(PO_4)_2$; c-$FePO_4$; d-$Mg_3(PO_4)_2$; e-$CaHPO_4$; p-$Ca_{10}(PO_4)_6(OH)_2$; q-$Mg(NH_4)PO_4 \cdot 6H_2O$.

found as early as 7 days after start of AD, whereas in chicken and dairy manure it was only found after 30 days. Furthermore, $Mg(NH_4)PO_4 \cdot 6H_2O$, as a kind of slow release fertilizer, was found in pig and dairy manure digestates after fermentation day 15, which was not observed in chicken manure digestate. In the mesophilic condition, the formation of struvite is related to the ionic solubility product and pH in the system (Le Corre *et al.*, 2009), which would be affected by Mg, N, P content of the substrate. But the low formation rate may be expected due to the lower pH in the range of

6.8-8.2 (Figure 3.2A) in the batch AD process instead of 9-10.5 which is the optimal pH condition to recycle struvite (Ryu *et al.*, 2008). However, in dairy manure digestate, $Mg_3(PO_4)_2$ formed after 15-day fermentation. With AD process, Fe-P partly changed from Fe^{3+}-P to Fe^{2+}-P in all three manures because of the reduction reaction by reducing agent (H_2S) in the digestates (Chen *et al.*, 2008a; Möller & Müller, 2012) or the alienated reducing bacteria in the digestates, such as dissimilatory metal reducing bacteria (Hao *et al.*, 2018). This means that metals had a significant influence on the P transformation because of their ability to combine with PO_4^{3-}/HPO_4^{2-}. Moreover, some studies indicated that the ions, including NH_4^+ and CO_3^{2-}, affected P turnover and precipitation in magnesium ammonium phosphate (MAP) or hydroxyapatite (HAP) reactions (Le Corre *et al.*, 2009; Möller & Müller, 2012).

3.3.4 Micro-shape of phosphate

Figure 3.6 presents the microstructures of three animal manure digestates captured by SEM technology. The depicted surface was rough with some heterogeneous particles that were composed of undigested organic matter (Kataki *et al.*, 2017). The conversion of organic matter to biogas during AD resulted in some cavities in the digestate surface, explaining the loose crystal structure of digestate (Sri Bala Kameswari *et al.*, 2014). Most crystals were amorphous because the presences of phosphates in digestates were in generally in the form of complex polymers, which combined with each other and other metal ions, such as Fe^{3+}, Al^{3+} and some heavy metal ions, especially after several days of AD reaction. The formed crystals were classified into three major shapes including rod, block and ball (Figure 3.6). As reported by (Ge *et al.*, 2014b), the rod- and block-phosphates are struvite ($Mg(NH_4)PO_4{\cdot}6H_2O$) and ball-phosphate is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Ball-phosphate was detected in all three animal manure digestates, while rod- and block-phosphates were mainly found in

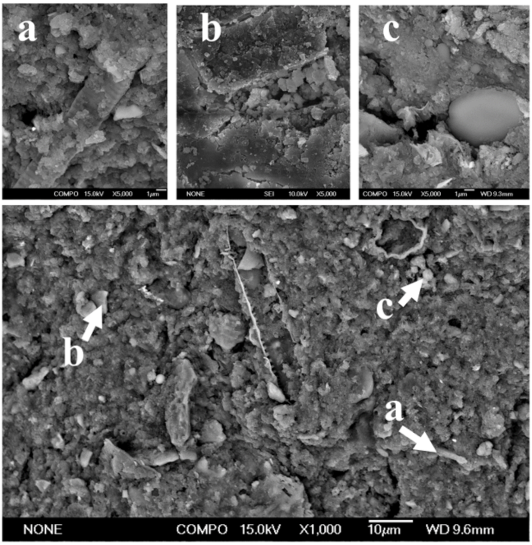

Figure 3.6: Microstructure of animal manure digestate and micro-shapes of phosphates from electron microscopy. a-rod-phosphate; b-block-phosphate; c-ball-phosphate.

dairy manure digestates. This may be because the dairy samples had a more reasonable ratio between Mg^{2+} and NH_4^+. In this study, the shape and distribution of phosphates were generally distinct for different digestates due to the different physicochemical characteristics of feedstock. It was worth noting that the detailed fractions of rod-, block- and ball-phosphates were not determined directly from SEM images, which are to be further analyzed in future research.

3.4 Conclusions

From the perspective of fertilizer, digestate application could avoid excessive Labile-P loss to the environment and provide readily available N and biologically stabilized organic matters for plants compared to direct application of animal manures on farmland. Moreover, metal ions are the important factors influencing the P transformation due to their ability to combine with PO_4^{3-}/HPO_4^{2-}. Ultimately, the structure of phosphates in the three manure digestates are different due to the different physicochemical characteristics of feedstock. During AD, the species of phosphates increased, $AlPO_4$, $FePO_4$, $Mg_3(PO_4)_2$, $CaHPO_4$, $Mg(NH_4)PO_4{\cdot}6H_2O$ and $Ca_{10}(PO_4)_6(OH)_2$ were the main phosphates present. The results and conclusions in this study were drawn from 30-day batch AD experiments fed with animal manure, so the effect continuous AD systems and especially of different substrates on P dynamics need further research. However, the results of this study can provide a deeper understanding of P turnover during AD of the investigated substrates and will be helpful for future research on P nutrient recycling from animal manure.

CRediT authorship contribution statement

Bowen Li: Conceptualization, Methodology, Investigation, Writing - original draft.**Konstantin Dinkler:**Writing - review & editing, Conceptualization. **Nan Zhao:** Formal analysis, Visualization.**Mostafa Sobhi:**Validation, Resources.**Wolfgang Merkle:**Writing - review & editing.**Shan Liu:**Writing - review & editing.**Renjie Dong:**Project administration.**Hans Oechsner:**Writing - review & editing, Funding acquisition.**Jianbin Guo:**Writing - review & editing, Funding acquisition, Supervision.

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.

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References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., & Johansen, C. (1990). Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science, 248(4954), 477–480. https://doi.org/10.1126/science.248.4954.477

Ajiboye, B., Akinremi, O. O., & Racz, G. J. (2004). Laboratory Characterization of Phosphorus in Fresh and Oven-Dried Organic Amendments. Journal of Environment Quality, 33(3), 1062–1069. https://doi.org/10.2134/jeq2004.1062

Akselsson, C., Westling, O., Alveteg, M., Thelin, G., Fransson, A. M., & Hellsten, S. (2008). The influence of N load and harvest intensity on the risk of P limitation in Swedish forest soils. Science of the Total Environment, 404(2-3), 284–289. https://doi.org/10.1016/j.scitotenv.2007.11.017

American Public Health Association. (2012). Standard Methods For the Examination of Water and Wastewater (22nd ed.).

Arthurson, V. (2009). Closing the Global Energy and Nutrient Cycles through Application of Biogas Residue to Agricultural Land – Potential Benefits and Drawback. Energies, 2(2), 226–242. https://doi.org/10.3390/en20200226

Bachmann, S., Gropp, M., & Eichler-Löbermann, B. (2014). Phosphorus availability and soil microbial activity in a 3 year field experiment amended with digested dairy slurry. Biomass and Bioenergy, 70, 429–439. https://doi.org/10.1016/j.biombioe.2014.08.004

Bai, Z., Ma, L., Jin, S., Ma, W., Velthof, G. L., Oenema, O., Liu, L., Chadwick, D., & Zhang, F. (2016a). Nitrogen, Phosphorus, and Potassium Flows through the Manure Management Chain in China. Environmental Science & Technology, 50(24), 13409–13418. https://doi.org/10.1021/acs.est.6b03348

Bai, Z., Ma, L., Ma, W., Qin, W., Velthof, G. L., Oenema, O., & Zhang, F. (2016b). Changes in phosphorus use and losses in the food chain of China during 1950–2010 and forecasts for 2030. Nutrient Cycling in Agroecosystems, 104(3), 361–372. https://doi.org/10.1007/s10705-015-9737-y

Bhattacharyya, P., Das, S., & Adhya, T. K. (2013). Root Exudates of Rice Cultivars Affect Rhizospheric Phosphorus Dynamics in Soils with Different Phosphorus Statuses. Communications in Soil Science and Plant Analysis, 44(10), 1643–1658. https://doi.org/10.1080/00103624.2013.769562

Bianco, F., Race, M., Papirio, S., & Esposito, G. (2020). Removal of polycyclic aromatic hydrocarbons during anaerobic biostimulation of marine sediments. Science of the Total Environment, 709. https://doi.org/10.1016/j.scitotenv.2019.136141

Cerrillo, M., Palatsi, J., Comas, J., Vicens, J., & Bonmatí, A. (2015). Struvite precipitation as a technology to be integrated in a manure anaerobic digestion treatment plant - removal efficiency, crystal characterization and agricultural assessment. Journal of Chemical Technology and Biotechnology, 90(6), 1135–1143. https://doi.org/https://doi.org/10.1002/jctb.4459

Chen, C. R., Condron, L. M., & Xu, Z. H. (2008a). Impacts of grassland afforestation with coniferous trees on soil phosphorus dynamics and associated microbial processes: A review. Forest Ecology and Management, 255(3-4), 396–409. https://doi.org/10.1016/j.foreco.2007.10.040

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008b). Inhibition of anaerobic digestion process: A review. Bioresource Technology, 99(10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

Chowdhury, R. B., Moore, G. A., Weatherley, A. J., & Arora, M. (2017). Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. https://doi.org/10.1016/j.jclepro.2016.07.012

Dalu, T., Wasserman, R. J., Magoro, M. L., Froneman, P. W., & Weyl, O. L. (2019). River nutrient water and sediment measurements inform on nutrient retention, with implications for eutrophication. Science of the Total Environment, 684, 296–302. https://doi.org/10.1016/j.scitotenv.2019.05.167

Dou, Z., Toth, J. D., Galligan, D. T., Ramberg, C. F., & Ferguson, J. D. (2000). Laboratory procedures for characterizing manure phosphorus. Journal of Environmental Quality, 29(2), 508–514. https://doi.org/10.2134/jeq2000.00472425002900020019x

Gahoonia, T. S., Asmar, F., Giese, H., Gissel-Nielsen, G., & Erik Nielsen, N. (2000). Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. European Journal of Agronomy, 12(3-4), 281–289. https://doi.org/10.1016/S1161-0301(00)00052-6

Ge, J., Lin, Y., Qian, F., Song, Y., & Wang, Y. (2014a). Interactive effects of Ca^2 and CO_3^{2-} on phosphorus removal from anaerobic digestion liquor by struvite crystallization. Huanjing Kexue Xuebao/Acta Scientiae Circumstantiae, 34(12), 2998–3005. https://doi.org/10.13671/j.hjkxxb.2014.0606

Ge, J., Qian, F., Song, Y., Wang, Y., & Lin, Y. (2014b). Process of phosphorus removal from wastewater by dolomite fluidized crystallization. Huanjing Kexue Xuebao/Acta Scientiae Circumstantiae, 34(6), 1480–1488. https://doi.org/10.13671/J.HJKXXB.2014.0539

Güngör, K., & Karthikeyan, K. G. (2008). Phosphorus forms and extractability in dairy manure: A case study for Wisconsin on-farm anaerobic digesters. Bioresource Technology, 99(2), 425–436. https://doi.org/10.1016/j.biortech.2006.11.049

Gurmessa, B., Pedretti, E. F., Cocco, S., Cardelli, V., & Corti, G. (2020). Manure anaerobic digestion effects and the role of pre- and post-treatments on veterinary antibiotics and antibiotic resistance genes removal efficiency. Science of the Total Environment, 721. https://doi.org/10.1016/j.scitotenv.2020.137532

Hao, X., Zhou, J., & Wang, C. (2018). Vivianite formed in anaerobic digestion of excess sludge: verification and analysis. China. Water. Wastewater., (34), 7–13. www.watergasheat.com%20(in%20chinese)

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science

Society of America Journal, 46(5), 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Kataki, S., Hazarika, S., & Baruah, D. C. (2017). Investigation on by-products of bioenergy systems (anaerobic digestion and gasification) as potential crop nutrient using FTIR, XRD, SEM analysis and phyto-toxicity test. Journal of Environmental Management, 196, 201–216. https://doi.org/10.1016/j.jenvman.2017.02.058

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. Waste Management, 31(8), 1737–1744. https://doi.org/10.1016/j.wasman.2011.03.021

Le Corre, K. S., Valsami-Jones, E., Hobbs, P., & Parsons, S. A. (2009). Phosphorus recovery from wastewater by struvite crystallization: A review (Vol. 39). https://doi.org/10.1080/10643380701640573

Leytem, A. B., Smith, D. R., Applegate, T. J., & Thacker, P. A. (2006). The Influence of Manure Phytic Acid on Phosphorus Solubility in Calcareous Soils. https://doi.org/10.2136/sssaj2006.0003

Li, R., Volenec, J. J., Joern, B. C., & Cunningham, S. M. (1998). Effects of phosphorus nutrition on carbohydrate and protein metabolism in alfalfa roots. Journal of Plant Nutrition, 21(3), 459–474. https://doi.org/10.1080/01904169809365417

Ma, L., Ma, W. Q., Velthof, G. L., Wang, F. H., Qin, W., Zhang, F. S., & Oenema, O. (2010). Modeling Nutrient Flows in the Food Chain of China. Journal of Environmental Quality, 39(4), 1279–1289. https://doi.org/10.2134/jeq2009.0403

Marti, N., Bouzas, A., Seco, A., & Ferrer, J. (2008). Struvite precipitation assessment in anaerobic digestion processes. Chemical Engineering Journal, 141(1-3), 67–74. https://doi.org/10.1016/j.cej.2007.10.023

McDowell, R. W. (2012). Minimising phosphorus losses from the soil matrix. Current Opinion in Biotechnology, 23(6), 860–865. https://doi.org/10.1016/j.copbio.2012.03.006

McLaughlin, J., Ryden, J., & Syers, J. (1977). Development and evaluation of a kinetic model to describe phosphate sorption by hydrous ferric oxide gel. Geoderma, 18(4), 295–307. https://doi.org/10.1016/0016-7061(77)90038-6

Meals, D. W., Fay, J., & Barsotti, M. (2008). Effects of water treatment residual addition on phosphorus in liquid dairy manure. https://doi.org/10.1002/j.1551-8833.2008.tb09610.x

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242–257. https://doi.org/10.1002/elsc.201100085

Möller, K., Schulz, R., & Müller, T. (2010). Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutrient Cycling in Agroecosystems, 87(2), 307–325. https://doi.org/10.1007/s10705-009-9340-1

Möller, K., & Stinner, W. (2009). Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides).

European Journal of Agronomy, 30(1), 1–16. https://doi.org/10.1016/j.eja.2008.06.003

Möller, K., & Stinner, W. (2010). Effects of organic wastes digestion for biogas production on mineral nutrient availability of biogas effluents. Nutrient Cycling in Agroecosystems, 87(3), 395–413. https://doi.org/10.1007/s10705-010-9346-8

Nabel, M., Temperton, V. M., Poorter, H., Lücke, A., & Jablonowski, N. D. (2016). Energizing marginal soils - The establishment of the energy crop Sida hermaphrodita as dependent on digestate fertilization, NPK, and legume intercropping. Biomass and Bioenergy, 87, 9–16. https://doi.org/10.1016/j.biombioe.2016.02.010

Pradel, M., & Aissani, L. (2019). Environmental impacts of phosphorus recovery from a “product” Life Cycle Assessment perspective: Allocating burdens of wastewater treatment in the production of sludge-based phosphate fertilizers. Science of the Total Environment, 656, 55–69. https://doi.org/10.1016/j.scitotenv.2018.11.356

Rehim, A., Farooq, M., Ahmad, F., & Hussain, M. (2012). Band placement of phosphorus improves the phosphorus use efficiency and wheat productivity under different irrigation regimes. International Journal of Agriculture and Biology, 14(5), 727–733.

Ryden, J. C., McLaughlin, J. R., & Syers, J. K. (1977). Mechanisms of phosphate sorption by soils and hydrous ferric oxide gel. Journal of Soil Science, 28(1), 72–92. https://doi.org/10.1111/j.1365-2389.1977.tb02297.x

Ryu, H. D., Kim, D., & Lee, S. I. (2008). Application of struvite precipitation in treating ammonium nitrogen from semiconductor wastewater. Journal of Hazardous Materials, 156(1-3), 163–169. https://doi.org/10.1016/j.jhazmat.2007.12.010

Selig, U., Hübener, T., & Michalik, M. (2002). Dissolved and particulate phosphorus forms in a eutrophic shallow lake. Aquatic Sciences, 64(1), 97–105. https://doi.org/10.1007/s00027-002-8058-9

Sobhi, M., Guo, J., Cui, X., Sun, H., Li, B., Aboagye, D., Shah, G. M., & Dong, R. (2019). A promising strategy for nutrient recovery using heterotrophic indigenous microflora from liquid biogas digestate. Science of the Total Environment, 690, 492–501. https://doi.org/10.1016/j.scitotenv.2019.06.487

Sri Bala Kameswari, K., Kalyanaraman, C., Umamaheswari, B., & Thanasekaran, K. (2014). Enhancement of biogas generation during co-digestion of tannery solid wastes through optimization of mix proportions of substrates. Clean Technologies and Environmental Policy, 16(6), 1067–1080. https://doi.org/10.1007/s10098-013-0706-3

State Environmental Protection Administration (SEPA). (2002). Water and wastewater monitoring and analysis methods (4th ed.). China Environmental Science Press, China (in Chinese).

Ström, L., Owen, A. G., Godbold, D. L., & Jones, D. L. (2002). Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. Soil Biology and Biochemistry, 34(5), 703–710. https://doi.org/10.1016/S0038-0717(01)00235-8

Tittonell, P., Rufino, M. C., Janssen, B. H., & Giller, K. E. (2010). Carbon and nutrient losses during manure storage under traditional and improved practices in smallholder crop-livestock systems-evidence from Kenya. Plant and Soil, 328(1), 253–269. https://doi.org/https://doi.org/10.1007/s11104-009-0107-x

Toor, G. S., Peak, J. D., & Sims, J. T. (2005). Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. Journal of Environmental Quality, 34(2), 687–697. https://doi.org/10.2134/jeq2005.0687

Turner, B. L., & Leytem, A. B. (2004). Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure. Environmental Science and Technology, 38(22), 6101–6108. https://doi.org/10.1021/es0493042

U.S. Geological Survey. (2019). Mineral Commodity Summaries 2019. https://doi.org/10.3133/70202434

Ye, Y., Ngo, H. H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., & Jia, H. (2017). Insight into chemical phosphate recovery from municipal wastewater. Science of the Total Environment, 576, 159–171. https://doi.org/10.1016/j.scitotenv.2016.10.078

Yin, H., Kong, M., & Fan, C. (2013). Batch investigations on P immobilization from wastewaters and sediment using natural calcium rich sepiolite as a reactive material. Water Research, 7. https://doi.org/10.1016/j.watres.2013.04.044

Zheng, Z., Zhang, T., Wen, G., Kessel, C., Tan, C., O'Halloran, I., Reid, D. K., Nemeth, D., & Speranzini, D. (2014). Soil Testing to Predict Dissolved Reactive Phosphorus Loss in Surface Runoff from Organic Soils. Soil Science Society of America Journal, 78(5), 1786–1796. https://doi.org/10.2136/sssaj2014.02.0065

Zhou, J., You, X., Niu, B., Yang, X., Gong, L., Zhou, Y., Wang, J., & Zhang, H. (2020). Enhancement of methanogenic activity in anaerobic digestion of high solids sludge by nano zero-valent iron. Science of the Total Environment, 703(40), 135532. https://doi.org/10.1016/j.scitotenv.2019.135532

4 Adapted Hedley fractionation for the analysis of inorganic phosphate in biogas digestate

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Abstract

The major share of phosphate in biogas digestate is inorganic. For optimized nutrient recovery, inorganic phosphate must be analyzed adequately. Therefore, the photometric spectra of extracts from Hedley fractionation were measured and analyzed for their peaks using the molybdenum blue method. The ideal wavelength was 709 nm. The lower calibration limit needed to be raised from 15 $\mu g\, L^{-1}$ to 50 $\mu g\, L^{-1}$ to avoid underestimation of phosphate concentration. Drying digestate before extraction increased H_2O-P by 78.4% and $NaHCO_3$-P by 44.9% compared to undried digestate. The filter paper of the filtration between extractions was added to the next extraction to avoid phosphate losses. This made it necessary to rinse the samples with 30 mL deionized H_2O after the H_2O extraction, with 60 mL $NaHCO_3$ after $NaHCO_3$ extraction and 60 mL NaOH after the NaOH extraction. Ultimately, the results showed that the phosphate concentration in extracts was independent of extraction time.

