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Development of a Process for the Enhanced Phosphorus Recovery from the Organic Matrix of Agricultural Residues

Fraunhofer-Institut für
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Von der Fakultät Energie-, Verfahrens- und Biotechnik der Universität Stuttgart

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Stuttgart, 8 Juli 2014

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Abstract

The use of phosphorus (P) fertilizers in arable farming is indispensable to achieve the sufficient crop yields needed by the ever-growing world population. However, the established exploitation and production processes for synthetic phosphorus fertilizers have negative environmental, economic and geopolitical consequences. Alternately, P can be recovered and recycled from a more sustainable source, such as agricultural residues (e.g. animal manure and anaerobic digestate), which are rich in nutrients.

Agricultural residues contain refractory phosphorus ($P_{refract}$) and inorganic orthophosphate (P_i). From these two types of phosphorus compounds, only the inorganic form is readily available for crops and for its recovery as phosphate salts. Thus, current state of the art technologies can only recover P_i in the liquid fraction after solid-liquid separation of these residues, which can be as low as 20% of the total P content. The rest of the P is present as organic P, insoluble phosphate minerals or it is adsorbed to the organic matrix. Hence, the objective of this thesis was to develop a process to increase the P_i content in the liquid fraction, after the solid-liquid separation of these residues. For this, three types of process were tested: an enzymatic process and two chemical processes, namely acidification and addition of carbonate ions. Finally, a concept for the integrated nutrient recovery from pig manure was proposed.

As a first step, various samples of animal manure and anaerobic digestate were characterized regarding their total nutrient content. Subsequently, P_i was determined photometrically after water and $NaHCO_3$ (0.5 M) extractions. Finally, the mass balances and element distribution after the solid-liquid separation of pig manure and anaerobic digestate were determined. With this analysis, it was confirmed that most of the phosphorus in agricultural residues (e.g. 80% for pig manure, 60% for anaerobic digestate) remained as refractory phosphorus in the solid fraction. Therefore, it cannot be recovered by current technologies.

The enzymatic treatment was first carried out with P model compounds (phosphate monoesters, phosphate diesters, condensed polyphosphate), which are commonly present in agricultural residues. The activity of different analytical grade and industrial phosphatases was tested at 37°C and various incubation time (1-6 h) and pH value (pH 5-9) conditions for the individual model P compounds and a mixture of them. It was demonstrated that analytical grade wheat phytase and alkaline phosphatase hydrolysed a wider range of P compounds. Subsequently, batch experiments with wheat phytase and industrial fungal phytase were carried out using dry and fresh real agricultural residues samples. As result, these enzymes did not enhance significantly the P_i release from these samples, possibly due to the low

organic P content in the samples or simultaneous acidic hydrolysis of refractory P. Moreover, inhibition of the enzyme and precipitation/adsorption of the released P_i on the residue components could occur.

The acidification process of fresh pig manure samples was carried out in batch and continuous mode using 6M sulphuric acid (pH 5). It was determined that acidification could increase the P_i content in the liquid fraction from up to 80%. Moreover, the acidified liquid contained high concentrations of calcium, magnesium and ammonium. For this reason, the phosphate ions could be precipitated only by increasing the pH value to 9, without the necessity to add these ions by external chemicals. The product obtained was rich in phosphorus (13.8%) and complied with German regulations for fertilizers.

The addition of carbonate experiments were carried out using fresh pig manure samples and $NaHCO_3$ as carbonate source. The samples were mixed at different conditions of time (1h, 3h, 6h), temperature (21°C, 37°C), pH value (8.5, 9.2) and carbonate-calcium ratio (0, 60, 110, 160). The process successfully increased the P_i released in more than 65% at the optimal combination of parameters: pH value of 9.2 and carbonate-calcium ratio 60.

An integrated nutrient recovery concept was proposed for treating pig manure. The process includes an acidification step for the enhanced P_i release in the liquid fraction. The main product of the process is the precipitated phosphate salts. Moreover, organic soil conditioner and nitrogen and potassium salts could be recovered.

With this thesis, it was successfully confirmed that it is possible to increase the quantity of P_i in the liquid fraction after solid-liquid separation by acidification, and in certain conditions by the enzymatic process. Moreover, phosphate salts were successfully precipitated from the liquid fraction as a valuable fertilizer product.

Zusammenfassung

Phosphor (P)-Dünger im Ackerbau sind unersetzlich, um die Erträge zu erzielen, die für eine steigende Weltbevölkerung notwendig sind. Die bestehenden Herstellungsprozesse für synthetische P-Dünger haben jedoch negative geopolitische, ökonomische und ökologische Auswirkungen. Andererseits kann P aus einer regenerativen Quelle, nämlich aus nähstoffreichen Reststoffen aus der Landwirtschaft, wie Gülle und Gärrest wiedergewonnen werden.

Landwirtschaftliche Reststoffe enthalten refraktäres P ($P_{refract}$) und anorganisches Orthophsophat (P_i). Von diesen zwei P-Formen ist nur P in der anorganischen Form gut für Pflanzen und zur Wiedergewinnung als Phosphatsalz verfügbar. Deswegen sind die Technologien auf dem Stand der Technik nur in der Lage, Pi aus der Flüssigphase dieser Reststoffe nach einer Fest-Flüssig-Trennung zu gewinnen, was nur 20% der gesamt-P-Menge ausmachen kann. Der Rest des P liegt als in organischen Verbindungen vor, als unlösliche P-Mineralien oder ist adsorbiert an die Matrix der Biomasse. Deswegen war das Ziel dieser Arbeit, einen Prozess zu entwickeln, der den Pi-Gehalt der Flüssigphase nach der Fest-Flüssig-Trennung dieser Reststoffe erhöht. Dazu wurden drei Verfahren getestet: ein enzymatischer und zwei chemische Prozesse, nämlich Ansäuerung und Zugabe von Carbonat. Am Ende wurde ein integriertes Konzept zur Nährstoffrückgewinnung aus Schweinegülle entwickelt.