Keywords: Phosphate, Hedley fractionation, Pretreatment, Anaerobic digestion, Digestate

4.1 Introduction

Anaerobic digestion generates energy in form of biogas and nutrient- rich digestate. Although nutrient-rich, biogas digestate has a high water content, making transportation over long distances uneconomical. Therefore, it is applied to agricultural land around the facilities. In case of overloading, this can lead to environmental problems such as eutrophication and ground water contamination (Möller & Müller, 2012; Nkoa, 2014; Rehl & Müller, 2011; Severin *et al.*, 2016). Consequently, recent research has focused on the recovery of nutrients to increase their concentration and, therefore, their transportability (Ma *et al.*, 2018; Wang & Lee, 2021). Struvite precipitation with its simultaneous recovery of nitrogen and phosphate (Dong *et al.*, 2020; Günther *et al.*, 2018; Lin *et al.*, 2015) and solid – liquid separation with a focus on phosphate binding in the solid fraction (Lin *et al.*, 2015; Tambone *et al.*, 2017), show great potential. Analyzing the different phosphate types in digestate and their solubility is important for effective phosphate recovery on a larger scale (Xu *et al.*, 2015). For example, in struvite recovery the use of chemicals can be optimized if the solubility of phosphate is known.

For the analysis of phosphate solubility and classification, phosphate salts must be extracted from the sample by different solvents. For this, methods from soil science are applied. An overview and comparison of various soil phosphorus extraction methods is given in the study of Wuenscher *et al.* (2016). The methods in this study are mostly limited to labile-P. This fraction is declared to be plant available phosphate but shows crop and soil dependencies (Kamh *et al.*, 2002; Recena *et al.*, 2015). The Hedley fractionation, developed in 1982, is a sequential extraction method that includes but is not limited to labile-P. It analysis the entirety of phosphate compounds in soil by sequentially using alkaline and acidic solvents to group them into fractions. The original method differentiates between Resin-P, $NaHCO_3$-P, $CHCl_3$/$NaHCO_3$-P, NaOH-P, Sonicate/NaOH-P, HCl-P and Residual-P (Hedley *et al.*, 1982). Resin-P, or H_2O-P as used by Dou *et al.* (2000), and $NaHCO_3$-P are considered labile-P whereas NaOH-P and HCl-P are considered non- labile-P (Ngo *et al.*, 2013). The fractions have been adapted several times in literature (Bachmann *et al.*, 2011; Dou *et al.*, 2000; Tiessen & Moir, 1993; Turner & Leytem, 2004). The study of Dou *et al.* (2000) showed that the original extraction time of 16 h could be reduced when extracting phosphate from manure because the extraction time had a minor effect on the extracted phosphate concentration. In this work, it is investigated if the same applies for digestate.

Nevertheless, research on phosphate compounds in digestate from agricultural biogas plants is limited. In the available references, samples of manure and digestate were dried before the extraction. Drying the samples creates soil-like properties and makes it easier to weigh them. However, there is no cross-laboratory consensus on an adequate drying temperature. Turner and Leytem (2004) used freeze drying but other authors such as Dou *et al.* (2000) or Toor *et al.* (2005) used elevated temperatures of 65 °C and 55 °C, respectively. Distortion of phosphate fractions by sample drying has been discussed in multiple studies on various soils (Bünemann *et al.*, 2013; Turner & Haygarth, 2001; Wang *et al.*, 2020). The results coincidently state that drying leads to an increase in water-soluble phosphate. The authors draw the conclusion that microbial biomass is the main source of distortion. For digestate, this is likely to be significant because it contains large amounts of microbial biomass. If this is the case and if this error can be avoided will be investigated in this work.

The Hedley fractionation can be considered a pretreatment for the subsequent analysis of the extract. There is increasing interest in conducting this analysis by ICP-OES (Wuenscher *et al.*, 2016) and ICP-MS (Kruse *et al.*, 2015). Furthermore, ICP and photometric methods can be combined to analyze organic and inorganic phosphates. However, ICP analysis is cost intensive and requires advanced equipment. Additionally, 82% to 88% of the total phosphate in digestate of agricultural biogas plants is inorganic (Bachmann *et al.*, 2016). Therefore, this work will focus on the photometric analysis of inorganic phosphate with the coloring agent developed by Murphy and Riley (1962). The method ofMurphy and Riley (1962) has been adjusted multiple times and to date wavelengths ranging from 680 to 890 nm and different reducing agents are used (Nagul *et al.*, 2015). The appropriate wavelength depends on the equipment, the reducing agent used for the color forming reaction and the sample matrix. The salts in the sample matrix can interfere with the chemical reaction and the actual absorption and lead to the so-called salt error. This has already been described by Murphy and Riley (1962) and is addressed by (APHA, 2012). Hence, the choice of an adequate wavelength is not trivial and, for a new sample matrix, it is advisable to analyze the spectrum for its optimal peak. It is the goal of this research to (1) specify the adequate wavelength for colorimetric phosphate analysis in digestate extracts when using the Murphy and Riley (1962) coloring agent. (2) Evaluate if drying has an effect on the Hedley fractions. (3) If the extraction time can be reduced and (4) if the Hedley fractionation needs to be adjusted for the fractionation of digestate.

4.2 Material and methods

Samples were taken from the digestate storage at the biogas research plant Unterer Lindenhof of the University of Hohenheim (Naegele *et al.*, 2012). At the time, the reactor was working under thermophilic conditions between 48 °C and 54 °C and the digestate storage was unheated. The reactor feed consisted of approximately ten metric tons liquid manure (mixture of liquid pig, dairy manure and rain water), two metric tons solid manure (mixture of solid dairy, pig, horse, sheep and chicken manure), and eight metric tons energy crops (maize silage, grass silage, cereal, and sugar beet) (Naegele *et al.*, 2012).

4.2.1 Sample pretreatment

Samples were dried at 50 °C (D50), 105 °C (D105) and freeze-dried (FZD) until the weight was constant. Afterwards, the samples were ground and sieved to 2 mm. A total of 0.3 g sieved sample was added to 250 mL Polypropylene bottles with four repetitions each. Additionally, four bottles were filled with undried digestate (UD) equivalent to approximately 0.3 g dry matter (DM). The DM content of D50, D105 and FZD samples after grinding was measured by subsequent drying of subsamples at 105 °C. Afterwards the samples were incinerated at 550 °C for volatile solid (VS) determination, according to DIN EN 12880 (2001b) and DIN EN 12879 (2001a), respectively. The DM content after grinding was used to correct the amount of dry sample added to each bottle. For the DM and VS of UD samples, fresh digestate was dried and burned as described.

4.2.2 Identification of optimal wavelength

For the determination of the appropriate wavelength a sample of each pretreatment (FZD, D50, D105, UD) group was randomly selected. A dilution series from 1:100 to 1:600 with a stepwise dilution increase of 50 was prepared of each filtrate from H_2O to HCl (filtrates are explained in section 4.2.4). The series were analyzed in the spectrum of 600 nm to 1100 nm in steps of 1 nm with a photometer (DR 3900, Hach Lange GmbH, Düsseldorf, Germany). Samples were measured in fourfold repetition. A polynomial regression of the fourth order between 670 nm and 750 nm as well as 850 nm and 930 nm was calculated with the *lm* function of the *car* package (Fox & Weisberg, 2019) for the mean values of each repetition. The absorbance peak of each polynomial regression was calculated with the optimize function of the stats package (R Core Team, 2020). The wavelengths of the peaks were compared to evaluate the optimal wavelength for measurement. Plotting was done with the *ggplot2* package (Wickham, 2016). In total, eleven series with eight standards each were analyzed, resulting in 88 spectral lines with a polynomial regression at each peak, meaning 176 polynomial regressions. For the filtrates 176 spectral lines where measured, resulting in 352 polynomial regressions. The filtrates contained colloids, making a baseline correction in accordance to the procedure of APHA (2012)necessary. The resulting wavelength was used for further measurements. For the wavelength determination, the phosphate concentration was given as $\mu g\ L^{-1}$. Standards were prepared with a phosphorus standard solution (1000 mg L^{-1}P, 1001.0 mg L^{-1} ± 3.6 mg L^{-1}, Grade: AVS Titrinorm, VWR International bvba, Leuven, Belgium)

4.2.3 Measurement of phosphate concentration

In accordance with Murphy and Riley (1962) the coloring agent for the photometric analysis in this work consisted of potassium antimonyl oxide tartrate trihydrate (purity $\geq$ 99%; Bernd Kraft GmbH, Duisburg, Germany), ascorbic acid (purity $\geq$ 99%, Grade: AVS Titrinorm, Sigma-Aldrich, St. Louis, Missouri, United States of America), ammonium heptamolybdate tetrahydrate (purity $\geq$ 99%, analytical grade, Merck KGaA, Darmstadt, Germany) and sulfuric acid (2.5 mol L^{-1} ± 0.005 mol L^{-1}, Grade: AVS Titrinorm, VWR International S.A.S., Fontenay-sous- Bois, France). Analysis limits were set from 15 to 250 μg L^{-1} for a 50 mm light path and baseline correction was done in accordance with APHA (2012). Considering the different dry matter contents of the samples, the results of the fractionation were normalized to g kg_{DM}^{-1}.

4.2.4 Hedley fractionation

In accordance with the work of Dou *et al.* (2000) deionized H_2O, 0.5 M $NaHCO_3$ (purity $\geq$ 99%, analytical grade, Merck KGaA, Darmstadt, Germany), 0.1 M NaOH (0.1 mol L^{-1} ± 0.0015 mol L^{-1}, Grade: AVS Titrinorm, AppliChem GmbH, Darmstadt, Germany) and 1 M HCl (1 mol L^{-1} ± 0.002 mol L^{-1}, Grade: AVS Titrinorm, VWR International S.A.S., Fontenay-sous-Bois, France) were added subsequently. The corresponding phosphate concentrations will be called H_2O-P, $NaHCO_3$-P, NaOH-P and HCl-P in the following. For each extraction step, the bottles were shaken for 16 hours on an orbital shaker (IKA 260 Control, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 250 rpm. Afterwards, the samples were filtered by gravity filtration using a 110 mm folded filter paper (MN 619 G, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The filtered solution will heron after be called filtrate and, depended on the solvent, either H_2O-Fil, $NaHCO_3$-Fil, NaOH-Fil or HCl-Fil. The filtrates were stored at 4 °C for later analysis. The filter paper contained small particles, which were associated with phosphate (Peters *et al.*, 2011).

To avoid the loss of these small particles, the filter cake along with the filter was cut into pieces and added to a bottle for the next extraction step. The ratio of extraction media to sample weight (dry base) was 1:100 in the H_2O and $NaHCO_3$ extraction, coinciding with the work of Dou *et al.* (2000). In the NaOH and HCl extraction, the ratio was increased to 1:200 to compensate the growing size of the filter cake because of the added filter paper. During extraction, the samples D105, D50 and FZD served as the controls with conventional pretreatment and were compared to UD.

4.2.5 Variation of extraction time and relict determination

Parallel to the extraction of dried and undried samples as described in section 4.2.1 and 4.2.4, undried samples were extracted in fourfold repetition with four different extraction time, viz. 1 h, 2 h, 4 h and 16 h. The extraction times coincide with the work of Dou *et al.* (2000). These samples are heron after called UD-1h, UD-2h, UD-4h and UD-16h. After filtration the filter and filter cake were rinsed 3 times with the extractant of the foregoing extraction. The liquid of each rinse was collected and stored at 4 °C for later analysis. The rinses are heron after called Rinse 1, Rinse 2 and Rinse 3. The volume used for each rinse was the same as the volume used during the extraction (30 mL H_2O, 30 mL $NaHCO_3$, 60 mL NaOH). As a reference value for each rinse the mean of UD-1h to UD-16 h of each extraction (H_2O-P, $NaHCO_3$-P and NaOH-P) was calculated. The sample UD served as a control to see the difference between rinsed and unrinsed samples. Die difference can be traced back to the filter paper added to the extraction.

4.2.6 Analysis of solved phosphate

Part of the sample collected at the biogas plant was centrifuged at 20,000 x g in four 85 mL round bottom tubes for 15 min. Afterwards, the supernatant was transferred to 2 mL conical bottom Eppendorf tubes with a threefold repetition each. The Eppendorf tubes were centrifuged at 10,000 x g for 10 min. 100 µL of each supernatant from these tubes was diluted 1:20 and transferred to 2 mL Eppendorf tubes. The tubes where then centrifuged again for 10 min at 10,000 x g. The resulting phosphate concentration in the supernatant was considered the solved phosphate in the prevailing equilibrium in the digestate and is hereon after called CEN.

4.2.7 Analysis of total phosphate

Part of the fresh sample was incinerated at 550 °C and subsequently boiled with 6 M HCl for 3 min for the analysis of total phosphate (P_{tot}). According to Yokota *et al.* (2003), 550 °C is adequate to mineralize all organic phosphates. The acid-ash mixture was passed through a 110 mm folded filter (619 G MN, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) into a 250 mL beaker and brought to volume. The P concentration in the filtrate was considered total phosphate (P_{tot}).

4.2.8 Statistical analysis

Outliers were detected with the function *geom_boxplot* of the *ggplot2* package and excluded from the data. The criteria for outlier determination was the interquartile range multiplied by 1.5 (Wickham, 2016). Afterwards, the data was tested for normal distribution with the Shapiro-Wilk test by using the *shapiro.test* function of the *stats* package (R Core Team, 2020) and for homogeneity of variance using Levene's test (*leveneTest* function of the *car* package (Fox & Weisberg, 2019)). Statistical significance ($p < 0.05$) was tested by one of the following methods: If the data showed normal distribution and homogeneous variance it underwent one-way ANOVA (*anova* function of the *agricolae* package (de Mendiburu, 2020)) and a subsequent Tukey test (*HSD.test* function of the *car* package (Fox & Weisberg, 2019)). In all other cases Welch-ANOVA (*oneway.test* function of the *stats* package (R Core Team, 2020)) followed by a Games-Howell test (*posthocTGH* function of the *userfriendlyscience* package (Peters, 2017)) was applied.

4.3 Results and discussion

4.3.1 Spectral analysis

Spectral analysis of standards

The spectra of eight standards between 15 and 250 µg L^{-1} were analyzed. In Figure 4.1 A, the spectral lines of eight standards are depicted. Two profound peaks around 709 nm and 889 nm can be distinguished. Figure 4.1 B shows the linear regression of the absorbance of these standards at 709 nm and 889 nm. The linear regression shows a good fit with an $R^2 > 0.99$ at both wavelengths the average peak wavelengths of all analyzed standards are 709 nm and 889 nm. The sensitivity of the method is higher at 889 nm, which is expressed in the higher gradient of 1.9 at this wavelength compared to the 1.38 at 709 nm. This shows that the response of the absorbance to a concentration increase is higher at 889 nm. Figure 4.1 C shows the relation between the two peaks including the data of all analyzed standards. There is a good linear correlation with an $R^2 > 0.99$. The ordinate intercept is close to zero and can be neglected. Therefore,

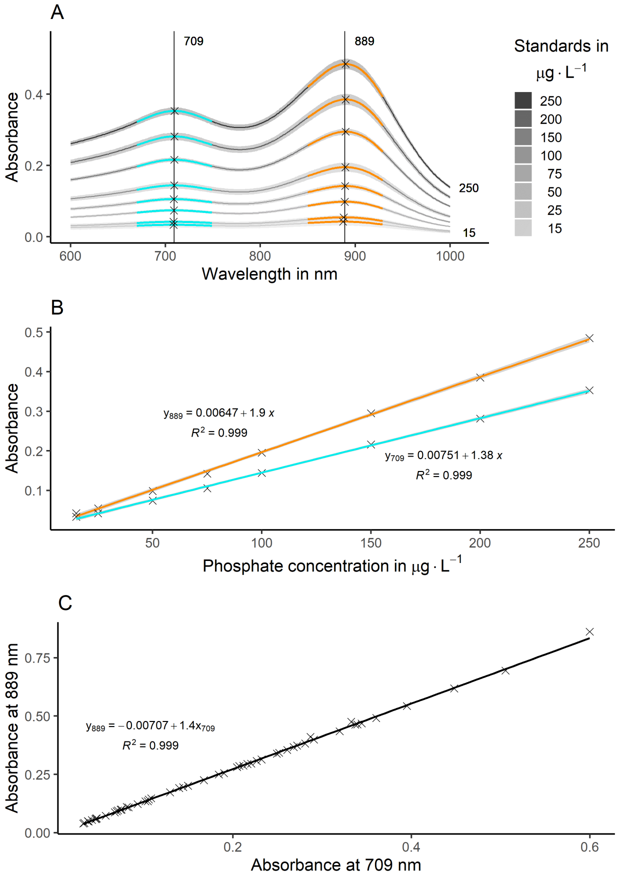

Figure 4.1: A: Spectral absorbance of a set of 8 standards in concentrations from 15 to 250 µ g L^{-1}. Each line marks a mean of four repetitions and the underlying shade marks the standard deviation. The cyan and orange lines mark the polynomial regressions of the fourth order from 670 to 750 nm and from 850 to 930 nm, respectively. The vertical lines mark the wavelengths of 709 nm and 889 nm. B: Linear regression of absorbance peaks of the 8 standards in A at 709 nm (cyan line) and 889 nm (orange line) over phosphate concentration of the standards. C: Correlation between absorbance at 709 nm and 889 nm of all analyzed standards.

for phosphate solutions without potential interferences, the measurement wavelength with the Hach Lange DR 3900 and vials with a 50 mm light path can be either 709 nm or 889 nm. The 709 nm values can be converted into the 889 nm values with the factor 1.41 (equation in Figure 4.1 C). In conclusion, it can be stated that the method is adequate and both peaks are equally suited for the analysis of aqueous phosphate solutions that do not contain interfering elements.

Spectral analysis of filtrates

It was tested if the filtrates would show a peak shift due to interfering salts in the sample matrix. Figure 4.2 A shows the spectrum of a set of eleven dilutions of HCl-Fil. The wavelengths 709 nm and 889 nm are marked with vertical lines. The selected sample shows the peak shift towards higher wavelengths around 709 nm and towards lower wavelengths around 889 nm at low absorbance. The shift is more profound at 889 nm. As a result, the lower calibration limit was increased from 15 µg L^{-1} to 50 µg L^{-1} to avoid measurements at low absorbance.

The linear regression in Figure 4.2 B proofs that the two peaks have a good correlation with an $R^2 > 0.99$. The gradients of the linear regression in Figure 4.1 C (1.41) and in Figure 4.2 C (1.37) are almost equal. Furthermore, the intercept is close to zero in all regressions and can be neglected. This shows that the correlation of the peaks is the same in the sample matrix and the standards and the sample matrix does not affect the two peaks differently.

Lastly, the absorbance in Figure 4.2 C is partially out of range of the upper calibrated absorbance limit in Figure 4.1 C. The highest absorbance at 889 nm with 2.41 (Figure 4.2 C) was almost 2.8 times higher than the highest standard at 889 nm with an absorbance of 0.86 (Figure 4.1 C). Nevertheless, no peak shift was detected and a good linear regression with

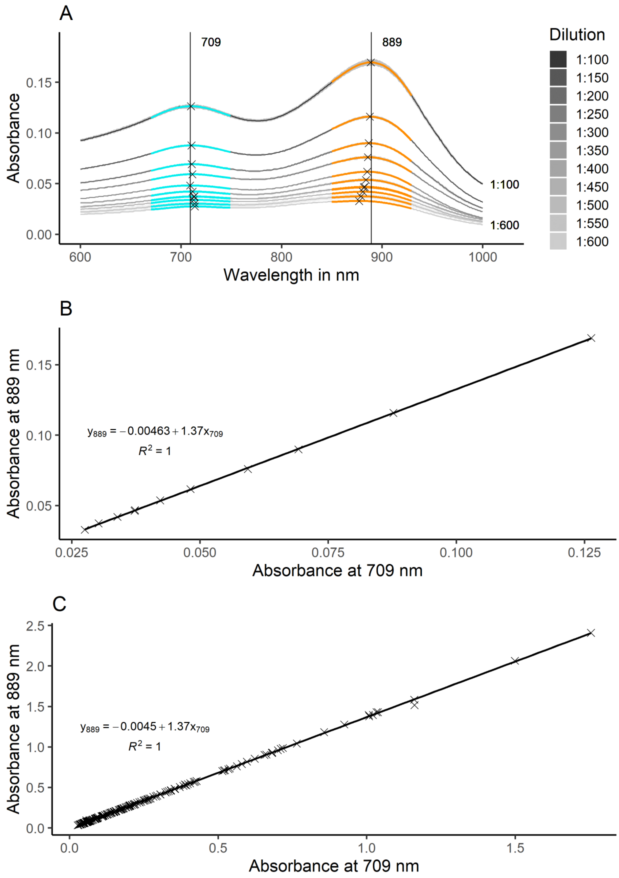

Figure 4.2: A: Spectral absorbance of 11 dilutions from 1:100 to 1:600 of an $HCl_{filtrate}$. Each line marks a mean of four analyzed spectra and the underlying shade marks the standard deviation. The cyan and orange lines mark the polynomial regressions of the fourth order from 670 to 750 nm and from 850 to 930 nm, respectively. The vertical lines mark the wavelengths of 709 nm and 889 nm. B: Correlation between absorbance at 709 nm and 889 nm of the 11 dilutions in A. C: Correlation between absorbance at 709 nm and 889 nm of all analyzed filtrates.

$R^2 > 0.99$ can be achieved when including these values. This shows, that it might be possible to increase the upper calibration limit by far. The same is true for the values at 709 nm. However, more data points are needed for verification.

In conclusion, both wavelengths are adequate for the present sample matrix. It is possible that the measured absorption is reduced by chemical binding of parts of the reactant through interaction with the sample matrix (Nagul *et al.*, 2015). This effect is considered to be minimal, because of the excess of reactant used (2000 µL reactant to 10 µL sample). While the change in the calibration range should solve the issue of peak shifting, 709 nm was used for sample analysis in the following due to the higher peak shift at 889 nm (Figure 4.2 C).

4.3.2 Hedley fractionation

Figure 4.3 shows the four extraction steps as described in section 4.2.4. The data depicted shows a significant ($p < 0.05$) increase of 78.4% of the H_2O-P concentration in the controls FZD, D50, D105 (Figure 4.3 A). Compared to the undried (UD) sample with a concentration of 3.24 g kg_{DM}^{-1}, the D105 samples had a concentration of 4.28 g kg_{DM}^{-1}, the D50 samples 5.78 g kg_{DM}^{-1} and the FZD samples 4.75 g kg_{DM}^{-1}. This indicates that drying temperature and H_2O-P seem to have an inverse correlation. This possibly results from the higher residual moisture content within the samples dried at lower temperatures. The mean residual moisture content was 15.45% for FZD, 9.41% for D50 and 1.81% for D105 samples after grinding. The increase of the H_2O-P concentration in dried samples, compared to the undried sample as seen in Figure 4.3 A, is most likely related to the release of microbial P and the destruction of labile organic phosphate bonds as observed in soil samples (Bünemann *et al.*, 2013; Wang *et al.*, 2020). Additionally, a significant increase of H_2O-P is noticeable between extractable H_2O-P (with water addition for extraction) from UD and the centrifugation (CEN)

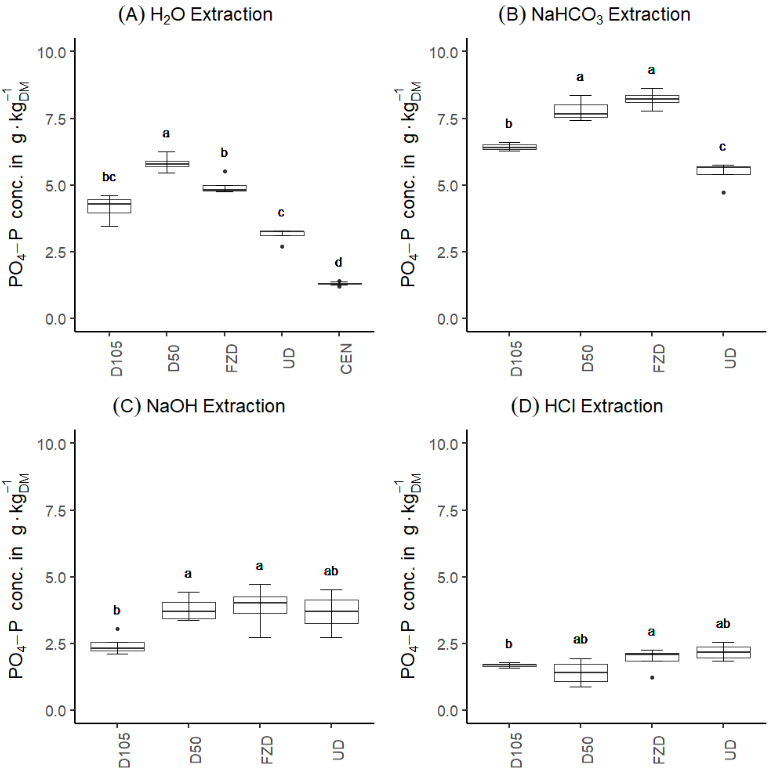

Figure 4.3: Phosphate concentrations in subsequent fractionation steps as influenced by sample preparation such as drying at 105 °C (D105) at 50 °C (D50), freeze drying (FZD) and untreated, undried (UD). The sample CEN (A) did not undergo extraction but solid liquid separation through centrifugation. Significance levels are based on one-way-ANOVA with Tukey test or Welch-ANOVA with Games-Howell test depending on the data distribution and homogeneity of variance. Different letters indicate significant differences ($p < 0.05$)

(without water addition (Figure 4.3 A)). The added water for the extraction shifts the chemical equilibrium and, therefore, dissociates exchangeable phosphate (Li *et al.*, 2013; Price *et al.*, 2010), leading to higher H_2O-P. For phosphate recovery, this could be interesting since the amount of solved and therefore reactive phosphate increases by 150% (Figure 4.4 A) through simple water addition. These phosphates could be removed easily by chemical precipitation.

The results of $NaHCO_3$ extraction in Figure 4 B show no significant difference between the samples FZD and D50 with concentrations of 8.2 g kg_{DM}^{-1} and 7.7 g kg_{DM}^{-1}, respectively. The concentration in the D105 extract is lower with 6.41 g kg_{DM}^{-1}, but the $NaHCO_3$-P of the UD sample is lowest at 5.66 g kg_{DM}^{-1}, showing an increase of $NaHCO_3$-P after drying of up to 44.88% in FZD. In the NaOH-P and HCl-P (Figure 4.3 C, D), this difference of dried and undried samples is no longer visible. In reference to their respective fraction sums, the sum of H_2O-P and $NaHCO_3$-P made up 67.72% of P-SumFZD, 72.33% of P-SumD50, 71.86% of P-SumD105 and 59.63% of P-SumUD, therefore representing the largest share of phosphate in all treatments.

4.3.3 Inherent error of the method

The sum of P concentrations of all concentrations shown in Figure 4 exceeded the analyzed P_{tot} by as much as 101.67% for FZD. The other treatments surpassed P_{tot} by 47.10% (UD) and 50.39% (D105), respectively. This error arises because the filters used are added to the next extraction step. This inevitably leads to an increase of the filter cake and, therefore, a higher retention of solved P during filtration. Therefore, after each extraction relicts from prior extractions underlie the real value. To evaluate the scale of this error, the filter and filter cakes of the undried samples UD-1h, UD-2h, UD-4h, and UD-16h were rinsed three times as described in section 4.2.5 and compared to the unrinsed control UD. The

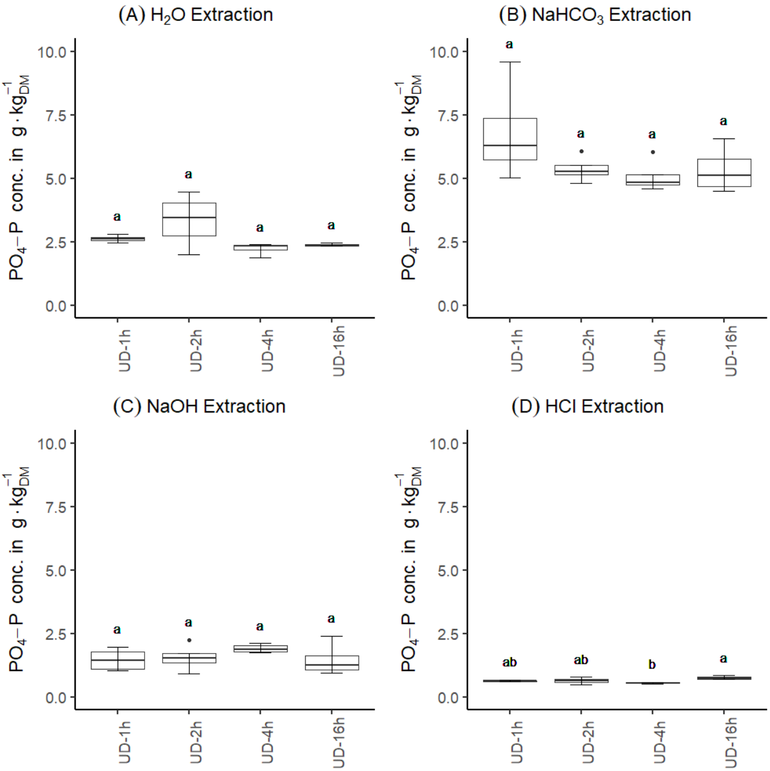

Figure 4.4: Phosphate concentrations in subsequent fractionation steps as influenced by shaking time of 1h (UD-1h), 2h (UD-2h), 4h (UD-4h) and 16 h (UD-16h). Between each extraction step, the samples were rinsed three times with the former solvent. Significance levels are based on one-way-ANOVA with Tukey test or Welch-ANOVA with Games-Howell test depending on the data distribution and homogeneity of variance. Different letters indicate significant differences ($p < 0.05$).

collected filtrates of each rinse were analyzed and the results are discussed further in section 4.3.4.

The resulting fractions of the samples UD-1h, UD-2h, UD-4h, and UD-16h are depicted in Figure 4.4. The control value of UD shows no significant difference ($p > 0.05$) to the samples of UD-1h to UD-16h in H_2O-P and $NaHCO_3$-P concentration (Figure 4.4 A and B). Therefore, the error through relicts is neglectable. In the NaOH and HCl extraction this error becomes statistically significant with UD at an NaOH-P of 3.71 g kg_{DM}^{-1} and HCl-P of 2.16 g kg_{DM}^{-1} and the rinsed samples between 1.25 g kg_{DM}^{-1} (UD-16h) to 1.88 g kg_{DM}^{-1} (UD-4h) for NaOH-P and 0.55 g kg_{DM}^{-1} (UD-4h) to 0.73 g kg_{DM}^{-1} (UD-16h) for HCl-P (Figure 4.4 C, D). One reason for the error becoming significant in the NaOH extraction is the increased amount of solvent. For $NaHCO_3$ extraction 30 mL are used used during but during NaOH extraction 60 mL are added to ensure sufficient movement of the samples in the solution during shaking. This leads to a higher dilution of NaOH extractable phosphate and a greater impact of the relicts from the prior extraction.

4.3.4 Reduction of relict error by rinsing

As described in section 4.2.5, the filtrate of each rinse was collected and analyzed. Table 4.1 shows the mean P concentrations in the filtrates of each rinse. For the calculation of all means, the values of UD-1h to UD-16h were combined because they were not significantly different ($p > 0.05$) (Figure 4.4). It is evident that the P concentration declines with each rinse. After H_2O extraction, the concentration in Rinse 2 is 63% and in Rinse 3 90% below the reference value. After $NaHCO_3$ extraction, however, the concentration of Rinse 2 and 3 are 58% and 70%, respectively, below the reference. It is evident from the data in Table 4.1 that the sample should be rinsed once with 30 mL deionized H_2O after the H_2O extraction, with 60 mL (twice with 30 mL) after $NaHCO_3$ extraction as well as 60 mL after the NaOH extraction. These steps are depicted in Figure 4.5. Additional rinses increase the accuracy but also the duration of the fractionation. Since $NaHCO_3$ and NaOH are both alkaline solvents, NaOH-P likely has the highest inaccuracy because of the remaining relicts from $NaHCO_3$ extraction. The HCl fraction will have a higher accuracy again due to the low amounts of relicts after one rinse with NaOH (Table 4.1).

Table 4.1: Mean P concentration in g kg_{DM}^{-1} (± standard deviation) in the filtrate of each rinse and their respective reference P concentrations. For mean calculation, the values of all samples UD-16h to UD-1h where combined. Means in the same column with different superscript letters are significantly different ($p < 0.05$, one-way-ANOVA with Tukey test or Welch-ANOVA with Games-Howell test).

	H_2O	$NaHCO_3$	NaOH
Reference	2.64 ± 0.67^a	5.64 ± 1.26^a	1.59 ± 0.48^a
Rinse 1	2.28 ± 0.33^a	6.01 ± 1.19^a	1.02 ± 0.68^b
Rinse 2	1.03 ± 0.35^b	2.39 ± 0.69^b	0.12 ± 0.67^c
Rinse 3	0.29 ± 0.08^c	1.60 ± 0.86^b	0.04 ± 0.01^c

The sum of all four extractions exceeded P_{tot} by 5.68% for UD-1h, 10.98% for UD-2h and 5.26% for UD-16h, whereas for UD-4h it was 0.54% lower than P_{tot}, which assumed a random measurement divergence without any correlation to the extraction time. When comparing this to the values of the control without rinsing (UD) in section 4.3.3, this is significantly more accurate. However, due to the exceedance of P_{tot} the calculation of residue-P was impossible. It must be considered that the real exceedance of P_{tot} is higher because organic P and residue P were not measured. However, according to literature they represent a small share of total phosphates (Bachmann *et al.*, 2016; Dou *et al.*, 2000; Li *et al.*, 2020).

Another error that arises is the reaction of the former extractant with the later one. While this error is partially accounted for by an increased solvent concentration (0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl) the reaction can lead a more neutral pH. In the first three stages, this effect will not be as profound, but in the HCl extraction this might have a significant effect, since the prior extractant is NaOH. Additionally, it might lead to a volume change because of NaCl flocculation. These effects could be counteracted to some degree by rinsing the sample with H_2O before the next extraction as done in a different sequential extraction by European Commission *et al.* (2001). In any case, the boxplots of UD-1h to UD-16h in Figure 4.4 show low variation, indicating reproducible results of the method.

4.3.5 Variation of extraction time

The boxplots UD-1h to UD-16h in Figure 4.4 A-D show that the extraction time (shaking time) can be significantly reduced to as low as 1 h, because the different durations of extraction show no significant difference in P concentration. However, the filtration of the samples took >24 h sometimes. This time can also contribute to the establishment of the chemical equilibrium and negates the difference in extraction time.

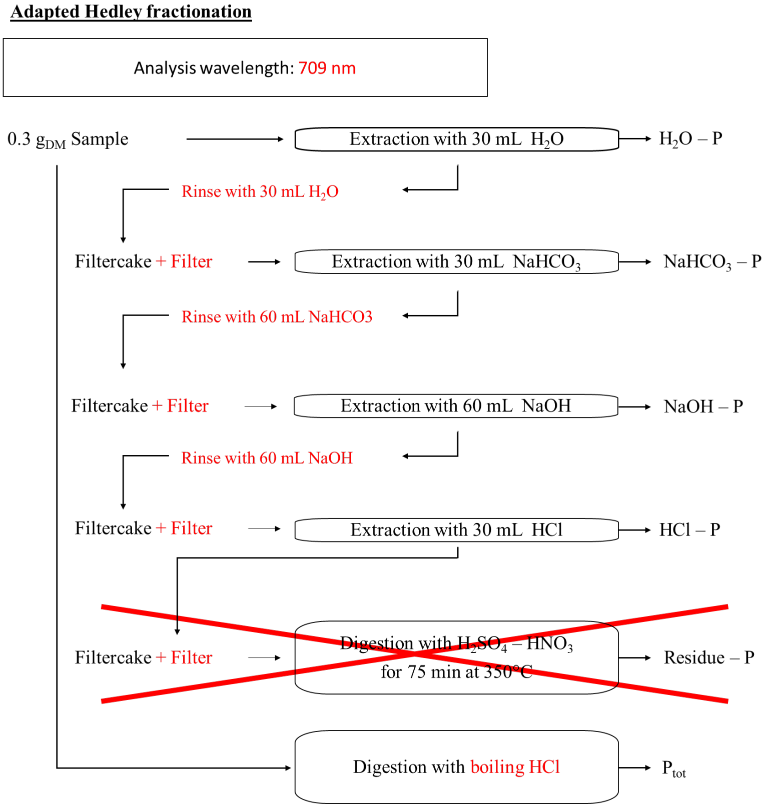

Figure 4.5: Flow diagram of the adapted Hedley fractionation for the analysis of inorganic phosphate in digestate from anaerobic digestion used in this work. The adaptations are marked in red. P_{tot} is total phosphate, H_2O-P, $NaHCO_3$-P, NaOH-P and HCl-P are the inorganic phosphate fractions based on their respective solvents. Residue-P is the P fractions that remains after all former extractions.

4.3.6 Advantages and drawbacks of the adapted method

It was observed that the conducted baseline correction is unnecessary if the absorbance is high because only the third decimal was affected if the sample dilution was 1:100 or higher. A problem in the extraction of undried digestate is that the dry matter content of the digestate has to be estimated before the extraction. This estimation inevitably leads to a different dry matter to extractant ratio than the one used in this work. However, if phosphate is not extracted directly after sampling and the sample is frozen or cooled before extraction, the fractions will shift (Jarvie *et al.*, 2002). The presented method leads to a better representation of the H_2O-P and $NaHCO_3$-P fraction than the common method with dried samples. This is especially important considering that these two fractions make up 70% of the total phosphate. The results are reproducible and reasonable for a sequential extraction when compared to P_{tot}. Ultimately, the aspects described in this study conclude in the method depicted in Figure 4.5. The changes compared with the conventional method are marked in red.

4.4 Conclusion

In conclusion, 709 nm is the ideal wavelength for phosphate analysis in digestate extracts using a 50 mm light path and the molybdenum blue method. The lower calibration limit needs to be raised to 50 $\mu g\ L^{-1}$ to avoid underestimation of phosphate concentration. Furthermore, base-line correction is unnecessary. Phosphate should be extracted from undried digestate because drying increases H_2O-P and $NaHCO_3$-P. Samples must be rinsed with 30 mL deionized H_2O, 60 mL $NaHCO_3$ and 60 mL NaOH after their respective extractions. The adapted Hedley fractionation provides a tool for the investigation of phosphate changes during AD and phosphate in digestate.

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.

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Supplementary material

Testing of solid – liquid separation method

In pre-trials it was tested, if the solid-liquid separation after each extraction step can be applied as described by Dou *et al.* (2000). In this study, the extract was centrifuged and vacuum-filtered. The regained filter cakes were further extracted and the filter was discarded. In the trials the centrifugation proved to be highly inefficient for digestate because of a high amount of suspended solids with low density. As a result, the filter retained all these solids. Parts of the solids were caught in the filter material and impossible to rinse or scratch out. These particles where lost to the extraction after filter disposal. This led to a high P loss with fraction sums of only about 50% P of total P. Because of the inefficient centrifugation, the samples were filtered by gravity filtration instead. Vacuum filtration was considered for a shorter filtration time but proved unfeasible. After each extraction, the filter cake was added to the next extraction along with the cut up filter. This way, the particles caught in the filter material were returned to the extraction. To tackle the increase in filter cake volume, the volume of the added extractant was increased in the NaOH and HCl extraction from 1:100 to 1:200 to ensure sufficient movement during shaking.