Als erster Schritt wurden Proben von Gülle und Gärrest auf ihren Nährstoffgehalt hin charakterisiert. Danach wurde der Gehalt an Pi photometrisch unter Zuhilfenahme von Wasser und 0,5 M $NaHCO_3$-Lösung bestimmt. Am Ende wurden die Massenbilanzen und Elementverteilungen nach der Fest-Flüssig-Trennung von Schweinegülle und Gärrest bestimmt. Mit dieser Analyse konnte bestätigt werden, dass der meiste P in Reststoffen aus der Landwirtschaft (z. B. 80% bei Schweinegülle, 60% beim Gärrest) als refraktäres P in der Festphase blieb. Dieses P kann somit mit den vorhandenen Technologien nicht wiedergewonnen werden.

Die enzymatische Behandlung wurde zuerst mit P-haltigen Modellsubstanzen als Substrat durchgeführt, die in Restoffen aus der Landwirtschaft häufig vorkommen, wie Phosphatmono- und -diester und kondensierte Polyphosphate. Die Aktivität analytischer und industrieller Phosphatasen wurde für die Einzelsubstanzen und eine Mischung bei 37°C, Inkubationszeiten (1-6 h) und pH-Werten (5-9) überprüft. Es konnte gezeigt werden, dass die Weizenphytase und die alkalische Phosphatase mehr P-Komponenten hydrolisierten. Danach wurden diskontinuierliche Experimente durchgeführt mit Weizenphytase und industrieller pilzlicher Phytase und mit echten trockenen und frischen Reststoffen aus der

Landwirtschaft. Ergebnis dieses Versuches war, dass keines der eingesetzten Enzyme die P_i-Freisetzung aus diesen Proben signifikant verstärkte. Ursache dafür war wahrscheinlich der geringe Anteil an organisch gebundenem P in den Proben oder die gleichzeitige Hydrolyse von refraktärem P durch Ansäuerung, Inhibierung des Enzyms und Ausfallen/Adsorption des freigesetzten Pi an den Reststoffsubstanzen.

Die Ansäuerung der Proben mit frischer Schweinegülle wurde in diskontinuierlichen und kontinuierlichen Versuchen mit 6 M Schwefelsäure durchgeführt. Dabei wurde festgestellt, dass die Ansäuerung den P_i –Gehalt auf 80% erhöhen kann. Außerdem enthielt die angesäuerte Flüssigkeit hohe Konzentrationen an Kalzium, Magnesium und Ammonium. Deswegen konnten die Phosphationen einfach durch das Erhöhen des pH-Wertes auf 9 ausgefällt werden. Eine Zugabe weitere Chemikalien war nicht notwendig. Das erhaltene Produkt war reich an Phosphor (13,8%) und erfüllte die deutschen Vorschriften für Düngemittel.

Die Experimente mit Zugabe von Karbonat wurden mit frischer Schweinegülle und mit $NaHCO_3$ als Karbonatquelle durchgeführt. Beim Mischvorgang wurden die Parameter Zeit (1h, 3h, 6h), Temperatur (21°C, 37°C), pH-Wert (8.5, 9.2) und das Karbonat-Kalzium Verhältnis (0, 60, 110, 160) variiert.

Ein integriertes Nährstoffrückgewinnungskonzept wurde für die Behandlung von Schweinegülle vorgeschlagen. Das Verfahren beinhaltet einen Ansäuerungsschritt zur erhöhten Pi-Freisetzung aus organischen Verbindungen der Flüssigphase. Das Hauptprodukt dieses Verfahrens sind die ausgefallenen Phosphatsalze. Außerdem konnten ein organischer Bodenverbesserer und Stickstoff- und Kalium-enthaltene Salze wiedergewonnen werden.

Durch die vorliegende Dissertation konnte erfolgreich bestätigt werden, dass es möglich ist die Menge an P_i in der Flüssigphase nach einer Fest-Flüssigtrennung zu erhöhen, und zwar durch Ansäuerung und unter Umständen durch enzymatische Prozesse. Außerdem konnten die Phosphatsalze aus der Flüssigphase erfolgreich als wertvolles Düngerprodukt ausgefällt werden.

Acknowledgement

Content

Abbreviations

AAM	Acetone-acid-molybdate solution
ACP	Amorphous calcium phosphate
AMP	Adenosine 5′-monophosphate monohydrate
AcPhos	Acid phosphatase
AlkPhos	Alkaline phosphatase
AsPhy	*Aspergillus Niger* phytase
ATP	Adenosine 5′-triphosphate
Ch-	Chicken manure sample
Dig-	Anaerobic digestate sample
DM	Dry matter basis
DIN	German Institute for Standarization (Deutsches Institut für Normung)
DNA	Deoxyribonucleic acid
DCPA	Dicalcium phosphate anhydrous or monetite
DCPD	Dicalcium phosphate dihydrate or brushite
EC	Enzyme commission
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
FM	Fresh matter basis
HAP	Hydroxylapatite
$Ks_{4.3}$	Acid neutralizing capacity to pH 4.3
Pig-	Pig manure sample
M	Molar concentration
MCPA	Monocalcium phosphate anhydrous
MCPM	Monocalcium phosphate monohydrate
N	Number of measurements
n.a	Not available
OCP	Octacalcium phosphate
P_i	Inorganic phosphorus
P_o	Organic phosphorus
PhosDie	Phosphodiesterase
N_{total}	Total nitrogen
RNA	Ribonucleic acid
rpm	Revolutions per minute
SI	Saturation index
RSM	Response surface methodology

TCP	Tricalcium phosphate
TS	Total solids
TTCP	Tetracalcium phosphate
Tris	Tris(hydroxymethyl)aminomethane
U	Enzyme units
V	Volume
WPhy	Wheat phytase
^{31}P-NMR	Phosphorus 31 nuclear magnetic resonance