The ideal solution would be a combination of centrifugation and filtration, which means first centrifuge the sample and filter the supernatant afterwards. However, as described, the filter still needs to be added to the next extraction to avoid losses. As a result, the filter cake itself already has a large volume without the extractant and a regular 50 mL falcon tube will not suffice. Additionally, for high throughput, many vials are necessary during extraction. As a result, a large capacity centrifuge is needed.

References

APHA. (2012). 4500-P Phosphorus. In E. W. Rice, a. B. Baird, A. D. Eaton, & L. S. Clesceri (Eds.), Standard methods for the examination of water and wastewater (22nd ed.). American Public Health Association, American Water Works Assiciation, Water Environmental Federation. https://doi.org/10.2105/SMWW.2882.093

Bachmann, S., Uptmoor, R., & Eichler-Löbermann, B. (2016). Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Scientia Agricola, 73(1), 9–17. https://doi.org/10.1590/0103-9016-2015-0069

Bachmann, S., Wentzel, S., & Eichler-Löbermann, B. (2011). Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. Journal of Plant Nutrition and Soil Science, 174(6), 908–915. https://doi.org/10.1002/jpln.201000383

Bünemann, E. K., Keller, B., Hoop, D., Jud, K., Boivin, P., & Frossard, E. (2013). Increased availability of phosphorus after drying and rewetting of a grassland soil: Processes and plant use. Plant and Soil, 370(1-2), 511–526. https://doi.org/10.1007/s11104-013-1651-y

de Mendiburu, F. (2020). agricolae: Statistical Procedures for Agricultural Research. https://cran.r-project.org/package=agricolae

DIN Deutsches Institut für Normung e. V. (2001a). DIN EN 12879:2001-02 Characterization of sludges - Determination of the loss on ignition of dry mass; German version EN 12879:2000. Deutsches Institut für Normung e.V.

DIN Deutsches Institut für Normung e. V. (2001b). DIN EN 12880:2001-02 Characterization of sludges - Determination of dry residue and water content; German version EN 12880:2000. Deutsches Institut für Normung e.V. https://doi.org/10.31030/9066387

Dong, D., Choi, O. K., Lee, K., & Lee, J. W. (2020). Pilot-scale demonstration of nitrogen recovery in the form of ammonium phosphate (AP) from anaerobic digestate. Bioresource Technology, 297, 122392. https://doi.org/10.1016/j.biortech.2019.122392

Dou, Z., Toth, J. D., Galligan, D. T., Ramberg, C. F., & Ferguson, J. D. (2000). Laboratory procedures for characterizing manure phosphorus. Journal of Environmental Quality, 29(2), 508–514. https://doi.org/10.2134/jeq2000.00472425002900020019x

European Commission, Innovation, Directorate-General for Research, Ruban, V., Rauret, G., López-Sánchez, J., Muntau, H., & Quevauviller, P. (2001). The certification of the extractable contents (mass fractions) of phosphorus in freshwater sediment following a five-step extraction procedure : BCR 684. (Tech. rep.). Publications Office.

Fox, J., & Weisberg, S. (2019). An R Companion to Applied Regression (3rd ed.). SAGE Publications, Inc. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Günther, S., Grunert, M., & Müller, S. (2018). Overview of recent advances in phosphorus recovery for fertilizer production. Engineering in

Life Sciences, 18(7), 434–439. https://doi.org/10.1002/elsc.201700171

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal, 46(5), 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Jarvie, H. P., Withers, J. A., & Neal, C. (2002). Review of robust measurement of phosphorus in river water: sampling, storage, fractionation and sensitivity. Hydrology and Earth System Sciences, 6(1), 113–131. https://doi.org/10.5194/hess-6-113-2002

Kamh, M., Abdou, M., Chude, V., Wiesler, F., & Horst, W. J. (2002). Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. Journal of Plant Nutrition and Soil Science, 165(5), 566–572. https://doi.org/10.1002/1522-2624(200210)165:5<566::AID-JPLN566>3.0.CO;2-O

Kruse, J., Abraham, M., Amelung, W., Baum, C., Bol, R., Kühn, O., Lewandowski, H., Niederberger, J., Oelmann, Y., Rüger, C., Santner, J., Siebers, M., Siebers, N., Spohn, M., Vestergren, J., Vogts, A., & Leinweber, P. (2015). Innovative methods in soil phosphorus research: A review. Journal of Plant Nutrition and Soil Science, 178(1), 43–88. https://doi.org/10.1002/jpln.201400327

Li, B., Dinkler, K., Zhao, N., Sobhi, M., Merkle, W., Liu, S., Dong, R., Oechsner, H., & Guo, J. (2020). Influence of anaerobic digestion on the labile phosphorus in pig, chicken, and dairy manure. Science of the Total Environment, 737, 140234. https://doi.org/10.1016/j.scitotenv.2020.140234

Li, M., Whelan, M. J., Wang, G. Q., & White, S. M. (2013). Phosphorus sorption and buffering mechanisms in suspended sediments from the Yangtze Estuary and Hangzhou Bay, China. Biogeosciences, 10(5), 3341–3348. https://doi.org/10.5194/bg-10-3341-2013

Lin, H., Gan, J., Rajendran, A., Reis, C. E. R., & Hu, B. (2015). Phosphorus Removal and Recovery from Digestate after Biogas Production. In Biofuels - status and perspective (p. 64, Vol. 2). InTechOpen. https://doi.org/10.5772/60474

Ma, H., Guo, Y., Qin, Y., & Li, Y. Y. (2018). Nutrient recovery technologies integrated with energy recovery by waste biomass anaerobic digestion. Bioresource Technology, 269, 520–531. https://doi.org/10.1016/j.biortech.2018.08.114

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242–257. https://doi.org/10.1002/elsc.201100085

Murphy, J., & Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters (A.-L. Müller & W. Reichmann, Eds.). Analytica Chimica Acta, 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Naegele, H.-J., Lemmer, A., Oechsner, H., & Jungbluth, T. (2012). Electric Energy Consumption of the Full Scale Research Biogas Plant “Unterer Lindenhof”: Results of Longterm and Full Detail Measurements. Energies, 5(12), 5198–5214. https://doi.org/10.3390/en5125198

Nagul, E. A., McKelvie, I. D., Worsfold, P., & Kolev, S. D. (2015). The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. Analytica Chimica Acta, 890(5), 60–82. https://doi.org/10.1016/j.aca.2015.07.030

Ngo, P. T., Rumpel, C., Ngo, Q. A., Alexis, M., Vargas, G. V., Mora Gil, M. d. l. L., Dang, D. K., & Jouquet, P. (2013). Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. Bioresource Technology, 148, 401–407. https://doi.org/10.1016/j.biortech.2013.08.098

Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. Agronomy for Sustainable Development, 34(2), 473–492. https://doi.org/10.1007/s13593-013-0196-z

Peters, G.-J. Y. (2017). Diamond Plots: a tutorial to introduce a visualisation tool that facilitates interpretation and comparison of multiple sample estimates while respecting their inaccuracy. PsyArXiv. https://doi.org/10.31234/osf.io/fzh6c

Peters, K., Hjorth, M., Jensen, L. S., & Magid, J. (2011). Carbon, Nitrogen, and Phosphorus Distribution in Particle Size–Fractionated Separated Pig and Cattle Slurry. Journal of Environment Quality, 40(1), 224. https://doi.org/10.2134/jeq2010.0217

Price, R. M., Savabi, M. R., Jolicoeur, J. L., & Roy, S. (2010). Adsorption and desorption of phosphate on limestone in experiments simulating seawater intrusion. Applied Geochemistry, 25(7), 1085–1091. https://doi.org/10.1016/j.apgeochem.2010.04.013

R Core Team. (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.r-project.org/

Recena, R., Torrent, J., del Campillo, M. C., & Delgado, A. (2015). Accuracy of Olsen P to assess plant P uptake in relation to soil properties and P forms. Agronomy for Sustainable Development, 35(4), 1571–1579. https://doi.org/10.1007/s13593-015-0332-z

Rehl, T., & Müller, J. (2011). Life cycle assessment of biogas digestate processing technologies. Resources, Conservation and Recycling, 56(1), 92–104. https://doi.org/10.1016/j.resconrec.2011.08.007

Severin, M., Fuß, R., Well, R., Hähndel, R., & Van den Weghe, H. (2016). Greenhouse gas emissions after application of digestate: short-term effects of nitrification inhibitor and application technique effects. Archives of Agronomy and Soil Science, 62(7), 1007–1020. https://doi.org/10.1080/03650340.2015.1110575

Tambone, F., Orzi, V., D'Imporzano, G., & Adani, F. (2017). Solid and liquid fractionation of digestate: Mass balance, chemical characterization, and agronomic and environmental value. Bioresource Technology, 243, 1251–1256. https://doi.org/10.1016/j.biortech.2017.07.130

Tiessen, H., & Moir, J. O. (1993). Characterization of Available P by Sequential Extraction. In Martin R. Carter (Ed.), Soil sampling and methods of analysis (pp. 75–86). Lewis Publishers (A CRC Press Company).

Toor, G. S., Peak, J. D., & Sims, J. T. (2005). Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. Journal of Environmental Quality, 34(2), 687–697. https://doi.org/10.2134/jeq2005.0687

Turner, B. L., & Haygarth, P. M. (2001). Phosphorus solubilization in rewetted soils. Nature, 411(6835), 258–258. https://doi.org/10.1038/35077146

Turner, B. L., & Leytem, A. B. (2004). Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure. Environmental Science and Technology, 38(22), 6101–6108. https://doi.org/10.1021/es0493042

Wang, J., Wu, Y., Zhou, J., Bing, H., Sun, H., Luo, J., & Pu, S. (2020). Air-drying changes the distribution of Hedley phosphorus pools in forest soils. Pedosphere, 30(2), 272–284. https://doi.org/10.1016/S1002-0160(17)60456-9

Wang, W., & Lee, D. J. (2021). Valorization of anaerobic digestion digestate: A prospect review. Bioresource Technology, 323(December 2020), 124626. https://doi.org/10.1016/j.biortech.2020.124626

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag. https://ggplot2.tidyverse.org

Wuenscher, R., Unterfrauner, H., Peticzka, R., & Zehetner, F. (2016). A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. Plant, Soil and Environment, 61(No. 2), 86–96. https://doi.org/10.17221/932/2014-PSE

Xu, Y., Hu, H., Liu, J., Luo, J., Qian, G., & Wang, A. (2015). pH dependent phosphorus release from waste activated sludge: Contributions of phosphorus speciation. Chemical Engineering Journal, 267, 260–265. https://doi.org/10.1016/j.cej.2015.01.037

Yokota, T., Ito, T., & Saigusa, M. (2003). Measurement of total phosphorus and organic phosphorus contents of animal manure composts by the dry combustion method. Soil Science and Plant Nutrition, 49(2), 267–272. https://doi.org/10.1080/00380768.2003.10410006

5 Response of phosphorus speciation to organic loading rates and temperatures during anaerobic co-digestion of animal manures and wheat straw

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Abstract

The world is facing huge phosphate (P) shortage and anaerobic digestion (AD) is a recognized technology to promote nutrient (N and P) recycling. The composition of P speciation in the digestate is essential for the fertilizing effect. However, how P speciation in the digestates interacts with the AD process conditions is unknown. Therefore, interaction of P speciation in digestates with AD process conditions was investigated by using a chemical sequential extraction method (Hedley fractionation) and X-ray diffraction; specifically, the effects of organic loading rate (OLR), temperature, and substrate composition were investigated. The results showed that OLR and feedstock affected P speciation in the digestate significantly due to different ion species and ionic strengths. The H_2O-P concentration in chicken manure with straw (CMS) and dairy manure with straw (DMS) digestates decreased by 44.04–48.76% and 48.88–50.49%, respectively, as the OLR increased from 2 to 4 kg VS m^{-3} d^{-1}. Simultaneously, HCl-P increased by 38.02–44.01% in the CMS digestates due to Ca-P and Mg-P formation, indicating that Ca-P and Mg-P formation was positively correlated with OLR, whereas P mobility decreased. Further, thermophilic temperature conditions were more conducive for the formation of insoluble P than mesophilic temperature conditions in the digestates due to the thermodynamic driving force of the reactions. The results would facilitate the understanding of P transformation in the AD process under the influence of feedstock, OLR, and temperature. From the viewpoint of nutrient management, lower OLR and temperature are more beneficial for a fast P availability, whereas higher OLR and temperature are more helpful for storage and export because of P precipitated into solid phase of digestate.

Keywords: Phosphorus speciation; Anaerobic digestion; Organic loading rate; Temperature

5.1 Introduction

P is one of the most limited and unevenly distributed resources worldwide (Ye *et al.*, 2017). Moreover, large amounts of P are being lost due to the direct release of animal manure from livestock farms into water bodies (Bai *et al.*, 2016a). Anaerobic digestion (AD), an effective technology for converting agricultural waste into resources, is considered to be a promising solution to reduce P loss reduction and recycle P (Arthurson, 2009).

For optimizing digestate use, researches have focused on the phase distribution of nutrients during the solid-liquid separation of digestates. More than 80% of N is found to exist in the liquid fraction (Bachmann *et al.*, 2016), while most P is found in the solid phase after solid-liquid separation. Möller and Müller (2012) reported that approximately 60–95% of P remains in the thickened sludge after sedimentation of animal manure digestate.

Few studies investigated the P species in the digestate. Mazzini *et al.* (2020) used sequential fractionation methods for comprehensively classifying the P in the digestate from different biogas plants. The research indicated that P speciation in digestate depended on ingestate, especially those with more manure/slurry could result in P precipitation as Ca/Mg-P salts. Güngör and Karthikeyan (2008) analyzed the content of dissolved P component and ortho-P in the influent and effluent from biogas plants in different seasons and stated that water extractable P of dairy manure reduced by 22–47% during mesophilic AD process. Although previous studies described P speciation in both digestate and the operating parameters of the analyzed biogas plants, investigating and comparing the actual effect of parameters, such as feedstock, OLR, pH, and temperature, on P speciation is difficult because the biogas plants operate under multivariate conditions.

In the AD process, temperature can affect the microbial metabolic activities and chemical reactions (Li *et al.*, 2010; Strømme, 1994). Most industrialized biogas plants operate at either mesophilic (30–42 °C), or thermophilic conditions (50–60 °C) (Westerholm *et al.*, 2018; Yu *et al.*, 2014), which theoretically affects P speciation due to the different thermodynamic driving force at different temperatures (Rahman *et al.*, 2018; Song *et al.*, 2002). Moreover, changes in pH immediately affect the formation of phosphate salts. Nonetheless, pH is not considered as an adjustable parameter but a parameter resulting from the used feedstock and the corresponding ion equilibrium (Nikulina *et al.*, 2021). Moreover, an optimized organic loading rate (OLR) is necessary to achieve high biogas production and stable operation, which remain economic drivers for biogas plants (Bhatt & Tao, 2020). Changes in the OLR alter the nutrient concentration in the AD the process, and is thus, a critical parameter for exploring P speciation. The limited understanding of P behavior in the AD systems affected by all the above parameters is the motivation of this study. The objectives of the study were to elucidate the effects of temperature (mesophilic and thermophilic conditions), OLR (2, 3, and 4 kg VS m^{-3} d^{-1}), and substrate composition (high Ca content vs low Ca content) on P speciation fractionated by the Hedley fractionation method adapted by Dinkler *et al.* (2021) during the anaerobic co-digestion of chicken manure with straw (CMS) and dairy manure with straw (DMS). The study would assist in P management by providing insights on when to export or supply P through the AD process.

5.2 Materials and methods

5.2.1 Substrates and inoculum

Table 5.1: Characteristics of substrates and inoculum. Total solids (TS) and volatile solids (VS) values are shown as value ± standard deviation. Elemental analysis was done with inductively coupled plasma-optical emission spectrometry.

Characteristics	**TS**	**VS**	**P_{tot}**	**N_{tot}**	**K**	**Ca**	**Mg**	**Al**	**Fe**
Unit	% FM	% TS			$g\ kg^{-1}TS$				
Chicken manure	49.67 ± 5.91	75.20 ± 1.47	14.14	60.87	24.67	68.39	5.48	0.54	0.79
Dairy manure slurry	6.48 ± 0.06	80.14 ± 0.03	8.38	58.17	32.50	13.99	6.42	0.92	1.05
Straw	88.82 ± 0.10	95.96 ± 0.33	0.81	7.75	6.119	2.88	0.67	0.16	0.18
Reactor sludge start of the experiment	5.95 ± 0.39	68.58 ± 1.76	10.36	69.99	50.15	18.49	6.73	2.57	3.10
Inoculum mixture reactor start	6.88 ± 0.09	73.92 ± 1.11	/	/	/	/	/	/	/

Chicken manure was obtained from the Hagenauer Hof KG hen farm in Neuhausen, Germany, and dairy manure was acquired from the Markus Bauer dairy farm in Harthausen, Germany. Grated wheat straw (particle size < 4 mm) was obtained from a vendor located in Neufra, Germany. The inoculums necessary to start the reactions were produced by mixing the reactor sludge of two full-scale biogas plants to achieve a mixture of mesophilic and thermophilic organisms and to create the same starting conditions for both temperature variations. One plant is in Harthausen, Germany, and has been operational since 2005. It digests dairy manure and maize silage under mesophilic conditions. The other plant is a thermophilic biogas plant in Isny, Germany, which was commissioned in 2006 and is fed with a mixture of maize and grass silage, dairy and pig manure, and landscape maintenance material. The total solid (TS) and volatile solid (VS) analysis of the mixed inoculum and the substrates is shown in Table 5.1.

Two hybrid substrates were used in this study. Chicken manure was mixed with straw (CMS) to adjust the C:N ratio to 20:1, reducing the risk of inhibition from high ammonia in the chicken manure. This resulted in a 2:1 ratio of chicken manure to straw on a dry matter basis. The dry matter ratio was also applied to the hybrid substrate of dairy manure and straw (DMS) to increase the overall TS content of the mixture. The input was augmented with tap water to achieve the necessary volume for a constant hydraulic retention time (HRT). The substrates were stored at -18 °C and defrosted at 4 °C before feeding.

5.3 Experimental setup

5.3.1 Digester

Horizontal continuous stirred-tank reactor (CSTR) of 20 L total volume and 17 L working volume were used. The temperature was controlled using a water jacket connected to a hot water bath. Gas was released from the top of the reactors and passed a gas wash bottle before it was collected in sealed gas bags. Once a day, gas was sucked from the bag to a gas quantifier (Höntzsch FA MS40, Germany) and subsequently, to a gas analyzer, where CH_4 and CO_2 contents were measured with one sensor (D-AGM Plus, Sensors Europe, Germany) and H_2S with another (MembraporH2S/S-5000-S, Switzerland) (Haag et al., 2015).

5.3.2 Operational conditions

The two hybrid substrates were anaerobically digested at 40 ± 1 °C (i.e., under mesophilic conditions) and 53 ± 1 °C (i.e., under thermophilic conditions). Each treatment was performed in triplicate resulting in a total of 12 reactors. All reactors were fed daily at the same time. A constant HRT of 30 d was ensured throughout the experiment.

Table 5.2: Experimental treatments. CMS = Chicken manure + straw, DMS = Dairy manure + straw, OLR = Organic loading rate

Abbreviation	**Temperature**	**OLR (kg m^{-3} d^{-1})**					
		1	2	2.5	3	3.5	4
		Duration					
CMS 40	40 °C (Mesophilic)	5 d	45 d	5 d	45 d	5 d	45 d
CMS 53	53 °C (Thermophilic)						
DMS 40	40 °C (Mesophilic)						
DMS 53	53 °C (Thermophilic)						

5.3.3 Start up and operation

Mesophilic and thermophilic inoculums were mixed, and 17 L of the mixed inoculum were fed into the reactors to start their operation. The reactors were then fed with 0.5 L dairy manure slurry every day for one month to stabilize the systems and ensure their functionality. After this period, samples, which marked the reactors' sludge composition at the start of the formal experiment (Table 1), were collected for analysis. The reactors were operated at an OLR of 1.0 kg VS m^{-3} d^{-1} for 5 d, after which the OLR was raised to the first targeted OLR of 2.0 kg VS m^{-3} d^{-1}. After 45 d, the OLR was raised to 2.5 kg VS m^{-3} d^{-1} for 5 d and then to the second targeted 3.0 kg VS m^{-3} d^{-1} for another 45 d. Later, the OLR was raised to 3.5 kg VS m^{-3} d^{-1} for 5 d and ultimately, the third targeted OLR of 4.0 kg VS m^{-3} d^{-1} was maintained for 45 d. Details of the experimental design are presented in Table 2. Samples were taken during the last 15 d of each targeted OLR and were regarded as steady state of the targeted OLR.

5.3.4 Pretreatment of samples and analysis methods

A rubber tube was used to collect weekly samples from the inside of the reactor. The pH was measured using a pH meter WTW pH323 and pH electrode (SenTx®41, WTW, Weilheim, Germany) directly after sampling, to ensure that it was not affected by temperature changes in the samples. Subsequently, the samples were subjected to TS/VS determination (APHA, 2540), Hedley fractionation (Dinkler *et al.*, 2021), and total nitrogen (N_{tot}) analysis and NH_4^+ analysis by Kjeldahl extraction. Total P (P_{tot}) and K as well as the micronutrients Fe, Al, Mg, Ca were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, 5110 svdv, Agilent, Santa Clara, United States) after microwave digestion (UltraClave Fa. MLS Leutkirch, Deutschland). For process control, the ratio of volatile fatty acids to total inorganic carbonate (VFA/TIC) was analyzed using a 785 DMP Titrino (Metrohm, Herisau, Switzerland). Gas analysis and quantification were performed using a fully automated laboratory system at the State Institute of Agricultural Engineering and Bioenergy as described in section 5.3.1 Gas data were normalized to 0 °C and an ambient pressure of 1013.25 hPa. After the continuous experiment was completed, some sediments were found at the bottom of the CMS reactors. These samples were analyzed using X-ray diffraction (XRD; XD-2, Beijing Purkinje General Instrument Co., Ltd., China) with Cu K(α) radiation at a voltage of 36 kV and current of 20 mA over a scanning angular range from 10° to 80° at a rate of 4°/min. The chemical compositions were analyzed using the phase retrieval function of MDI Jade 5.0 software (Materials Data, Inc., Livermore, CA).

5.3.5 Statistical analysis

The data were analyzed for normal distribution and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. Significance was tested using one-way analysis of variance (ANOVA). The Tukey test was used if the data showed a normal distribution and homogeneous variance with an equal sample size. In all other cases, Welch-ANOVA was used, followed by the Games-Howell test. Analysis was conducted using SPSS version 13.0 and R Studio. Pearson's correlation analysis and all data visualizations were carried out using Origin software 2018.

5.4 Results and discussion

5.4.1 Process stability

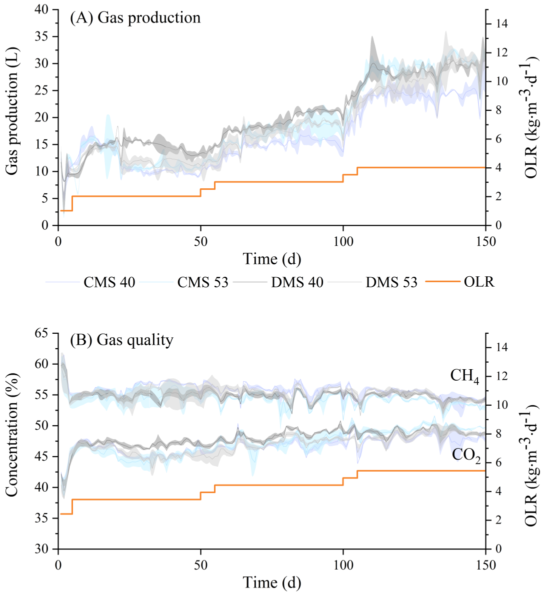

Figure 5.1: Gas production (A) and gas quality (B) of the substrates at different temperatures. The line plots mark the means and the underlying shades represent the standard deviations.

The daily gas production over the experimental period of 150 d is shown in Figure 5.1A It is evident that each OLR increase was accompanied by a gas production increase. Gas production was stable within each group over the last 15 d. During the stable phase, the average gas productions of CMS with OLR at 2, 3, and 4 kg VS m^{-3} d^{-1} were 9.56 ± 1.06, 15.63 ± 1.17, and 24.81 ± 2.19 L d^{-1}, respectively, under mesophilic conditions, and 10.68 ± 1.25, 18.71 ± 1.87, and 30.55 ± 2.11 L d^{-1}, respectively, under thermophilic conditions. Further, the average gas production of DMS with OLR at 2, 3, and 4 kg VS m^{-3} d^{-1} were 13.43 ±1.62, 21.08 ± 0.71, and 29.79 ± 1.63 L d^{-1}, respectively, under mesophilic conditions, and 11.59 ± 1.82, 18.90 ± 1.07, and 30.27 ± 2.14 L d^{-1}, respectively, under thermophilic conditions. Figure 5.1 B shows that the CH_4 content over all four treatments was between 54.2% (DMS 40 °C, OLR 4) and 56.7% (CMS 40 °C, OLR 2) during the experimental period with a slightly decreasing trend as OLR increased. The declining CH_4 content coincided with an increasing CO_2 content. The results showed that mesophilic conditions were optimal for gas production from DMS, but CMS produced more gas under thermophilic conditions. However, previous studies showed contrasting findings. Bi *et al.* (2019) found that thermophilic conditions had a negative effect on the CH_4 yield of chicken manure at high OLRs because of the high concentration of free ammonia nitrogen. The difference to the presented results is likely related to the straw that was used to increase the C:N ratio. Rahman *et al.* (2018) reported that thermophilic conditions were advantageous for biogas production using a mixture of poultry droppings and straw. In conclusion, from the viewpoint of gas production, enhancing the C:N ratio of high-nitrogen substrates could eliminate the negative influence of thermophilic AD.

Table 5.3: Volatile solid (VS) content, pH, ratio of volatile organic acids to total inorganic carbonate (VFA/TIC), and concentration of NH_4^+ in the CMS and DMS digestates at different OLRs and temperatures. All values are shown as mean value ± standard deviation.

		CMS 40	**CMS 53**	**DMS 40**	**DMS 53**
VS (% FM)	**OLR2**	$2.07^{Bb} \pm 0.23$	$2.01^{Bb} \pm 0.09$	$2.83^{Ca} \pm 0.29$	$3.29^{Ca} \pm 0.43$
	OLR3	$2.05^{Bc} \pm 0.58$	$2.13^{Bc} \pm 0.69$	$3.23^{Bb} \pm 0.11$	$4.40^{Ba} \pm 0.30$
	OLR4	$4.31^{Ab} \pm 0.11$	$4.24^{Ab} \pm 0.20$	$5.45^{Aa} \pm 0.08$	$6.34^{Aa} \pm 0.39$
pH	**OLR2**	$7.70^{Ac} \pm 0.07$	$8.00^{Aa} \pm 0.08$	$7.50^{Ad} \pm 0.05$	$7.82^{Ab} \pm 0.07$
	OLR3	$7.59^{Bb} \pm 0.02$	$7.76^{Ca} \pm 0.03$	$7.28^{Cd} \pm 0.02$	$7.49^{Cc} \pm 0.01$
	OLR4	$7.73^{Ab} \pm 0.06$	$7.93^{Ba} \pm 0.05$	$7.40^{Bc} \pm 0.06$	$7.69^{Bb} \pm 0.03$
VFA/TIC	**OLR2**	$0.158^{Ab} \pm 0.003$	$0.176^{Ba} \pm 0.006$	$0.177^{Ba} \pm 0.003$	$0.184^{Ba} \pm 0.015$
	OLR3	$0.150^{Bc} \pm 0.008$	$0.176^{Bab} \pm 0.007$	$0.181^{Bb} \pm 0.008$	$0.186^{Ba} \pm 0.009$
	OLR4	$0.155^{ABc} \pm 0.007$	$0.189^{Ab} \pm 0.007$	$0.195^{Ab} \pm 0.006$	$0.206^{Aa} \pm 0.006$

Note: Different capital letters indicate significant differences within columns and lowercase letters indicate significant differences between rows ($p < 0.05$).

Gas production provides important information about the stability of the AD process, whereas the volatile solid (VS), VFA/TIC, and pH of digestates are crucial indicators to monitor the running status of the system. Table 5.3 shows the VS, VFA/TIC, and pH during the last 15 d of each phase. The results indicated that the VS of the DMS digestate under mesophilic conditions was lower than that under thermophilic conditions, showing a higher degradation rate under mesophilic conditions. In contrast, the VS in the CMS digestate displayed an opposite pattern (5.3). The gas production results were consistent with the degradation rate of organic matter in the digestates (Figure 5.1 A and Table 5.3). Simultaneously, the pH of the digestates fluctuated in the ranges of 7.59–7.73 in the mesophilic CMS digestate, 7.76–8.00 in the thermophilic CMS digestate, 7.28–7.50 in the mesophilic DMS digestate, and 7.49–7.82 in the thermophilic DMS digestate. The initial pH values of the mesophilic digestate and thermophilic digestate were 7.95 and 8.25, respectively. This shows a slight drop in pH during the experiment, which was related to the formation of organic acids during the process. The CSTR systems normally operate at close to neutral or slightly alkaline pH, and the pH is affected by several factors, including the buffering action of carbonates and NH_4^+ and the transforma-

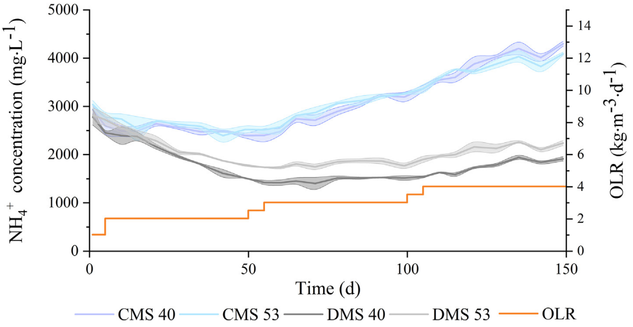

Figure 5.2: NH_4^+ concentrations of chicken manure + straw (CMS) and dairy manure + straw (DMS) digestates at different temperatures. The line plots mark the means and the underlying shades represent the standard deviations.

tion speed of VFA (Ali *et al.*, 2019). In this experiment, the changes in pH occurred gradually without a sudden increase or decrease, thus, showing good stability in the operation of the reactors. In the digestates, the pH was normally higher under thermophilic conditions than under mesophilic conditions, which was consistent with the findings of Rahman *et al.* (2018) and partly related to the chemical equilibrium of NH_3 and NH_4^+, which shifts towards NH_3 at higher temperature. Additionally, the pH in the CMS digestate was higher than in the DMS digestate, which could be associated with the higher nitrogen content in CMS. Moreover, VFA/TIC in the CMS digestate was generally lower than in the DMS digestate under the same conditions. The maximum VFA/TIC values in all treatments were below 0.4 mg CH_3COOH $CaCO_3$ mg^{-1}, which is the threshold that indicates that the AD processes are at risk of becoming unstable (Zhou *et al.*, 2021).

In the CMS group, the average concentration of NH_4^+ increased as the OLR increased. In the DMS group, the average concentration of NH_4^+ remained stable when the OLR changed from 2 to 3 kg VS m^{-3} d^{-1}, and a significant increase in NH_4^+ concentration was observed when the OLR reached 4 kg VS m^{-3} d^{-1}. The average NH_4^+ concentrations of the DMS digestate at an OLR of 4 kg VS m^{-3} d^{-1} were 1874 ± 69 mg L^{-1} under mesophilic conditions and 2171 ± 87 mg L^{-1} under thermophilic conditions (Figure 5.2), both of which were lower than 3000 mg L^{-1}, which is the critical point of ammonia inhibition (Morozova et al., 2020). The NH_4^+ concentration increased to 4156 ± 160 mg L^{-1} by the end of the experiment in the mesophilic CMS digestate and to 3958 ± 162 mg L^{-1} in the thermophilic CMS digestate. However, the high NH_4^+ concentration in the CMS digestate did not have any negative influence on AD as can be seen in the stable gas quality and quantity. This lack of obvious inhibition of gas production was related to the adaptation of microorganisms to gradually increasing NH_4^+ concentration because of increasing OLR (Morozova *et al.*, 2020; Nie *et al.*, 2015) and optimal C:N ratio due to the co-digestion of chicken manure and straw (Rahman *et al.*, 2018).

5.4.2 Effect of organic loading rate on P speciation in the digestates of chicken manure with straw and dairy manure with straw

Figure 5.3 shows the concentrations of different phosphate species in the CMS and DMS digestates at different OLRs and temperatures. In the CMS digestates, the content of H_2O-P decreased by 44.03% (from 5.11 ± 0.98 g kg^{-1} TS (OLR 2) to 2.86 ± 0.24 g kg^{-1} TS (OLR 4)) and 48.90% (from 4.54 ± 0.71 g kg^{-1} TS (OLR 2) to 2.32 ± 0.31 g kg^{-1} TS (OLR 4)) under mesophilic and thermophilic conditions, respectively (Figure 5.3 A). The content of HCl-P increased by 44% (from 1.25 ± 0.44 (OLR 2) to 1.80 ± 0.53 g kg^{-1} TS (OLR 4)) and 38.06% (from 1.55 ± 0.39 (OLR 2) to 2.14 ± 0.43 g kg^{-1} TS (OLR 4)) under mesophilic and thermophilic conditions, respectively ($p < 0.05$) (Figure 5.3 D). The $NaHCO_3$-P content increased from 5.13 ± 1.24 (OLR 2) to 5.93 ± 1.62 g kg^{-1} TS (OLR 4), that is, it increased slightly with increasing OLR in the mesophilic digestate (Figure 5.3 B). Simultaneously, the NaOH-P content was not significantly affected by OLR (Figure 5.3 C). In the DMS digestates, the content of H_2O-P decreased by 48.90% (from 3.64 ± 0.86 g kg^{-1} TS (OLR 2) to 1.86 ± 0.34 g kg^{-1} TS (OLR 4)) and 50.45% (from 3.37 ± 0.38 g kg^{-1} TS (OLR 2) to 1.67 ± 0.37 g kg^{-1} TS (OLR 4)) under mesophilic and thermophilic conditions, respectively (Figure 5.3 A). Further, the contents of NaOH-P and HCl-P increased marginally (from 3 to 4 kg VS m^{-3} d^{-1}) with increasing OLR, indicating that they were not significantly affected by OLR (Figure 5.3 C and D). Moreover, the $NaHCO_3$-P concentration increased slightly (from 3.64 ± 0.57 g kg^{-1} TS (OLR 2) to 4.73 ± 0.49 g kg^{-1} TS (OLR 4)) in the mesophilic DMS digestate, and similar results were observed in the mesophilic CMS digestate (Figure 5.3 B). According to Hedley *et al.* (1982), H_2O-P, $NaHCO_3$-P, NaOH-P, and HCl-P are ortho-P, exchangeable P (adsorbed on the surface of particles), Al-P/Fe-P, and Ca-P/Mg-P, respectively. Therefore, the results indicated that as the OLR increased, ortho-P mainly transformed to Ca-P/Mg-P in both mesophilic and thermophilic CMS digestates and partly transformed to the exchangeable P in mesophilic CMS and DMS digestates. This is likely related to the accumulation of materials in the reactor with increasing OLR, especially the accumulation of metal ions that can combine with P to produce insoluble P. Moreover, the increased OLR provided more particles that could adsorb H_2O-P to form $NaHCO_3$-P (Güngör & Karthikeyan, 2008; Möller & Müller, 2012).

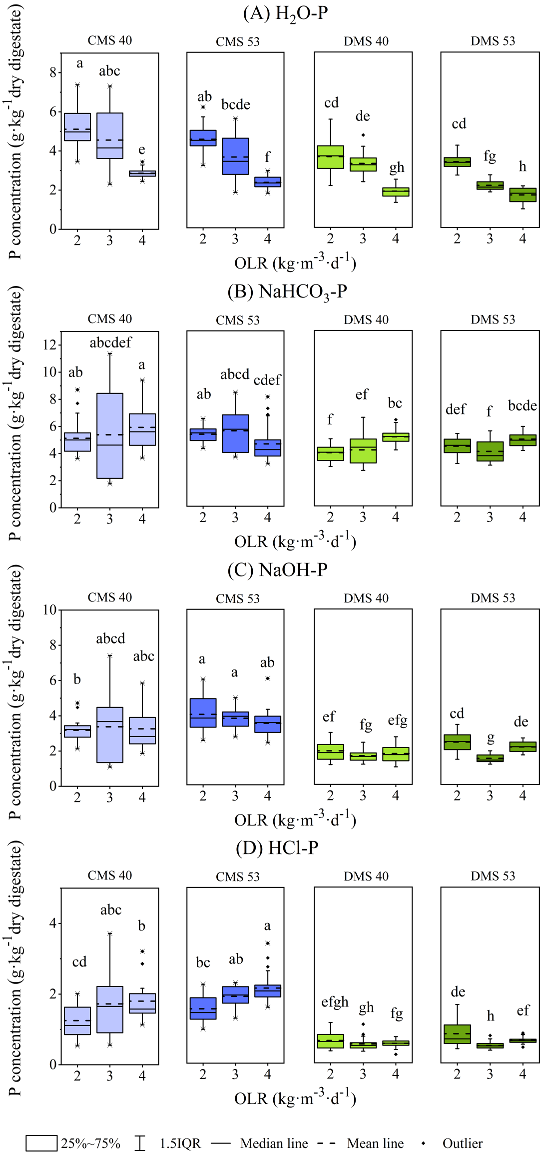

Figure 5.3: Phosphate concentration of each extraction step in the CMS and DMS digestates at different organic loading rates (OLRs) and temperatures.

These results agreed well with those of previous studies. Güngör and Karthikeyan (2008) indicated that ortho-P precipitated as an inorganic P in the solid phase of the digestate or became associated with particulate solids ($NaHCO_3$-P) during the AD process of dairy manure. Li *et al.* (2020) reported that P is transformed from soluble-P (H_2O-P and $NaHCO_3$-P) to insoluble P (NaOH-P and HCl-P) during batch AD process. Kumar and Pal (2015) stated that the formation of struvite ($(NH_4)MgPO_4$ $6H_2O$) is promoted by the ionic strength and ion activity of Mg^{2+}, NH_4^+, and PO_4^{3-} ions. Song *et al.* (2002) indicated that a higher ion concentration leads to a lower value of Gibb's free energy (ΔG), thus, causing a stronger thermodynamic driving force to promote Ca-P precipitation (Song *et al.*, 2002). The chicken manure used in this experiment contained 68.39 g kg^{-1} TS calcium (Table 5.3), thereby, providing advantages for Ca-P precipitation in the CMS digestate. Further, the calcium concentration in the used chicken manure was 127 times higher than the aluminum concentration and 86 times higher than the iron concentration and 12.5 times higher than the magnesium concentration. This led to a significant thermodynamic driving force towards Ca-P formation, which is the reason why the content of HCl-P increased and that of H_2O-P decreased while the NaOH-P content remained unchanged, with increasing OLR in the CMS digestate. Previous studies have reported a decrease in H_2O-P and increase in HCl-P in the digestate during AD, but the behaviors of P corresponding to the increase in OLR were not clearly reported 5.5. A previous study (Güngör & Karthikeyan, 2008), which reported that the content of ortho-P in digestate increased from 7 to 15 mg/L with an increase in OLR from 3 to 4 kg VS m^{-3} d^{-1}, provides limited information. However, the the effect of seasonal variation was investigated but OLR and temperature were not the targeted parameters for variation. Therefore, it is difficult to summarize the effects of process parameters on P speciation during AD according to the previous studies. The present study provided comprehensive information that facilitated an in-depth understanding on the correlation of OLR and P.