Chemical Formulas

Al	Aluminum
$AlCl_3$	Aluminum chloride
$AlPO_4$	Aluminum phosphate
Ca^{2+}	Calcium ion
$CaCO_3$	Calcium carbonate
$CaHPO_4$	Monetite (dicalcium phosphate anhydrous)
$CaHPO_4 \cdot 2H_2O$	Brushite (dicalcium phosphate dehydrate)
$Ca_8H_2(PO_4)_6 \cdot 6H_2O$	Octacalcium phosphate
$Ca_{10}(PO_4)_6(OH)_2$	Hydroxylapatite
$Ca(OH)_2$	Calcium hydroxide
$Ca_3(PO_4)_2$	Tricalcium phosphate
$Ca_xH_y(PO4)_z$	Any of the variations of calcium phosphate
Cd^{2+}	Cadmium ion
Cl^-	Chlorine ion
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
CH_3COO^-	Acetate
$C_6H_{11}O_9PNa_2 \cdot xH_2O$	Myo-Inositol hexakisphosphate disodium salt (phytic acid)
$C_6H_{11}O_9PNa_2 \cdot xH_2O$	α-D-Glucose-1-phosphate disodium salt
$C_6H_{12}NaO_9P$	D-Glucose 6-phosphate sodium salt
$C_{10}H_{14}N_5O_7P \cdot H_2O$	AMP
$C_{10}H_{14}N_5O_{13}P_3Na_2 \cdot xH_2O$	ATP
Fe	Iron
$FeCl_3$	Iron chloride (III)
$FePO_4$	Iron phosphate
H^+	Hydron
HCl	Hydrochloric acid

III

Formula	Name
H_2O	Water
H_2SO_4	Sulfuric acid
$H_2NCH_2CH_2P(O)(OH)_2$	2-Aminoethylphosphonic acid
K	Potassium
K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
Mg	Magnesium
Mg^{2+}	Magnesium ion
$MgCO_3$	Magnesium carbonate (Magnesite)
$MgCO_3 \cdot CaCO_3$	Dolomite
$MgCl_2$	Magnesium chloride
$MgNH_4PO_4 \cdot 6H_2O$	Magnesium ammonium phosphate hexahydrate (struvite)
$MgSO_4 \cdot 7H_2O$	Magnesium sulfate heptahydrate
MgO	Magnesium oxide
$Mg(OH)_2$	Magnesium hydroxide
$Mg_3(PO_4)_2$	Magnesium phosphate
N	Nitrogen
N_2	Molecular nitrogen
NaOH	Sodium hydroxide
Na_3PO_4	Sodium phosphate
$Na_3C_6H_5O_7$	Trisodium citrate
$Na_5P_3O_{10}$	Pentasodium tripolyphosphate
NH_3	Ammonia
NH_4^+	Ammonium
$NH_4^+ - N$	Nitrogen in form of ammonium
$(NH_4)_3PO_4,$	Ammonium phosphate
NO_3^-	Nitrate
NO_2-	Nitrite
O_2	Molecular oxygen
OH-	Hydroxic
P	Phosphorus
PO_4^{3-}	Phosphate
$PO_4^{3-}-P$	Phosphorus in form of phosphate
$P_3O_9^{3-}$	Metaphosphates
$P_2O_7^{4-}$	Pyrophosphates
Zn^{2+}	Zinc ion

List of Figures

List of Tables

1 Introduction

1.1 Motivation

The use of phosphorus (P) fertilizers in arable farming is indispensable to achieve the sufficient crop yields needed by the ever-growing world population. So that the increasing demand on food, biofuels and biomaterials can be fulfilled [1].

The raw material for the production of synthetic P fertilizers is phosphate rock, which is a limited non-renewable resource. The mining and upgrading of phosphate rock are highly energy demanding and environment pollutant processes [2]. Moreover, during mining around 33% of phosphate is lost and additional 10% losses occurred in transportation and handling [3].

Phosphate rock has only 5% to 40% phosphate content [4]. Yet it contains high concentrations of hazardous elements, such as cadmium, arsenic, chromium, led, mercury and uranium [5] [6]. The predominant production process, the so-called "wet process", uses sulphuric acid to produce an impure phosphoric acid and a radioactive waste called phospho-gypsum [2]. The phosphoric acid is used for industry requiring further purification, whereas the waste is landfilled or discharged to the sea [3, 7].

The use of phosphate rock as solely source for phosphorus fertilizer has also economic and geopolitical drawbacks. Only a few countries control 80 percent of the world's reserves of usable phosphate, among them China (37%), Morocco (32%), South Africa (8%), United States (7%) and Jordan (5%) [3, 8]. Thus, the rest of the world is completely dependent on phosphorus imports from these countries. In the case of Europe, an important part of its phosphate rock is imported from Middle East and North Africa countries, such as Morocco, Tunisia, Jordan and until recently Syria [9]. However, political instability and internal conflicts in these regions have affected the exports to the rest of the world. In addition, other producing countries, such as China, have started to restrict the export of phosphate rock to safeguard the future availability to meet their own needs [9].

To reduce the use of synthetic mineral fertilizers, arable farmers use organic residues, such as animal manure from livestock farming and anaerobic digestate from biogas plants. This results in economic advantages, because the fertilizer costs decrease. Nevertheless, contrary to synthetic mineral fertilizers, the nutrient content in organic residues (i.e. N: P: K ratio) is not balanced for optimal plant utilization. When manure application is based on nitrogen-crop needs, which is the usual case in Europe, P usually exceeds the crop uptake

up to 500% [10]. Moreover, for the application of the residues a large agricultural area is needed. For example, an average agricultural biogas plant (500 kW_{el}), which produces around 100 tonnes nitrogen annually, would need around 440 hectares of agricultural area to spread the digestate produced [11]. Due to this limitation, agricultural residues have to transport to other regions or disposed, which means further costs to the farmers [12].

Livestock farming has developed significantly in the last century into a very specialized system that is characterized by high output of animal products per unit of land [13]. This intensification has caused an increasing pressure on the environment due to the quantities and composition of the residues produced. Only in Europe more than 1.4 billion tonnes of animal manure were produced in 2009 [14]. This represents a phosphorus recovery potential of more than 25 million tonnes phosphorus annually. This amount could theoretically cover the European phosphorus consumption, which would reduce the dependency on P imports from other countries.

P is present in agricultural residues as available inorganic phosphate (P_i) and as refractory phosphorus ($P_{refract}$). The current state of the art technologies, similar to those for wastewater treatment, can only recover P_i in the liquid fraction after solid-liquid separation of the residues. The processes consist in the precipitation of phosphates as insoluble salts, mainly calcium phosphates and struvite (magnesium ammonium phosphate). For this purpose the pH is increased to values between 8 and 9.5, where the solubility of phosphate salts has a minimum. If the concentration of cations, such as Mg^{2+}, is not enough respect to phosphate for the precipitation to occur, they have to be added chemically (e.g. $MgCl_2$ addition) or electrochemically (e.g. Mg anode) [15, 16].

The disadvantage of these existing technologies is that the P recovery is limited by the solubility of the P_i in watery solutions. After solid-liquid separation of agricultural residues, less than 20% of P remains in the liquid fraction. This behaviour of phosphorus differs from other macro nutrients, namely nitrogen and potassium, which are much more soluble in water. The reason of the poor P availability is, on the first place, the presence of organic P compounds. The organic P fraction, which mainly consists of phosphomonoesters, inositol phosphate, phospholipids and nucleic acids [17] is only available after its mineralisation to phosphate. Secondly, organic residues contain phosphate salts (e.g. calcium phosphates) with very low solubility at the typical pH values of organic residues (6.5-9.5)[18]. Finally, phosphate compounds can be adsorbed to the biomass matrix of the residues, reducing its availability in the liquid fraction.

As a consequence, for the effective recycling and use of P in agriculture, it is imperative the development of new processes to overcome the limitations of state of the art technologies. The necessary innovations must include the increase in the available phosphate concentration in solution by means of: i) Mineralization of the organic phosphorus compounds to free available phosphate, ii) Dissolution of insoluble phosphate minerals and iii) Solubilization and release of the adsorbed phosphates. In this way, phosphorus recovery from organic residues, by means of precipitation, will considerable increase.

1.2 Objectives

The general objective of this thesis is to develop a process to increase the inorganic phosphate (PO_4^{3-}) content in the liquid fraction, after the solid-liquid separation of agricultural residues to recover phosphorus as phosphate salts.

Subsequently, three specific objectives were identified:

1. Investigation of the enzymatic mineralization process of organic phosphorus into inorganic phosphate in model P compounds and in real agricultural residues

2. Investigation of the chemical dissolution and desorption of refractory phosphate in real agricultural residues by acidification and addition of carbonate ions

3. Development of a concept for the integrated nutrient recovery from agricultural residues

1.3 Thesis structure

This thesis is divided into four parts (Figure 1.1). Part I includes the characterization of agricultural residues. For this purpose, different types of animal manure and anaerobic digestate were collected and analyzed for total nutrient and phosphorus fractions. Part II investigates the mineralization of organic phosphorus, using model compounds and pig manure by means of phosphatases. Part III looks into the dissolution of insoluble and adsorbed phosphate minerals by means of chemical processes, namely acidification and addition of bicarbonate ions. Finally, in part IV a mass balance and design of the plant for treating 100 kg·h^{-1} pig manure was proposed.

I. Agricultural residues characterization

| Total nutrient determination | Parallel and sequential extraction | Mass balance and nutrient distribution after solid-liquid separation |

II. Enzymatic process for the mineralization of organic phosphorus compounds

| Enzyme selection and enzyme activity assay | Enzyme specificity assay using model P compounds | Batch enzymatic process using real residues | Continuous enzymatic process using real residues |

III. Chemical processes for the release of adsorbed phosphorus and dissolution of phosphate minerals

| Acidification process using real residues | Carbonate addition process using real residues |

IV. Developement of a integrated nutrient recovery concept

| Mass balances and requirement especification | Pilot plant design |

Figure 1.1 Thesis structure

2 Background Information

2.1 Generation and management of agricultural residues

2.1.1 Animal production systems

Animal production systems have changed significantly in the last half-century into a more specialized farming with increased number of animal head per unit of area [19]. This has led to a two inevitable consequences: First, a separation of livestock farming (residues producers) from arable farming (residues users) took place. The second consequence is that intensive livestock production leads to a very high manure amount, which exceeds the local land capacity [12]. This means, that the residues have to be transported, sometimes for long distances, to be used as nutrient source.

According to Sims *et al.* [20] concentrated animal feeding operation (CAFOs) are animal production systems with high stocking density (>1000 animal units). These systems are characterized by having little or no available crop land for the efficient recycling of the residue nutrients [13, 20]. They can be further divided into mixed systems and industrial systems. Mixed systems import only one part of the animal feed and the rest comes from other agricultural activities. Industrial systems depend on external feed supply. If more than 90% is imported to the farm, they are classified as "landless" systems. This kind of systems are common in regions with highly specialized pig and poultry production, for example in the Netherlands and some regions of France, Italy and Germany [13].

   i.   **Classification according manure collection system**

Livestock farming system can be characterized according the manure collection system of the farm. This can vary from region to region depending on different factors like storage and transport costs, water availability, animal welfare considerations, farm size and structure, etc. [12, 13]. The main collection systems are described as follow:

Indoor animal production

- *Liquid manure (slurry)*

Manure, which is a mixture of animal excreta, urine and some wash water, is collected in liquid form [12, 21]. This type of system is normally used for swine manure handling. The animals are kept on sloping floors (with the size of the slop depending on the size and age of the animal) and the manure is collected in a gutter or a concrete pit. In some operations the

manure falls into a shallow pit, which is regularly flushed and pumped into a larger outside tank or lagoon [12, 22]

- ***Solid manure***

Animals are kept on bedding material and the manure is collected as a solid mixture of excreta and bedding. To absorb urine and keep the floors dry, bedding material is added regularly [12, 22]. An example of this collection system used for swine manure is the "hoop structure" or "deep litter" concept: animals are confined in a layer of fibrous bedding material such as sawdust or ground corn stalks. The manure is deposited in the layer, absorbed and mixed by the animal themselves. The mixture is then removed and can be spread on fields. The quantity of bedding material needed is about 9 to 15 kilograms per pig [23]. Some of the advantages of these systems include low costs and no needs of management of liquids. The disadvantages are a low animal performance compared with traditional confinement buildings [23].