Overall, higher OLR led to lower H_2O-P and higher HCl-P in the digestate with high Ca feedstocks. However, H_2O-P also decreased significantly in the DMS digestate but with an increase in $NaHCO_3$-P. From the viewpoint of nutrient export, higher OLR could assist P export in the solid phase of the digestate. Additionally, the feedstock substrates played a role in P speciation in the digestates. This coincided with the findings of Mazzini *et al.* (2020), who reported that a substrate switch could be desirable for specific fertilizing requirements. On the other hand, low OLR increases direct plant availability, enhancing fertilizing effects when applying unseparated digestate or the liquid fraction of digestate from low OLR operation.

5.4.3 Effect of temperature on P speciation in the digestates of chicken manure with straw and dairy manure with straw

At the same OLR, temperature affected P speciation moderately in both CMS and DMS digestates (Figure 5.3 A). At an OLR of 2, 3, and 4 kg VS m^{-3} d^{-1} and thermophilic conditions, the H_2O-P concentrations in the CMS digestate were 4.54, 3.63, and 2.33 g kg^{-1} TS, respectively. This was 11.15% (OLR 2 kg VS m^{-3} d^{-1})–20.39% (OLR 3 kg VS m^{-3} d^{-1}) lower than that under mesophilic conditions (5.11, 4.56, and 2.86 g kg^{-1} TS, respectively). At the same time, the concentrations of NaOH-P and HCl-P increased by 7.66–26.02% and 10.34–23.72% when comparing mesophilic to thermophilic conditions in CMS digestate (Figure 5.3 C and D). A similar influence of temperature on P speciation was found in the DMS digestate. In the mesophilic DMS digestate, the H_2O-P concentrations were 3.64, 3.28, and 1.86 g kg^{-1} TS at OLRs of 2, 3, and 4 kg VS m^{-3} d^{-1}, respectively, while the corresponding H_2O-P concentrations were 3.37, 2.16, and 1.67 g kg^{-1} TS, respectively, in the thermophilic DMS digestate, which were 7.42–34.15% lower. Changes in temperature showed no statistically significance effect on the $NaHCO_3$-P content in the DMS digestate (Figure

5.3 B). However, NaOH-P and HCl-P were slightly higher in the thermophilic digestates with an exception at an OLR of 3 kg VS m^{-3} d^{-1} in the DMS digestate (Figure 5.3 C and D). In conclusion, the thermophilic conditions supported the transformation from H_2O-P to insoluble P (both NaOH and HCl-P) in the AD system. This is connected to the higher pH at thermophilic conditions in this study (Table 5.3), which promoted the formation of Ca-P and Mg-P. Previous studies indicated that the efficiency of P recovery increased as pH increased from 8.0 to 10.5 (Muhmood *et al.*, 2018). Möller and Müller (2012) stated that increasing the pH moves the chemical equilibrium toward the formation and subsequent precipitation of Ca-P and Mg-P. Furthermore, temperature influences the direction and rate of the reaction (Song *et al.*, 2002). Higher temperature promotes endothermic reactions. Song *et al.* (2002) reported a positive correlation between Ca-P precipitation and temperature, and stated that the precipitation of Ca-P is an endothermic process. NaOH-P was slightly higher in thermophilic compared to mesophilic treatments. Results similar to the previous study on the adsorption kinetics of phosphate on goethite (NaOH-P was considered as the P adsorbed on amorphous iron-aluminum oxides) wherein an increase in the adsorption of phosphate was observed with increasing temperature (Luengo *et al.*, 2007). Additionally, the effect of temperature was related to the endothermic process of adsorption of phosphate on goethite. In conclusion, thermophilic conditions moderately supported the transformation from H_2O-P to insoluble P (both NaOH and HCl-P) due to higher pH and promotion of endothermic reactions in the AD system.

Some sediments were found in the reactors of the CMS group at the end of the continuous experiment. The XRD results of these sediments showed that the main components were similar at different temperatures; they mainly consisted of $CaCO_3$, $AlPO_4$, Fe_2O_3, SiO_2, $(NH_4)MgPO_4$ $6H_2O$, and $Ca_{10}(PO_4)_6(OH)_2$ (Figure 5.4). However, between the temperatures, there was a shift in the peak intensities. As the peaks with major changes were all affiliated with calcium, among others, the observed shifts indicated that increasing the temperature influenced the deposits of calcium. This substantiates the results from the Hedley fractionation, where the calcium-containing HCl-P was moderately increased by thermophilic conditions. However, based on the sediment results, no conclusion can be drawn regarding the enhanced formation of specific salts at either of the two temperatures.

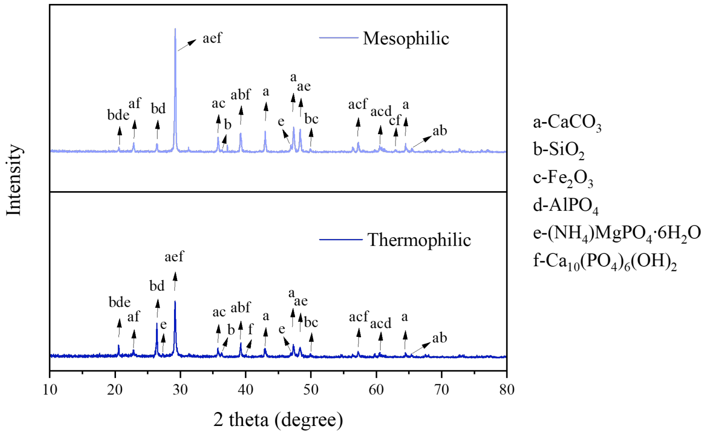

Figure 5.4: X-ray diffraction patterns of mesophilic and thermophilic CMS digestate. a: $CaCO_3$; b: $AlPO_4$; c: Fe_2O_3; d: SiO_2; e: $(NH_4)MgPO_4$ $6H_2O$; and f: $Ca_{10}(PO_4)6(OH)_2$.

5.4.4 Relationships between metal and P concentration in the digestates

The results described in the preceding sections suggest that the ion species and ionic strength were important influencers of P speciation in the digestates, while temperature played a role in promoting the transformation of P.

Correlation analysis among the main reaction partners for P-salt formation could provide some information for understanding the behavior of P in digestates. The Pearson's correlation analysis results (Figure 5.5) show that there were strong associations between phosphates and metals. In the CMS digestate, P content was significantly associated with Ca and Mg contents ($p < 0.05$), with Pearson's correlation coefficient values being 0.81 for Mg and 0.93 for Ca. This is likely to be related to the high content of calcium compared to the other metals in chicken manure (Table 5.1). This again emphasizes that calcium was the main reaction partner for phosphate during CMS digestion. In DMS digestion, this dominance of calcium could not be seen. Instead, phosphate correlated well with all the investigated metals, and therefore, no conclusions could be drawn from this analysis. However, it was evident that although calcium was predominant over the other metals in the DMS, its concentration was not sufficiently high to negate the affiliation of phosphate with iron and aluminum.

5.5 Conclusions

This study provides an in-depth understanding of the effect of process parameters on P behavior. The OLR increase during AD of CMS and DMS significantly decreased H_2O-P and moderately increased $NaHCO_3$-P in the mesophilic treatments. Additionally, a significant increase in HCl-P was observed with increasing OLR in both CMS treatments due to high Ca content in CMS. Thermophilic digestates showed slightly lower H_2O-P and moderately higher NaOH-P and HCl-P concentrations than mesophilic digestates, this, proving that temperature changed the thermodynamic driving force for P precipitation. However, OLR and substrate composition remained the main drivers for P precipitation with high OLR leading to high ionic concentrations, resulting in stronger P binding. From the viewpoint of nutrient management, higher OLR and temperature would

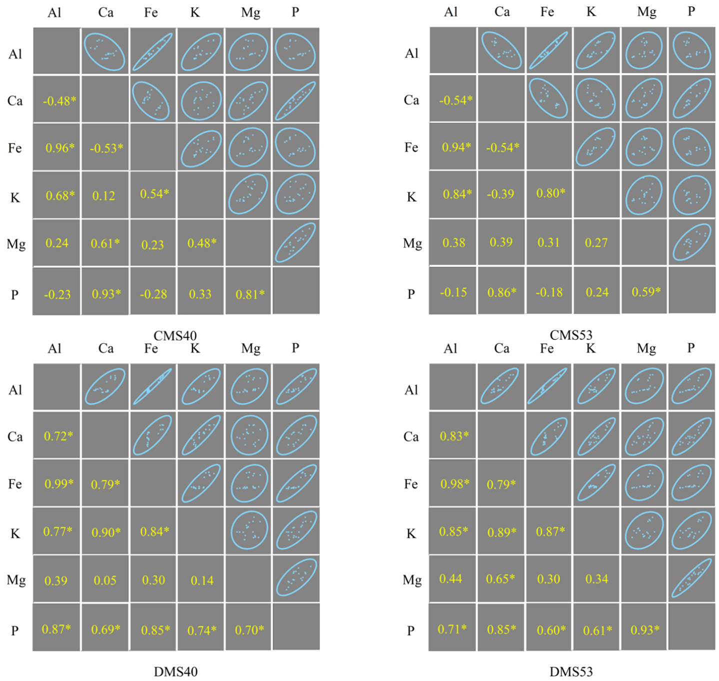

Figure 5.5: Pearson's correlation matrixes and significance values among the measured metals (Al, Ca, Fe, K, and Mg) and phosphorus (($*$ $p < 0.05$)

result in P precipitation in form of HCl-P, which could assist P export from the solid phase of the digestate. However, this study only focused on the P speciation at the steady state of each OLR. The dynamic transformation of P speciation under microbial action through the AD process needs further investigation.

CRediT authorship contribution statement

Bowen Li: Conceptualization, Methodology, Investigation, Writing - Original Draft, Formal analysis, Visualization, Validation and Editing. **Konstantin Dinkler:** Methodology, Investigation, Resources, Writing - Original Draft, Formal analysis, Visualization, Validation and Editing. **Nan Zhao:** Formal analysis, Visualization. **Xueling Ran:** Validation. **Mostafa Sobhi:** Writing- Reviewing and Editing. **Renjie Dong:** Project administration. **Joachim Müller:** Writing- Reviewing and Editing, Supervision. **Wei Xiong:** Writing- Reviewing and Editing. **Guangqun Huang:** Writing- Reviewing and Editing. **Jianbin Guo:** Writing- Reviewing and Editing, Funding acquisition, Supervision. **Hans Oechsner:** Writing- Reviewing and Editing, Funding acquisition, Supervision.

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.

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Supplementary material

Table 5.4: Concentrations of different P speciation in the CMS and DMS digestate at different OLRs and temperatures (40 °C and 53 °C). All values are shown as mean value ± standard deviation. Significance levels compare data of each solvent. CMS = Chicken manure + straw; DMS = Dairy manure + straw; OLR = Organic loading rates. Lower case letters indicate significance levels at $p < 0.05$ within each solvent group.

		H_2O-P in g kg^{-1} TS	**$NaHCO_3$-P in g kg^{-1} TS**	**NaOH-P in g kg^{-1} TS**	**HCl-P in g kg^{-1} TS**
OLR2	**CMS 40**	5.11 ± 0.98^{a}	5.13 ± 1.248^{ab}	3.18 ± 0.618^{b}	1.25 ± 0.448^{cd}
	CMS 53	4.54 ± 0.718^{ab}	4.93 ± 0.578^{ab}	4.01 ± 1.018^{a}	1.55 ± 0.398^{bc}
	DMS 40	3.64 ± 0.868^{cd}	3.64 ± 0.578^{f}	1.92 ± 0.498^{ef}	0.63 ± 0.238^{efgh}
	DMS 53	3.37 ± 0.388^{cd}	4.09 ± 0.558^{def}	2.42 ± 0.538^{cd}	0.83 ± 0.358^{de}
OLR3	**CMS 40**	4.56 ± 1.508^{abc}	5.39 ± 3.088^{abcdef}	3.38 ± 1.788^{abcd}	1.72 ± 0.918^{abc}
	CMS 53	3.63 ± 1.178^{bcde}	5.17 ± 1.438^{abcd}	3.79 ± 0.558^{a}	1.90 ± 0.318^{ab}
	DMS 40	3.28 ± 0.588^{de}	3.83 ± 0.968^{ef}	1.63 ± 0.338^{fg}	0.54 ± 0.188^{gh}
	DMS 53	2.16 ± 0.278^{fg}	3.73 ± 0.728^{f}	1.49 ± 0.248^{g}	0.49 ± 0.108^{h}
OLR4	**CMS 40**	2.86 ± 0.248^{e}	5.93 ± 1.628^{a}	3.26 ± 1.118^{abc}	1.80 ± 0.538^{b}
	CMS 53	2.33 ± 0.318^{f}	4.27 ± 1.228^{cdef}	3.51 ± 0.798^{ab}	2.14 ± 0.438^{a}
	DMS 40	1.86 ± 0.348^{gh}	4.73 ± 0.498^{bc}	1.75 ± 0.478^{efg}	0.56 ± 0.128^{fg}
	DMS 53	1.67 ± 0.378^{h}	4.57 ± 0.498^{bcde}	2.14 ± 0.308^{de}	0.65 ± 0.108^{ef}

Table 5.5: The summaries of previous studies and current work.

Achievements from previous studies	**Findings in current work**	**Sources**
P transformed from soluble-P (H_2O-P, $NaHCO_3$-P) to insoluble-P (NaOH-P and HCl-P) during AD process.	High OLR promoted the process of Ca-P/Mg-P precipitation.	Li *et al.*, 2020
The formation of struvite (a type of HCl-P) is promoted by the ionic strength and ion activity of magnesium, ammonium, and phosphate ions; A higher ion concentration leads to a lower value of Gibb's free energy (ΔG), thus, causing a stronger thermodynamic driving force to promote precipitation of Ca-P.	With the OLR increase, the substrates were gradually accumulated in the reactors, the concentration of ion increased, and the interaction and exchange among materials in digestate were enhanced. High concentration calcium in the feedstock led to high ion concentration, reducing Gibb's free energy in the reactor, thereby promoting Ca-P formation.	Kumar and Pal, 2015, Song *et al.*, 2002
The feedstock substrates played a role in P speciation in the digestates.	HCl-P concentrations increased with OLR rising in CMS digestate, while DMS digestate showed no effect. Substrate composition played a major role in P speciation.	Mazzini *et al.*, 2020
Temperature influences the direction and rate of the reaction; A positive correlation between Ca-P precipitation and temperature. The precipitation of Ca-P is an endothermic process	The content of H2O-P was lower and the content of insoluble-P (both NaOH and HCl-P) was higher in thermophilic digestate than mesophilic digestate. However, of the investigated parameters temperature had the lowest effect.	Song *et al.*, 2002
An increase in the adsorption of phosphate was observed with increasing temperature.	A moderate increase of NaOH-P and HCl-P under thermophilic conditions was found in CMS and DMS. P adsorption increased with OLR in DMS which is visible in an increase of $NaHCO_3$-P and decrease of H_2O-P at a constant concentration of NaHO-P and HCl-P. This effect was not significantly affected by temperature.	Luengo *et al.*, 2007
Raising the pH moves the chemical equilibrium toward the formation of phosphate and subsequent precipitation as Ca-P and Mg-P.	The pH values under thermophilic condition were higher than mesophilic condition (Table 3), coincided with the increase of insoluble-P under thermophilic condition.	Möller and Müller, 2012
Güngör and Karthikeyan (2008) indicated that ortho-P precipitated as an inorganic P in solid phase of digestate or became associated with particulate solids (NaHCO3-P) during AD process of dairy manure.	As the OLR increased, ortho-P mainly transformed to Ca-P/Mg-P in both mesophilic and thermophilic CMS digestate and partly transformed to the exchangeable P in mesophilic CMS and DMS digestate.	Güngör and Karthikeyan, 2008

References

Ali, S., Hua, B., Huang, J. J., Droste, R. L., Zhou, Q., Zhao, W., & Chen, L. (2019). Effect of different initial low pH conditions on biogas production, composition, and shift in the aceticlastic methanogenic population. Bioresource Technology, 289(April), 121579. https://doi.org/10.1016/j.biortech.2019.121579

Arthurson, V. (2009). Closing the Global Energy and Nutrient Cycles through Application of Biogas Residue to Agricultural Land – Potential Benefits and Drawback. Energies, 2(2), 226–242. https://doi.org/10.3390/en20200226

Bachmann, S., Uptmoor, R., & Eichler-Löbermann, B. (2016). Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Scientia Agricola, 73(1), 9–17. https://doi.org/10.1590/0103-9016-2015-0069

Bai, Z., Ma, L., Jin, S., Ma, W., Velthof, G. L., Oenema, O., Liu, L., Chadwick, D., & Zhang, F. (2016a). Nitrogen, Phosphorus, and Potassium Flows through the Manure Management Chain in China. Environmental Science & Technology, 50(24), 13409–13418. https://doi.org/10.1021/acs.est.6b03348

Bhatt, A. H., & Tao, L. (2020). Economic Perspectives of Biogas Production via Anaerobic Digestion. Bioengineering 2020, Vol. 7, Page 74, 7(3), 74. https://doi.org/10.3390/BIOENGINEERING7030074

Bi, S., Qiao, W., Xiong, L., Ricci, M., Adani, F., & Dong, R. (2019). Effects of organic loading rate on anaerobic digestion of chicken manure under mesophilic and thermophilic conditions. Renewable Energy, 139, 242–250. https://doi.org/10.1016/j.renene.2019.02.083

Dinkler, K., Li, B., Guo, J., Hülsemann, B., Becker, G. C., Müller, J., & Oechsner, H. (2021). Adapted Hedley fractionation for the analysis of inorganic phosphate in biogas digestate. Bioresource Technology, 331, 125038. https://doi.org/10.1016/j.biortech.2021.125038

Güngör, K., & Karthikeyan, K. G. (2008). Phosphorus forms and extractability in dairy manure: A case study for Wisconsin on-farm anaerobic digesters. Bioresource Technology, 99(2), 425–436. https://doi.org/10.1016/j.biortech.2006.11.049

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal, 46(5), 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Kumar, R., & Pal, P. (2015). Assessing the feasibility of N and P recovery by struvite precipitation from nutrient-rich wastewater: a review. Environmental Science and Pollution Research 2015 22:22, 22(22), 17453–17464. https://doi.org/10.1007/S11356-015-5450-2

Li, B., Dinkler, K., Zhao, N., Sobhi, M., Merkle, W., Liu, S., Dong, R., Oechsner, H., & Guo, J. (2020). Influence of anaerobic digestion on the labile phosphorus in pig, chicken, and dairy manure. Science of the Total Environment, 737, 140234. https://doi.org/10.1016/j.scitotenv.2020.140234

Li, N., Ren, N.-Q., Wang, X.-H., & Kang, H. (2010). Effect of temperature on intracellular phosphorus absorption and extra-cellular phosphorus removal in EBPR process. Bioresource Technology, 101(15), 6265–6268. https://doi.org/10.1016/j.biortech.2010.03.008

Luengo, C., Brigante, M., & Avena, M. (2007). Adsorption kinetics of phosphate and arsenate on goethite. A comparative study. Journal of colloid and interface science, 311(2), 354–360. https://doi.org/10.1016/J.JCIS.2007.03.027

Mazzini, S., Borgonovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., & Adani, F. (2020). Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. Science of The Total Environment, 734, 139284. https://doi.org/10.1016/j.scitotenv.2020.139284

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242–257. https://doi.org/10.1002/elsc.201100085

Morozova, I., Nikulina, N., Oechsner, H., Krümpel, J., & Lemmer, A. (2020). Effects of increasing nitrogen content on process stability and reactor performance in anaerobic digestion. Energies, 13(5). https://doi.org/10.3390/en13051139