- ***Mixed manure***

The manure is collected as a mixture of solid and liquid fractions. The animals are kept on bedding material, but the fluid stream is drained from the bedding and is collected in a storage container [12].

Outdoor animal production

Besides housing production systems, animals can also be raised in an outdoor or pasture production system. This type of system is characterized by large area of land, where the animals have contact with soil and growing plant and minimum capital and labor costs [24].

Outdoor production systems can be divided into three types [12]:

1. Animals are kept outside all the time with mobile shelters. This system is used for sheep and pigs.
2. Animal are kept on houses but have access to grassland surface for part of the day. Normally it is use for poultry production.
3. Animals are kept in houses in winter time and outside in summer time. This system is normally used for cattle

In this type of system manure is distributed as the animal graze and its collection is unrealizable. A more uniformly distribution can be achieved by rotation grazing. One of the main environmental concerns is the overloading of manure, especially in specific protected

areas. Moreover, the uncontrolled application of manure could damage soil and affect the growing of plants.

ii. Classification according type of animal

Livestock systems can also be classified according to the type of animal. The most important systems in Europe are for pigs, cattle and poultry manure [12]:

- ***Pig manure***

Pigs are normally indoor-housing raised and the manure is collected as slurry. The annual pig manure production is about 1 to 2 cubic meter of slurry per fattening pig [13]. However, there are differences in the residues composition and quantity, depending on the stages of pig production and the different regions in the world. In the farrowing and nursery stages, the animals are moved to confinement systems, where manure is stored in a pit or lagoon. The manure of finishing and gestation sows is normally handled as solid [23].

- ***Cattle manure***

Large differences exist between the various cattle farming systems, but in general they can be divided into three categories: veal, beef and milk production systems. A further characterization takes into account the integration of feed production and the way animals are kept. They can be raised in houses, in fields or in a mixture of both [25]. It is estimated that 15 to 25 cubic meters of undiluted slurry are produced annually per dairy cow, depending on the milk yield. If dilution of water is considered, these volume can increase in a factor of 1.5 to 10 [13].

- ***Poultry manure***

The main poultry production system includes the raise of laying hens (for eggs production) and broilers (for meat production). The animals are kept either in closed housed systems or in open systems. In closed systems, the manure can be collected in deep litter system or in containers located under the house. To keep the moisture at low levels, bedding material is added. In free-range systems, birds are typically kept indoors on a littered floor but they have access to an outdoor area [25].

2.1.2 Anaerobic digestion

Anaerobic digestion is a process in which organic matter breaks down naturally in the absence of oxygen to produce biogas and digestate. If offers several benefits by the reduction of emissions, odors and pathogens and producing renewable fuel [26].

Different types of organic substrates can be used for biogas production [11, 26]. The substrate can be added to the process as a single input or as a mixture of two or more feedstock types (co-digestion) [26]. Based on the type of substrate, biogas plants can be classified into four groups [11]:

a. Energy crop plants: The main purpose of this kind of plant is the production of energy. The main raw materials used are maize and grass silages. These substrates can also be mixed with animal manure, but the manure quantity is kept below 30% of the total feedstock.

b. Agricultural plants: In these types of biogas plants the treatment of animal manure is the main goal, which represents more than 30% of the feedstock. Additionally crop residues and energy plant silage can be added to increase the biogas yield.

c. Organic waste treatment plants: Among the substrates used in this type of plant are green waste, household organic waste, food waste, expired foodstuff, etc.

d. Industrial waste plants: This includes different types of organic waste from the food industry, for example, brewery residues, slaughterhouse waste, potato pulp,etc

Concerning the anaerobic digestion process, the most common units operate under mesophyllic conditions; this means at temperature of about 35°C. They produce a mixture gas with 50-75%$_{vol}$ methane and 25-50%$_{vol}$ carbon dioxide [27]. In thermophyllic digesters, which operate at about 70°C, the gas production is significantly increased. Additionally, the process can be also classified as "wet digestion", when the dry matter of the feedstock is below 15% or "dry digestion" when it is above this level [26].

Besides biogas, one important product of the anaerobic digestion process is digestate. The quantity of digestate produced depends on the substrate and digestion process. For example, Fuchs and Drosg [11] estimated that an 500 KW$_{el}$ agricultural biogas plant (which substrates are 50% cow manure, 43% maize silage, 3% pressed pulp from sugar production), produces around 23,800 tonnes digestate per year. The nutrient composition of digestate is presented in the following section.

2.1.3 Type and quantity of manure and digestate produced in Europe

i.   Animal manure

The main animal species in intensive livestock production are cattle, pigs and poultry. The nutrient composition in the these types of manure varies according to different factors, including type and age of the animal, collection and handling systems, environmental

conditions, etc. [13, 20, 28]. A general overview of the nutrient composition of different animal manures is presented in Table 2.1.

Table 2.1 Nutrient composition of different manure types [12]

Manure type	Animal Type	Concentration [kg per m³ of manure]			
		Dry Matter	N_{total}	$N-NH_4^+$	$P-PO_4^{3-*}$
Liquid manure / Slurry	Cattle	15 – 123	2.0 – 7.0	1.0 – 4.9	0.1 – 2.6 (1.35)
	Pigs	15 – 92	1.2 – 8.2	1.9 – 6.1	0.1 – 2.2 (1.15)
	Poultry	10 – 300	2.0 – 18.0	1.9 – 7.8	0.4 – 6.5 (3.45)
Solid manure	Cattle	140 – 300	4.2 – 8.1	0.3 – 2.0	0.5 – 2.1 (1.30)
	Pigs	150 – 330	3.5 – 11.0	0.5 – 6.0	0.7 – 6.5 (3.6)
	Laying hens	220 – 550	5.1 – 25.0	37.0 – 60.0	3.5 – 11.8 (7.6)
	Broilers	450 – 850	22.0 – 40.0	2.0 – 15.0	3.0 – 10.9 (6.9)

* The numbers in parenthesis are the average values

According to the database FAOSTAT [29] in the EU Member States in 2009 approximately 153.2 million pigs, 88.5 million cattle and 1,285 million chicken heads were produced. Based on this information Foged *et al.* [14] estimated the amount of manure produced in the EU-27 (Table 2.2). This estimation shows that approximately 1.4 billion tonnes of manure are produced annually only in Europe. The quantity of phosphorus contained in each manure type was calculated from the average phosphorus content in Table 2.1.

Table 2.2 Manure produced from pigs, cattle and chickens in the EU-27 region [14]

Manure type	Manure amount [10^6 tonnes·year^{-1}]	P amount [10^6 tonnes P·year^{-1}]
Pig manure		
Source separated solid	14.151	0.509
Source separated liquid	8.845	0.102
Slurry	148.590	1.709
Deep litter	5.307	0.191
Cattle manure		
Source separated solid	294.870	3.833
Source separated liquid	54.606	0.737
Slurry	447.776	6.045
Deep litter	294.870	3.833
Poultry manure		
Slurry	3.387	0.117
Deep litter	109.518	7.995
TOTAL	*1,381.911*	*25.071*

ii.   Anaerobic digestate

Digestate has a high concentration of plant nutrients. The specific composition depends, on one hand, on the nutrient content of the feedstock and on the other hand, in the characteristic of the anaerobic digestion. For instance, most of the organic nitrogen present in the feedstock is transform into ammonium [26]. This increases the plant availability of this nutrient. An example of the nutrient composition of different types of digestate is present in Table 2.3.

Table 2.3 Nutrient composition of different anaerobic digestate types [11, 27, 30]

Digestate type	Concentration [kg·tonne$_{FM}$]			
	Dry Matter	N_{total}	$N-NH_4^+$	$P-PO_4^{3-}$
Agricultural	5.6 - 8.2	4.4 - 4.6	2.5 - 3.1	0.2 - 5.0
Organic waste	1.5 - 8.0	1.0 - 11.0	0.5 - 9.0	0.3 - 2.0
Energy crop	6.0 - 9.0	3.5 - 10.0	1.0 - 6.0	0.8 -1.0

In Table 2.4 information about the quantity of residues treated by anaerobic digestion in Europe is shown. The processing of about 88 million tonnes of residues is carried out on 5,256 installations. This is equal to 6.4 % of the entire livestock manure production in EU [14]. Regarding phosphorus, around 56,200 tonnes are treated annually.

Table 2.4 Residues treated in Europe by anaerobic digestion [14]

Anaerobic digestion technology	Raw manure* treated [1000 tonnes]	Other residuesɬ treated [1000 tonnes]	Phosphorus [tonnes]
Mesophilic	45887	35718	52564
Thermophilic	3174	3287	3653
TOTAL	49034	39005	56217

* "Raw manure" means that no previous process/treatment is carried out
ɬ "Other residues" means manure that is already processed or other organic wastes

The number of biogas plants in Europe is expected to increase in the following years due to the European Union incentives to increase the use of renewable energy. The EU policies concerning renewable energy systems (RES) have set forward a fixed goal of supplying 20% of the European energy demands from RES by the year 2020, from which at least 25% shall originate from biogas [31]. Thus, the volume of digestion residues will also increase in the future.

2.1.4 Environmental impact of agricultural residues and European regulations concerning agricultural residues management

Intensive livestock production contributes to the economies of many European countries (for example Netherlands, Belgium, Denmark and Ireland). However, excessive manure generation and uncontrolled application causes serious environmental problems. The main pollution problems resulting from agricultural activities are summarized in Figure 2.1.
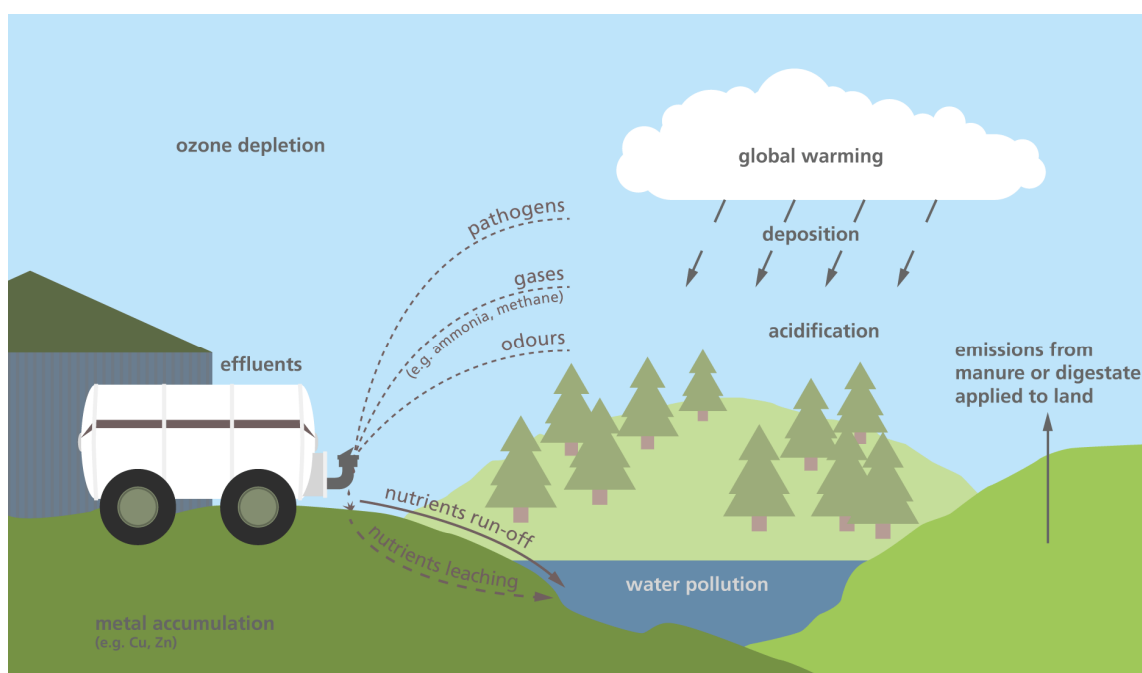
Figure 2.1 Environmental impacts from use of manure or digestate (modified from [12])

The environmental impact from agriculture activities can be divided into three areas: Soil, water and air pollution. Soil can be polluted by the residues application at high rates. Agricultural residues utilization results in economic advantages, because the use of mineral fertilizer is partially substituted and also the addition of organic matter maintains and improves soil fertility. However, farmers have limited control on the amount of nutrients excreted by their animals or obtained in digestion residues. In contrast to mineral fertilizers, the use of agricultural residues on fields has the disadvantage that the nutrient content is not optimal balanced for the crops. When manure application is based on nitrogen-crop needs (which is the usual case in Europe) P usually exceeds the crop uptake up to 500% [10]. Moreover, continuous application of high amounts of organic matter, coming from agricultural residues, can cause clogging of soil pores, which results in the reduction of water infiltration rate and oxygen diffusion [12].