Muhmood, A., Wu, S., Lu, J., Ajmal, Z., Luo, H., & Dong, R. (2018). Nutrient recovery from anaerobically digested chicken slurry via struvite: Performance optimization and interactions with heavy metals and pathogens. Science of the Total Environment, 635, 1–9. https://doi.org/10.1016/j.scitotenv.2018.04.129

Nie, H., Jacobi, H. F., Strach, K., Xu, C., Zhou, H., & Liebetrau, J. (2015). Mono-fermentation of chicken manure: Ammonia inhibition and recirculation of the digestate. Bioresource Technology, 178, 238–246. https://doi.org/10.1016/J.BIORTECH.2014.09.029

Nikulina, N., Uslu, S., Lemmer, A., Azbar, N., & Oechsner, H. (2021). Optimal conditions for high solid co-digestion of organic fraction of municipal solid wastes in a leach-bed reactor. Bioresource Technology, 331, 125023. https://doi.org/10.1016/j.biortech.2021.125023

Rahman, M. A., Møller, H. B., Saha, C. K., Alam, M. M., Wahid, R., & Feng, L. (2018). Anaerobic co-digestion of poultry droppings and briquetted wheat straw at mesophilic and thermophilic conditions: Influence of alkali pretreatment. Renewable Energy, 128, 241–249. https://doi.org/10.1016/j.renene.2018.05.076

Song, Y., Hahn, H. H., & Hoffmann, E. (2002). Effects of solution conditions on the precipitation of phosphate for recovery: A thermodynamic evaluation. Chemosphere, 48(10), 1029–1034. https://doi.org/10.1016/S0045-6535(02)00183-2

Strømme, K. O. (1994). Thermodynamic restrictions on the temperature and pressure dependence of the rate constant in chemical reaction kinetics. Thermochimica Acta, 237(2), 317–324. https://doi.org/10.1016/0040-6031(94)80189-4

Westerholm, M., Isaksson, S., Karlsson Lindsjö, O., & Schnürer, A. (2018). Microbial community adaptability to altered temperature conditions determines the potential for process optimisation in biogas production. Applied Energy, 226, 838–848. https://doi.org/10.1016/J.APENERGY.2018.06.045

Ye, Y., Ngo, H. H., Guo, W., Liu, Y., Li, J., Liu, Y., Zhang, X., & Jia, H. (2017). Insight into chemical phosphate recovery from municipal wastewater. Science of the Total Environment, 576, 159–171. https://doi.org/10.1016/j.scitotenv.2016.10.078

Yu, D., Kurola, J. M., Lähde, K., Kymäläinen, M., Sinkkonen, A., & Romantschuk, M. (2014). Biogas production and methanogenic archaeal community in mesophilic and thermophilic anaerobic co-digestion processes. Journal of Environmental Management, 143, 54. https://doi.org/10.1016/J.JENVMAN.2014.04.025

Zhou, L., Hülsemann, B., Cui, Z., Merkle, W., Sponagel, C., Zhou, Y., Guo, J., Dong, R., Müller, J., & Oechsner, H. (2021). Operating performance of full-scale agricultural biogas plants in germany and china: Results of a year-round monitoring program. Applied Sciences (Switzerland), 11(3), 1–17. https://doi.org/10.3390/APP11031271

6 General discussion

AD can contribute significantly to closing the nutrient cycle in agriculture and reduce the use of conventional fertilizers based on phosphate (P) rock and nitrogen gas from air. Nitrogen turnover during AD and its recovery have been thoroughly explored in literature. However, P turnover has, so far, not been investigated although its chemical state is essential for the fertilizing effect and potential recovery as marketable fertilizer. In this work, effects of process parameters on P-salt distribution in digestate were studied. Firstly, the kinetics of P turnover during AD were investigated. Afterwards, the Hedley fractionation, used for kinetics analysis, was optimized for the analysis of digestate and afterwards the effects of other process parameters, namely temperature, OLR and substrate composition were evaluated for a holistic evaluation of the influence of biogas plant operation on P turnover.

6.1 Reduction of labile P

Pig, chicken and dairy manure were digested in batch reactor systems and the change in Hedley fractions as well as P species over time was evaluated. The comparison of Hedley fractions in the input material and the final biogas digestate after 30 days showed that the decomposition of organic matter and corresponding release of kations into solution lead to a mineralization of P, meaning a reduction of H_2O-P. This coincides with the results of Möller and Müller 2012 and partly with Mazzini *et al.* 2020. According to Dinkler *et al.* 2021, sample drying before extraction leads to an increase in H_2O-P and $NaHCO_3$-P. Considering this, the share of mineral-P observed by Li *et al.* 2020, is even larger because the samples were dried before extraction leading to an overestimation of H_2O-P. However, the mineralization process observed over the digestion period is still accurate because all samples were treated equally, meaning that the input material underwent the same shift in fractions due to drying as the digestates.

Further, the results show that the reduction of H_2O-P took place rapidly in the beginning, after manure was mixed with activated sludge, which was likely related to the high concentration of ions in the inoculum. This can also be seen in the work of Li *et al.* (2022), where an increase of ion concentration in the reactor is directly related to reduced H_2O-P concentration. After the first two days, immobilization slowed down but continued until the end of the experiment in dairy manure. In addition, $NaHCO_3$-P decreased in all three digestates until day 30. A reduction of H_2O-P and $NaHCO_3$-P leads to a lower immediate fertilization effect because they are considered plant available. However, it also reduces the risk of run-off and leaching. Furthermore, a shift from the labile-P (sum of H_2O-P and $NaHCO_3$-P) fraction towards more stable forms also means that the applied P is not fixated in the soil through adsorption processes and chemical reactions but remains in the applied form. This can be advantageous if the form of P in digestate shows slow release properties, meaning that P salts dissociate over time, continuously replenishing the P concentration in soil solution and providing P to the crop over the growing period (Talboys *et al.*, 2016). This would be the case if especially struvite was formed because it shows good slow release properties (Talboys *et al.*, 2016). However, X-ray diffraction and Hedley fractionation did not show a significant tendency towards MAP formation (Li *et al.*, 2020, 2022). The fact that especially NaOH-P was formed during the batch experiments was likely related to the activated sludge used as inoculum because sewage sludge treatment plants frequently used iron and aluminum salt additives for P binding, resulting in the formation of Fe-P and Al-P salts (Roskosch & Heidecke, 2019).

6.2 Phosphate Analysis

For the analysis of P fractions in the batch systems the Hedley fractionation according to Dou *et al.* (2000) was used by Li *et al.* (2020). For this analysis samples were dried and afterwards sequentially extracted with different solvents. This can give conclusive results for the analysis of fertilizing characteristics of digestate. However, literature is not conclusive about the ideal drying temperature of the samples before extraction (Dou *et al.*, 2000; Toor *et al.*, 2005; Turner & Leytem, 2004). Some studies investigated the effect of drying on the extractable P concentration in soil samples and they concluded that drying could affect labile-P. Furthermore, they concluded that this is likely connected to microbial P being released (Bünemann *et al.*, 2013; Turner & Haygarth, 2001; Wang *et al.*, 2020). Since biogas digestate contains a substantial number of microorganisms, this effect needed further investigation in digestate. For this, digestate was dried at different temperatures and the P fractions were extracted. The temperatures were based on literature and feasibility for digestate drying (e.g. drying at 40 °C was not possible with digestate). Digestate samples were freeze dried and dried at 50 °C, 105 °C. Afterwards, undried digestate as well as the dried samples were extracted according to the Hedley fractionation. In the method by Hedley *et al.* (1982) the extracts would be centrifuged and the supernatant filtered. Subsequently, the extraction would continue with the retentate from centrifugation and the material from the filter paper. However, it was noted that in the undried sample particles remained in the filter paper and were impossible to scratch off or rinse out. Therefore, the entire paper was added to the subsequent extraction step to prevent losses. The results by Dinkler *et al.* (2021) show that this led to an overestimation of P concentration because the filter contained P that was solved in the prior extraction. The sum of all fractions was up to 101.67% higher than P_{tot} measured by incineration and P extraction from ash. Therefore, it was concluded that the filter would need to be rinsed with the former solvent to rinse remaining P from the filter. Rinsing three times after each extraction reduced the error to 5.26% to 10.98%. The solvent used for rinsing was analyzed for its P concentration to show the effect of several rinses. It was concluded that the filter needed to be rinsed with 30 mL H_2O after H_2O extraction and with 60mL $NaHCO_3$ or NaOH after $NaHCO_3$ and NaOH extraction, respectively (Dinkler *et al.*, 2021).

The analysis of the extracts was conducted colorimetrically. For coloring, the agent developed my Murphy and Riley in (1962) was used. However, the wavelength for analysis was questionable as the chemical composition of digestate samples differs significantly from soil. Additionally, the extracts from digestate samples showed high contents of colloids and the review by Ngo *et al.* (2013) showed that literature does not give a conclusive answer of an appropriate measurement wavelength in soil. These factors combined made it necessary to evaluate the ideal wavelength for analysis of digestate extracts. The measured spectra between 600 nm and 1000 nm showed two peaks, one at 709 nm and one at 889 nm. However, 709 nm showed to be more stable at low concentrations because the adsorption at 889 nm drifted from the mean value towards lower wavelengths. Therefore, 709 nm was found to be the desirable analysis wavelength for digestate extracts. Furthermore, it became clear that the colloids in the extracts did not interfere with the adsorption at this wavelength and therefore the correction measurement according to APHA (2012) was not necessary (Dinkler *et al.*, 2021).

The analysis of the extracts showed that the H_2O-P concentration in the dried samples was up to 78.40% higher than in the undried digestate. Furthermore, $NaHCO_3$-P increased by up to 44.88% after drying. Since these two fractions represent labile-P its accurate determination is important to estimate the fertilization effect and targeted P recovery. An overestimation of the two fractions may lead to an excessive use of chemicals during recovery. In conclusion, the adapted method as described by Dinkler *et al.*

(2021) should be used for future fractionations of digestate to achieve a proper representation of P fractions inside a biogas reactor without overestimation of H_2O-P and $NaHCO_3$-P. However, it needs to be considered that the method only analyse P_i. Additionally, the final number of rinses for good results also included considerations of laboratory capacities. For best results more rinses might be necessary, but this increases the overall time and space needed for extraction and filtration. The method as described leads to a twofold error in P concentration. On one hand, due to the nature of sequential extraction and the P relicts in the filters, the sum of fractions exceeds P_{tot} as described. On the other hand, the negligence of P_O means that the total overestimation is higher than calculated. However, P_O does not represent more than 18% of total P (Bachmann *et al.*, 2016). In any case, the analysis of P_O can be combined with the presented method by digestion of the individual extracts and ICP analysis of the digested liquid. The difference between the spectroscopic results and ICP results equals to P_O (Dou *et al.*, 2000). In conclusion, the method shows an inherent error and P_{tot} should always be measured separately instead of summing up the fractions.

In any case, the extraction of undried digestate proves to better represent the concentrations of H_2O-P and $NHCO_3$-P than the analysis of dried samples, which overestimates their concentrations. This is important for two reasons. Firstly, H_2O-P and $NHCO_3$-P not only represent up to 70% of P_{tot} but also define the labile-P fractions (Dinkler *et al.*, 2021). Therefore, measuring these values correctly is essential for the estimation of fertilizing effects when the digestate is incorporated into the soil instead of applied to the surface where it can dry faster. In connection to this the results imply that surface application of digestate leads to a higher risk of P run-off not only because it can be washed off easier but also because the water solubility increases when digestate dries on the soils surface. This shows that drying the samples before extraction can still be a valid sample pretreatment if run-off risks of surface application are to be evaluated.

The NaOH-P fraction of the adapted Hedley fractionation is likely the fraction with the highest error because it will include relicts from the first two extractions (H_2O-P and $NaHCO_3$-P). HCl-P will again be more accurate, firstly because the rinses of NaOH-P showed P-concentrations as low as (0.12 ± 0.67) g $\text{kg}_{\text{DM}}^{-1}$ and secondly because the high difference to the pH of NaOH means that different salts are solved and the ones solved by NaOH will precipitate.

Ultimately, the concentration of reactive P, which is mostly labile-P, is the decisive parameter for the amount of additive needed for P precipitation. If this value is overestimated it leads to higher operating costs due to higher additive consumption during P recovery.

6.3 Influence of process parameters on P species

The results by Li *et al.* (2020) showed that AD led to a gradual mineralization of P during AD. This means that HRT has a positive correlation with P mineralization, but the gradual changes show that HRT is not a decisive parameter to target specific P fractions. Therefore, horizontal, continuously stirred tank reactors were used for the investigation of other parameters, specifically temperature, OLR and substrate composition. The combination of manure with straw was used to firstly avoid process inhibition by high ammonia concentration in chicken manure digestion and secondly adjust the DM content in dairy manure to maintain a constant HRT when the OLR was increased.

The results of gas production and quality during the experiment showed that no inhibition of the process took place. Meanwhile, the measured NH_4^+ exceeded the threshold of 3000 mg L^{-1} concentration, at which inhibition can occur. This indicates that the microbial community adjusted to high ammonia concentration because of the slow increase in OLR as described by Morozova *et al.* (2020).

The P fractionation according to the method of Dinkler *et al.* (2021) showed that the P mobility decreased with increasing OLR. Temperature had a moderate effect and showed a tendency towards higher mineral-P concentrations at thermophilic conditions. Especially the H_2O-P fraction decreased significantly by up to 48.90% with increasing OLR. The negative correlation of OLR and H_2O-P was measurable in all treatments. The $NaHCO_3$-P fraction did not increase significantly, however, the fraction showed the trend to be higher at thermophilic conditions in all treatments. Additionally, $NaHCO_3$-P increased with increasing OLR in DMS treatments at mesophilic conditions. At thermophilic conditions the $NaHCO_3$-P concentration at an OLR of 4 kg_{VS} m^{-3} d^{-1} was also higher than at an OLR of 2 kg_{VS} m^3 d^{-1} but $NaHCO_3$-P at an OLR of 3 kg_{VS} m^3 d^{-1} was lower than at an OLR 2 kg_{VS} m^3 d^{-1}, showing no trend but confirming the higher $NaHCO_3$-P concentration at high OLR for the DMS treatment.

The NaOH-P fraction did not show any significant behavior with the change of either OLR or temperature. However, it was evident that NaOH-P concentration is moderately higher in the thermophilic treatments at each OLR compared to the corresponding mesophilic treatments in both CMS and DMS. The reason for this is that the pH in both, mesophilic and thermophilic systems was between 7 and 8 in all treatments but slightly higher pH in thermophilic systems, which is not favorable for NaOH-P formation (Al-P and Fe-P) but rather Ca-P and Mg-P (HCl-P) (Penn & Camberato, 2019). The concentration of HCl-P confirmed this with a positive correlation with OLR in CMS treatments.

Since H_2O-P correlates negatively with OLR this means that with increasing OLR especially H_2O-P is converted to HCl-P. This effect is especially significant in the CMS treatments because of the high calcium concentration in the feedstock, which accumulated in the reactor with increasing OLR. In conclusion, feedstock had a significant effect on P species, which coincides with the findings of Mazzini *et al.* (2020). This, in combination with the pH, shifts the thermodynamic equilibrium in the reactor towards the formation of Ca-P because Gibb's free energy is reduced with the increase of educts, favoring Ca-P formation. This also explains why the HCl-P concentration was systematically higher in the thermophilic systems in both CMS and DMS because the pH was slightly higher in these systems, therefore being closer to the ideal pH for Ca-P formation (Penn & Camberato, 2019). These results seem to partly contradict the finding in the batch experiments conducted by Li *et al.* (2020) where especially NaOH-P increased during the first three days in chicken manure. However, this experiment was a batch experiment with activated sludge as inoculum. As described, activated sludge contains high concentrations of Fe- and Al-ions (Wilfert *et al.*, 2016), which favors the formation of their corresponding salts.

The results of Li *et al.* (2020) and Li *et al.* (2022) coincide in the finding that a higher decomposition of substrate leads to higher P release from the substrate matrix. This can be seen in the higher grade of mineralization with increasing digestion time (Li *et al.*, 2020) and the higher H_2O-P concentration at low OLR (Li *et al.*, 2020) because a lower OLR leads to higher decomposition. The fact that H_2O-P does not mineralize at low OLR although higher degradation takes place is likely related to the low ion concentration inside the reactor.

The experiments showed that process parameters of AD can influence P species and that P-analysis in digestate needed an adaptation of the Hedley fractionation to better represent the labile-P fractions. Industrial biogas plants usually optimize the OLR to achieve the high gas yield. This usually means that they increase OLR to the point where system stability is still secure and gas yield maximized. The findings show that this contradicts high plant availability of P because high immediate plant availability requires low OLR. However, the findings also show that feedstock has a significant influence. Especially high calcium content can lead to fixation of P in the solid phase. This could be desirable for plant operators if their digestate application in biogas plant proximity is restricted by P concentration. Therefore, adding calcium rich substrates and low P content to the reactor feedstock could replace chemical additives for P binding.

6.4 Final conclusions and outlook

The results of this work show that P is mineralized during AD. Furthermore, the Hedley fractionation used in literature for the analysis of P fractions in digestate does not properly represent the P composition inside the biogas reactor. This can lead to wrong assumptions when using additives for P binding or mobilization. For example, the overestimation of H_2O-P concentration can lead to excessive use of magnesium salts for MAP precipitation. Additionally, the experiments have shown that feedstock and OLR have a significant influence on P salt formation, whereas temperature has a moderate influence. The readily available H_2O fraction decreases independent of the input material with increasing OLR during AD. However, the salts it converts to are dependent on feedstock and partially temperature.

Regarding plant operation, the results show that a trade-off must be made between fast plant availability of P and high biogas yield. Gas yield is driven by high OLR, whereas plant availability profits from low OLR. Currently, gas production is the financial driver for biogas plants. As a result, changing plant operation in order to increase P solubility seems to be an undesirable trade-off. However, German legislation aims for higher amounts of manure in biogas plants to decrease greenhouse gas emissions from livestock farming and reduce the use of energy crops. Simultaneously, the nutrient application limits with organic fertilizer were reduced in recent years. Additionally, worldwide AD is rather considered a waste treatment technology. For biowastes this includes liquids containing high loads of organics from production processes such as starch rich water from potato processing and whey or manures from animal husbandry. At the same time, the possible reduction of HRT is limited in the biogas process because if it is chosen to low the process acidifies, inhibiting methanogenic microorganisms. Additionally, in countries such as Germany, the legal framework prohibits retention times under a gas tight seal below 150 d to ensure that the substrate is fully degraded. All of these points can lead to a reduction in the TS content of substrate at constant HRT, which automatically leads to reduced OLR. The resulting higher mobility of P can lead to a lower P separation into the solid fraction by screw press filtration because only P associated with particles will be separated in the press. That means that the liquid fraction, applied in plant proximity, contains more P, which can lead to the exceedance of legal threshold values, making a recovery of P necessary. Therefore, the need for P removal from the liquid fraction by means of flocculation or precipitation might increase in the future. The positive aspect of this is that P can be precipitated with a reaction partner that results in desired fertilizer attributes. For example, for a slow release fertilizer magnesium salts could be used to form MAP.

Fertilizer is critical for food security and in times of political unrest, food security is a main concern for local governments making fertilizer independence important. Fertilizer imports from Russia to the EU accounted for 30% of total import (French Presidency, 2022) and 31% of P rock imports (Tulsidas *et al.*, 2019). At the same time 35.1% of P rock was derived from Morocco. Increasing this share to compensate reduced imports from Russian can only be a short-term solution not only due to political reasons but also because the P rock from Morocco has high concentrations of cadmium (Niño-Savala *et al.*, 2019). As a result, the use of local P reserves needs to be maximized to reduce dependencies.