Water pollution can be caused by phosphorus and nitrogen run-off or infiltration into water bodies, causing eutrophication. Specially in estuarine ecosystems, algae can efficiently

absorbed phosphorus and produced biomass blooms, making water environments very sensitive to even small phosphorus concentration [32].

Air pollution from gases (mainly nitrogen gases and methane), odors, dust, etc. is caused mainly in the farming building and during land application [12]. The impact in air contamination due to phosphorus is minimal, due to the low volatility of the phosphorus compounds.

European regulations concerning agricultural residues

The increased risk of an over application of manure in agricultural land has led to national and EU legislation that restricts the maximum application of manure or digestate per hectare land. The basic objective of EU waste policy is to prevent waste and to promote reuse, recycling and recovery to reduce negative environmental impact [33]. Recognizing this, the EU's Competitiveness Council in May 2007 called for „further actions to ensure cost-effective, reliable and environmentally responsible access and supply of raw materials for industry" [34].

Article 33 of the Treaty of Rome set out the specific objectives of the Common Agriculture Policy (CAP). In the latest reform agreed in December 2008, new objectives including the maintenance of a sustainable agricultural sector, incorporating environmental objectives were established [35]. The objectives and principles of the EC's environmental policy are also set out in the Treaty Article 174 consist in preventing, reducing and as far as possible eliminating pollution by giving priority to prevention at source and ensuring prudent management of natural resources.

At present, the most important directive concerning prevention of water pollution from agricultural sources is the Nitrates Directive (91/676/EC). The member states have to set up strategies for implementation and draw up action programs to improve the water quality across Europe by preventing nitrate runoff. The Nitrate Directive goals are the reduction and prevention of water pollution caused by nitrates form agricultural sources, in order to comply with the limit of 50 ppm nitrate in water. It also aims to reduce and prevent problems of eutrophication of coastal and marine waters caused by nitrate. Under the Nitrate Directive countries had to establish codes of good practice. These codes require that farming is practiced in a way that minimizes the pollution of water by nitrates. For examples, the codes should set up procedures for the land application of fertilizer and livestock manure that will maintain nitrate loss to water at an acceptable level. The nitrate directive does not cover phosphate. However, the European Commission has recognized the importance to establish regulations for the effective use and recovery and phosphorus. For this reason, it has

planned to publish a Green Paper on phosphorus, which should stimulate discussions about the phosphorus problematic in Europe [9].

According to Oenema [36], in the next decade the demanding environmental objectives for the prevention of phosphorus pollution in surface waters will have an even greater impact on agriculture than the Nitrates Directive has currently. Two countries in Europe, The Netherlands and Belgium, with highly intensive livestock production and consequently high phosphorus concentration in the agricultural soils have already recognized this problem and have implemented national manure policies based on phosphorus. In The Netherlands, quotas for manure production per farm and limits for manure application to land based on phosphorus were implemented (1984-1990) and lowered stepwise (1990 to 1998). From 1998 until present the Mineral Accounting System (MINAS) has been employed in response to the EU Nitrates Directive. MINAS is a farm-gate balance that records all inputs and outputs of N and P in a farm. This system includes levy for P and N surplus, where the levy charged for P surplus at the farm level is much higher than for N surplus (20.6 $€·kg^{-1}·ha^{-1}$ for P compared to 2.3 $€·kg^{-1}·ha^{-1}$ for N) [36]. In the region of Flanders, Belgium, restrictions for total phosphorus per hectare and for application of manure and organic fertilizers exist with regard to the period of spreading, the state of the soil and the distance to certain watercourses. Annually, each farmer has to declare his livestock, the surface of the farm cultivated acreage, geographical location and cropping plan, the transport of manure, the stock of manure at the end of the year, the use of chemical and organic fertilizers. All data are registered by the Flemish Manure Bank at the farm level and phosphorus balances are calculated for each farm to budget the use of P/ha. Farms with an annual phosphorus production over 10,000 kg P_2O_5 (7,500 kg in areas with a high P-pressure) are obliged to treat, process or transfer the excess manure to other regions [37].

A further legal system adopted for the European Union for pollution control is the "Integrated Pollution Control" (IPC). This is a principle for environmental protection and management, which aims to minimize the overall environmental impact of human activities and to identify the activities with high pollution potential. The system has been implemented by the EU as Integrated Pollution Prevention and Control (IPCC). This covers intensive animal rearing for farms with more than 40,000 animal units for poultry, 2,000 for fattening pigs and 750 for sows. The ICCP in the farm regulates the following activities: Farm management (including maintenance and cleaning of equipment), feed preparation and feeding strategy, rearing of animals, collection and storage of manure, on-site treatment of manure, land-spreading of manure, wastewater treatment, transport on site [12].