The results from this work show that AD can be considered a pretreatment for subsequent P recovery because mineralization facilitates the mobilization of P with acids and bases and, at the same time, process parameters can be adjusted to produce a designed digestate, fit for specific fertilization purposes. The work, however, does not define an optimal operating point for either P mineralization or mobilization. While the results show significant tendencies, the optimal operation must be evaluated on a case by case bases because it is influenced by the target parameters and reactor feedstock. In any case, the treatment for a design fertilizer or pretreatment for recovery lead to economic problems for plant operators if the production of fertilizer does not receive the required funding to compensate the reduced gas production. As long as fertilizer production through AD cannot be monetized, it will remain a side effect of energy production. The full potential of AD can only be unlocked if a market for the fertilizer products can be developed and CO_2 emission reduction through biological stabilization of organic waste and displacement of conventional fertilizer is rewarded, for example with CO_2 certificates. This would lead to the three potential income sources of energy, fertilizer and CO_2 certificates, making it viable to produce energy at higher cost than solar and wind, still be profitable and at the same time reduce emissions of agriculture and biological waste disposal. The combination of solar, wind and bioenergy is important for future energy systems because bioenergy can significantly reduce the need for energy storage.

Ultimately, processes that produce a mineral P fertilizer from digestate should always consider the recovery of nitrogen as well because in many cases this is the more critical parameter regarding legal thresholds of application. Therefore, future work should focus on the combined or at least subsequent recovery of P and nitrogen from biogas digestate. Such a process should be combined with adapted process parameters during AD as described in this work to facilitate recovery and reduce the need for chemicals. In current recovery technologies consumption of chemicals and electricity is still the main limiting factor for economic operation.

One approach for future research could be to use CO_2 from biogas to replace H_2SO_4 for pH reduction during P mobilization and part of the NH_3 recovered during nitrogen stripping from digestate to replace NaOH used for pH increase during P precipitation.

Further, future research should focus on an easy to implement low-tech approach with an optimized solid-liquid separation e.g. with a screw press. When combined with an adapted digestion process, the AD process could be used to produce digestate rich in labile-P and afterwards the addition of magnesium salts to the post digester could be used for the targeted production of MAP. During separation in the screw press MAP is separated into the solid fraction, enriching it with slow release P salts (MAP). This could lead to a quick reduction of nutrient loads while producing a designed organic fertilizer without the need of complex new equipment. In any case, the ideal operating point of such a process would also need to be evaluated.

Lastly, the fertilizers produced as described should be tested in pot trails and field trails to evaluate the fertilization effect on different crops such as maize that cannot mobilize P and chick pea that can mobilize P. These trails should evaluate the short and long term fertilization for a detailed evaluation of the fertilizer value.

References

APHA. (2012). 4500-P Phosphorus. In E. W. Rice, a. B. Baird, A. D. Eaton, & L. S. Clesceri (Eds.), Standard methods for the examination of water and wastewater (22nd ed.). American Public Health Association, American Water Works Assiciation, Water Environmental Federation. https://doi.org/10.2105/SMWW.2882.093

Bachmann, S., Uptmoor, R., & Eichler-Löbermann, B. (2016). Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Scientia Agricola, 73(1), 9–17. https://doi.org/10.1590/0103-9016-2015-0069

Bünemann, E. K., Keller, B., Hoop, D., Jud, K., Boivin, P., & Frossard, E. (2013). Increased availability of phosphorus after drying and rewetting of a grassland soil: Processes and plant use. Plant and Soil, 370(1-2), 511–526. https://doi.org/10.1007/s11104-013-1651-y

Dinkler, K., Li, B., Guo, J., Hülsemann, B., Becker, G. C., Müller, J., & Oechsner, H. (2021). Adapted Hedley fractionation for the analysis of inorganic phosphate in biogas digestate. Bioresource Technology, 331, 125038. https://doi.org/10.1016/j.biortech.2021.125038

Dou, Z., Toth, J. D., Galligan, D. T., Ramberg, C. F., & Ferguson, J. D. (2000). Laboratory procedures for characterizing manure phosphorus. Journal of Environmental Quality, 29(2), 508–514. https://doi.org/10.2134/jeq2000.00472425002900020019x

French Presidency. (2022). Potential consequences of the Russia-Ukraine conflict on the agricultural and agri-food sectors. https://www.politico.eu/wp-content/uploads/2022/03/09/Ukraine-food-security-french-presidency.pdf

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal, 46(5), 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Li, B., Dinkler, K., Zhao, N., Ran, X., Sobhi, M., Dong, R., Müller, J., Xiong, W., Huang, G., Guo, J., & Oechsner, H. (2022). Response of phosphorus speciation to organic loading rates and temperatures during anaerobic co-digestion of animal manures and wheat straw. Science of The Total Environment, 838, 155921. https://doi.org/10.1016/j.scitotenv.2022.155921

Li, B., Dinkler, K., Zhao, N., Sobhi, M., Merkle, W., Liu, S., Dong, R., Oechsner, H., & Guo, J. (2020). Influence of anaerobic digestion on the labile phosphorus in pig, chicken, and dairy manure. Science of the Total Environment, 737, 140234. https://doi.org/10.1016/j.scitotenv.2020.140234

Mazzini, S., Borgonovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., & Adani, F. (2020). Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. Science of The Total Environment, 734, 139284. https://doi.org/10.1016/j.scitotenv.2020.139284

Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242–257. https://doi.org/10.1002/elsc.201100085

Morozova, I., Nikulina, N., Oechsner, H., Krümpel, J., & Lemmer, A. (2020). Effects of increasing nitrogen content on process stability and reactor performance in anaerobic digestion. Energies, 13(5). https://doi.org/10.3390/en13051139

Murphy, J., & Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters (A.-L. Müller & W. Reichmann, Eds.). Analytica Chimica Acta, 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Ngo, P. T., Rumpel, C., Ngo, Q. A., Alexis, M., Vargas, G. V., Mora Gil, M. d. l. L., Dang, D. K., & Jouquet, P. (2013). Biological and chemical reactivity and phosphorus forms of buffalo manure compost, vermicompost and their mixture with biochar. Bioresource Technology, 148, 401–407. https://doi.org/10.1016/j.biortech.2013.08.098

Niño-Savala, A. G., Zhuang, Z., Ma, X., Fangmeier, A., Li, H., Tang, A., & Liu, X. (2019). Cadmium pollution from phosphate fertilizers in arable soils and crops: An overview. Frontiers of Agricultural Science and Engineering, 6(4), 419–430. https://doi.org/10.15302/J-FASE-2019273

Penn, C. J., & Camberato, J. J. (2019). A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. Agriculture (Switzerland), 9(6), 1–18. https://doi.org/10.3390/agriculture9060120

Roskosch, A., & Heidecke, P. (2019). Sewage Sludge Disposal in the Federal Republic of Germany. Umweltbundesamt (German Environment Agency).

Talboys, P. J., Heppell, J., Roose, T., Healey, J. R., Jones, D. L., & Withers, P. J. A. (2016). Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant and Soil, 401(1-2), 109–123. https://doi.org/10.1007/s11104-015-2747-3

Toor, G. S., Peak, J. D., & Sims, J. T. (2005). Phosphorus Speciation in Broiler Litter and Turkey Manure Produced from Modified Diets. Journal of Environmental Quality, 34(2), 687–697. https://doi.org/10.2134/jeq2005.0687

Tulsidas, H., Gabriel, S., Kiegiel, K., & Haneklaus, N. (2019). Uranium resources in EU phosphate rock imports. Resources Policy, 61, 151–156. https://doi.org/10.1016/j.resourpol.2019.02.012

Turner, B. L., & Haygarth, P. M. (2001). Phosphorus solubilization in rewetted soils. Nature, 411(6835), 258–258. https://doi.org/10.1038/35077146

Turner, B. L., & Leytem, A. B. (2004). Phosphorus Compounds in Sequential Extracts of Animal Manures: Chemical Speciation and a Novel Fractionation Procedure. Environmental Science and Technology, 38(22), 6101–6108. https://doi.org/10.1021/es0493042

Wang, J., Wu, Y., Zhou, J., Bing, H., Sun, H., Luo, J., & Pu, S. (2020). Air-drying changes the distribution of Hedley phosphorus pools in forest soils. Pedosphere, 30(2), 272–284. https://doi.org/10.1016/S1002-0160(17)60456-9

Wilfert, P., Mandalidis, A., Dugulan, A., Goubitz, K., Korving, L., Temmink, H., Witkamp, G., & Van Loosdrecht, M. (2016). Vivianite as an important iron phosphate precipitate in sewage treatment plants. Water Research, 104, 449–460. https://doi.org/10.1016/j.watres.2016.08.032

7 Abstract

Phosphate (P) is used extensively in agriculture, while its recovery from biogas digestate and manure is uncommon. This has led to a reliance on P imports. Meanwhile, the framework for fertilization with digestate and manure in the European Union has become more stringent in recent years. Therefore, nutrients should be recovered as fertilizer to reduce dependencies, redistribute nutrient and amplify the product portfolio of biogas plants.

Current nutrient recovery processes have in common that they are post digestion treatments of digestate, which neglect the phosphate behavior during digestion. It is necessary to closely evaluate P behavior during AD to optimize post digestion treatments of digestate by using digestion as a pretreatment for digestate. Additionally, the effect of fertilization with digestate can be enhanced. Therefore, it was the overall objective of this work to evaluate the turnover of P during anaerobic digestion in laboratory scale batch and continuous digestion systems.

In laboratory experiments with batch reactor systems three different manures, namely pig, dairy and chicken manure were digested. Activated sludge served as inoculum. A set of 120 mL batch digesters were filled and individual bottles were opened after defined times and discarded afterwards until the last reactors were opened on day 30. The results showed that H_2O-P and $NaHCO_3$-P decreased over the digestion period by up to 40.1%. Meanwhile, NaOH-P increased. Overall, it could be concluded that anaerobic digestion leads to a mineralization of P. The mineralization was especially profound during the first few days after the substrate was mixed with the inoculum, concluding that the ions in the inoculum played a significant role in this mineralization. In effect, AD reduces immediate plant availability but increases slow-release fertilization effects. At the

same time, it also reduces the risk of run-off compared to fertilization with manure because of lower water solubility.

Based on the experiences of the batch experiments as well as inconsistent methods for sample pretreatment in literature, it was concluded that the effect of different drying temperatures during pretreatment on P fractions needed to be analyzed. Furthermore, different analysis wavelengths for P analysis were found, while using the same coloring agent (Molybdenum blue method). Therefore, for a defined measurement wavelength for digestate the absorbance spectrum of digestate extracts needed to be analyzed. For the evaluation of these two aspects samples were convectively dried at 50°C and at 105°C and freeze dried. These samples and undried digestate were extracted by Hedley fractionation. The coloring agent was added to the extracts and the spectra between 600 nm and 1100 nm were measured. The spectral lines showed two peaks, one at 709 nm and another at 889 nm. The lower wavelength proved to be more stable at low absorbance, making this the better wavelength for analysis. The subsequent analysis of the Hedley extracts showed that drying increases the H_2O-P and $NaHCO_3$-P fraction by up to 70%.

Additionally, the extraction procedure needed to be adapted to prevent sample losses between the sequential extraction steps. The samples were rinsed with preceding solvent to increase accuracy. Overall, the adapted method achieved higher accuracy for H_2O-P, $NaHCO_3$-P than the former method.

The adapted fractionation was used for the analysis of samples during experiments in continuously stirred tank reactors. Chicken and dairy manure were each co-digested with straw and the parameters OLR and temperature were varied. The results showed that OLR had a negative correlation with H_2O-P, which decreased by up to 50.49%. Meanwhile, HCl-P increased significantly in chicken manure digestate, showing a positive correlation with OLR. This was related to increased ion concentration in the reactor at high OLR especially in chicken manure because of the high calcium content in the substrate. It was proven that temperature has a minor effect on P transformation with a slightly higher mineralization of P under thermophilic conditions. Furthermore, it could be concluded that substrate composition played a significant role in P transformation. Especially the high calcium concentration in chicken manure dominated the P turnover during the digestion, which can also be seen in the positive correlation of OLR with HCl-P as well as a high Pearson correlation coefficient above 0.85 for calcium and phosphate in chicken manure digestion.

The results of this work have proven that P changes its chemical composition significantly during anaerobic digestion. The parameters of the digestion process had a decisive effect on the final composition with OLR and substrate composition being the major drivers. The results further showed that gas production and high P solubility are in conflict because for increased H_2O-P OLR needs to be reduced. However, when nutrient recovery after digestion is desired, it would be feasible to reduce chemical use by adjusting the process parameters and using anaerobic digestion as a pretreatment. Future work should focus specifically on the combination of this anaerobic digestion and post-digestion treatments for cost effective recovery. However, its economic feasibly needs to be evaluated case by case and the legal framework needs to enforce the recirculation of local resources not only through restrictions on nutrient application but also reduction of carbon dioxide emission intensive fertilizer imports. This can play a key role for future profitability of biogas projects.

8 Zusammenfassung

Phosphat (P) wird in großem Umfang in der Landwirtschaft verwendet, während die Rückgewinnung aus Gärrest und Gülle selten ist. Das hat zu Anhängigkeiten von P importen geführt. Gleichzeitig wurde der rechtliche Rahmen zur Düngung mit Gärrest und Gülle in der Europäischen Union in den letzten Jahres strenger. Daher sollten Nährstoffe aus Gärresten zurückgewonnen werden um die Abhängigkeiten zu reduzieren, Nährstoffe neu zu verteilen und das Produktportfolie von Biogasanlagen zu erweitern.

Bei derzeitigen Verfahren zur Nährstoffrückgewinnung handelt es sich um Nachbehandlungen von Gärresten, die das Verhalten von Phosphat während der Vergärung vernachlässigen. Es erforderlich, das P-Verhalten während der anaeroben Vergärung genau zu untersuchen, um die Nachbehandlung von Gärresten zu optimieren, indem die Vergärung als Vorbehandlung genutzt wird. Außerdem kann der Düngungseffekt mit Gärrest dadurch verbessert werden. Daher war die Gesamtzielsetzung dieser Arbeit die P Umwandlung während der anaeroben Vergärung in Batch- und kontinuierlichen Vergärungssystemen im Labormaßstab zu analysieren.

In Laborexperimenten mit Batch-Reaktorsystemen wurden drei verschiedene tierische Exkremente, Schweine-, Rinder- und Hühnermist, vergoren. Belebtschlamm diente als Inokulum. Mehrere 120 mL Batch-Fermenter wurde gefüllt und einzelne Reaktoren wurden nach bestimmten Zeiten geöffnet und anschließend verworfen, bis die letzten Reaktoren am dreißigsten Tag geöffnet wurden. Die Ergebnisse zeigten, dass H_2O-P und $NaHCO_3$-P während der Vergärungszeit um bis zu 40,1% abnahmen. Gleichzeitig stieg NaOH-P an. Insgesamt ließ sich feststellen, dass die anaerobe Vergärung zu einer Mineralisierung von P führte. Die Mineralisierung war in den ersten Tagen nach der Vermischung des Substrats mit dem Inokulum besonders ausgeprägt, was darauf schließen lässt, dass die Ionen im Inokulum eine wichtige Rolle bei dieser Mineralisierung spielten. Daraus resultiert, dass AD die unmittelbare Pflanzenverfügbarkeit verringert, aber die Langzeitdüngewirkung erhöht. Gleichzeitig wird im Vergleich zur Düngung mit Gülle aufgrund der geringeren Wasserlöslichkeit das Risiko des Oberflächenabflusses verringert.

Auf der Grundlage der Erfahrungen aus den Batch-Experimenten sowie uneinheitlicher Methoden zur Probenvorbehandlung in der Literatur wurde festgestellt, dass die Auswirkungen unterschiedlicher Trocknungstemperaturen auf die P-Fraktionen während der Vorbehandlung analysiert werden müssen. Darüber hinaus wurden unterschiedliche Analysewellenlängen für die P-Analyse festgestellt, obwohl der gleiche Indikator (Molybdenum Blau Methode) verwendet wurde. Daher musste für eine definierte Wellenlänge für Gärresteanalysen das Absorptionsspektrum der Gärrestextrakte analysiert werden. Zur Beurteilung dieser beiden Aspekte wurden Proben konvektiv bei 50C und 105°C getrocknet und gefriergetrocknet. Anschließend wurden diese Proben und ungetrocknete Gärreste durch Hedley-Fraktionierung extrahiert. Die Indikatorlösung wurde den Extrakten zugesetzt und die Spektren zwischen 600 nm und 1100 nm gemessen. Die Spektrallinien zeigten zwei Maxima, ein bei 709 nm und ein weiteres bei 889 nm. Die niedrigere Wellenlänge war bei geringen Konzentrationen stabiler, weshalb diese die bessere Wellenlänge zur Analyse war. Die anschließende Analyse der Hedley-Extrakte zeigte, dass die Trocknung den Anteil an H_2O-P und $NaHCO_3$-P um bis zu 70‰ erhöht.

Außerdem musste das Extraktionsverfahren angepasst werden, um Probenverluste zwischen den aufeinanderfolgenden Extraktionsschritten zu vermeiden. Hinzu kommt, dass die Proben mit dem vorherigen Lösungsmittel gespült wurden, um die Genauigkeit zu erhöhen. Insgesamt erzielte die angepasste Methode eine höhere Genauigkeit für H_2O-P, $NaHCO_3$-P als die frühere Methode.

Die angepasste Fraktionierung wurde für die Analyse von Proben bei

Versuchen in kontinuierlich gerührten Tankreaktoren verwendet. Hühnerkot und Rindergülle wurden jeweils mit Stroh co-fermentiert und die Parameter Faulraumbelastung und Temperatur wurden variiert. Die Ergebnisse zeigten, dass die Faulraumbelastung eine negative Korrelation mit H_2O-P hatte, das um bis zu 50,49% abnahm. Gleichzeitig stieg der HCl-P-Gehalt in Gärresten aus Hühnerkot signifikant an und zeigte eine positive Korrelation mit der Faulraumbelastung. Dies hing mit der erhöhten Ionenkonzentration im Reaktor bei hoher Faulraumbelastung, insbesondere aufgrund des hohen Kalziumgehalts im Hühnerkot, zusammen. Es wurde nachgewiesen, dass die Temperatur einen geringen Einfluss auf die P-Umwandlung hat, wobei die P-Mineralisierung unter thermophilen Bedingungen etwas höher war. Außerdem konnte festgestellt werden, dass die Substratzusammensetzung eine wichtige Rolle bei der P-Umwandlung spielt. Insbesondere die hohe Kalziumkonzentration in Hühnerkot dominierte den P-Umsatz während der Vergärung, was sich auch in der positiven Korrelation der Faulraumbelastung mit HCl-P, sowie einem hohen Pearson-Korrelationskoeffizienten von über 0,85 für Kalzium und Phosphat in Hühnerkot zeigte.

Die Ergebnisse dieser Arbeit haben gezeigt, dass P seine chemische Zusammensetzung während der anaeroben Vergärung erheblich verändert. Die Parameter des Vergärungsprozesses haben einen entscheidenden Einfluss auf die endgültige Zusammensetzung, wobei die Faulraumbelastung und die Substratzusammensetzung die wichtigsten Faktoren waren. Die Ergebnisse zeigten außerdem, dass Gasproduktion und hohe P-Löslichkeit in Konflikt stehen, da für eine erhöhte H_2O-P Konzentration die Faulraumbelastung reduziert werden muss. Wenn jedoch eine Nährstoffrückgewinnung nach der Vergärung erwünscht ist, könnte der Einsatz von Chemikalien durch Anpassung der Prozessparameter und Verwendung der anaeroben Vergärung als Vorbehandlung reduziert werden. Zukünftige Arbeiten sollten sich speziell auf die Kombination dieser anaeroben Vergärung und Nachbehandlungen für eine kosteneffiziente Rückgewinnung konzentrieren. Die wirtschaftliche Durchführbarkeit muss jedoch von Fall zu Fall bewertet werden und der rechtliche Rahmen muss die Rückführung lokaler Ressourcen nicht nur durch Beschränkungen der Nährstoffausbringung, sondern auch durch die Verringerung der Kohlensoffdioxidemissionsintensiven Düngereinfuhren fördern. Dies kann eine Schlüsselrolle für die künftige Rentabilität von Biogasprojekten spielen.

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