2.2 Phosphorus forms in agricultural residues

In nature phosphorus is present in combination with four oxygen molecules as phosphate oxyanion. Due to its electron configuration, the phosphate ion can form different inorganic and organic structures, principally with carbon, nitrogen and metals [38]. Moreover, all P forms can be associated with other residues components like organic matter and other minerals [39, 40]. A representation of the phosphorus forms and interactions in the residues matrix is presented in Figure 2.2.
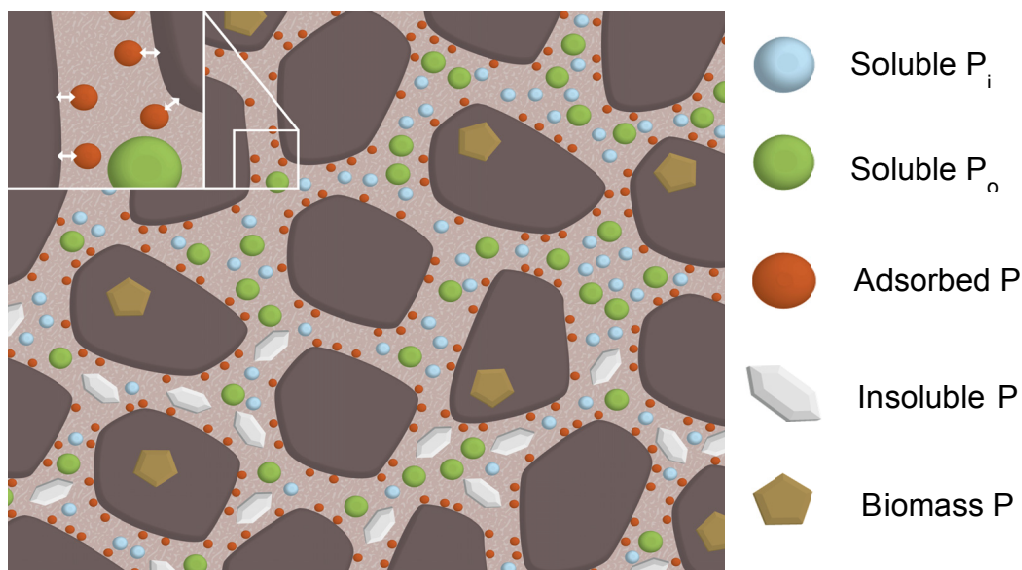
Figure 2.2 Phosphorus forms and interactions in agricultural residues

i.   Characterization of P compounds in agricultural residues

At present, a direct method for the quantification of the specific inorganic and organic P compounds in agricultural residues is not available. In soil sciences, diverse extraction methods have been developed to characterize soil P. This is achieved by using different solvents for the sequential solubilisation of the different P compounds. These methods have been used by different researchers for the characterization of agricultural residues such as cattle-, pig- and poultry manure (Table 2.5).

It is important to consider that sources of error by using extracting methods are inevitable. The most serious errors is the hydrolysis or inter-conversion of organic P form by strong extracting agents and also the incomplete extraction from the biomass matrix [39]. Moreover, the methods and observations of soil fractionation cannot be completely transferred to organic residues characterization. The reason is that the chemistry of soil (mainly controlled by iron and aluminum ions) differs distinctly from the chemistry of organic residues (mainly controlled by calcium and magnesium ions) [41].

Table 2.5 Characterization of animal manure by extraction methods

Type of manure	Total-P [g/kg DM]	Extracted phosphorus [% g $P_{released} \cdot$ g P_{total}^{-1}]						Ref.
		Water-P	NaHCO$_3$-P	NaOH-P	Acid-P	NaOH-EDTA-P	Residual-P	
Pig	1.62	11*	13	19	27	-	39	[42]
	33.60	66	12	8	16	-	1.1	[43]
	19.80	55	14	11	21	-	1.5	[43]
	23.10	-	6.9	12.1	-	-	81	[44]
	14.62	55	23	6	9	-	8	[41]
	14.62	-	-	-	-	92	8	[45]
Chicken	26.21	29	10	3	17	-	41	[42]
	13.31	22.8	7.5	18.8	50.8	-	0.1	[46]
	15.95	29	5	12	48	-	6	[41]
	15.95	-	-	-	-	97	3	[45]
Cow	2.94	50.3	-	-	-	-	49.7	[47]
	2.94	-	-	-	-	81.9	18.1	[47]
	6.60	-	18.2	12.1	-	-	69.7	[44]
	4.94	11	43	19	6	-	21	[41]
	4.94	-	-	-	-	90	10	[45]

* Resin was used instead of water

In the fractionation methods the P compounds are grouped in different fractions or pools: The water fraction contains readily soluble P compounds. The bicarbonate fraction contains additionally labile P that is loosely adsorbed on crystalline structures. Sodium hydroxide P considers P adsorbed to aluminum and iron oxides (definition for soil sciences). Acid soluble P includes slightly soluble P. Finally, the residual P can be assigned as insoluble mineral phases [42, 43, 46]. With the NaOH-EDTA solution it is possible to recover even poor soluble P in one step extraction because the EDTA form complex compounds with calcium and magnesium, and P can be released in the solution. Moreover, the alkaline solution improves the extraction of organic P compounds [41]. The differences in the values reported by the authors can be explained by the different in the analytical procedures and the types of samples analyzed.

The specific organic P compounds have been measured from the different extraction liquid fractions by enzymatic characterization (Table 2.6) and nuclear magnetic resonance spectroscopy ^{31}P-NMR (Table 2.7). In the enzymatic characterization, the extraction liquids are incubated with phosphatases. The concentration of inorganic P in the sample is measured before and after enzymatic incubation and this indicates the presence of a form of organic P [48]. In the solution ^{31}P-NMR spectroscopy method multiple P compounds can be quantified simultaneously [45]. The identification is based on the determination of the chemical shift of each compound related to a H_3PO_4 standard [49].

Table 2.6 Organic phosphorus determination by enzymatic hydrolysis

Type of manure	Total-P [g·kg$_{DM}^{-1}$]	Extractants	P extracted [% total P$_{manure}$]	Organic P content [% total P$_{manure}$]					Ref
				P$_{inorg}$	Phytate	Simple monoesters	Poly-nucleotide	Non-Hydrolizable P	
Cow	6.9	0.25 M NaOH +0.05 mM EDTA	100	67	3.8	2.9	0.3	26	[48]
	6.9	Water	60	46	2.0	4.0	1.0	7.0	[50]
Poultry	19.0	0.25 M NaOH +0.05 M EDTA	65	45	9.5	1.9	2.8	5.8	[48]
	13.3	Sequential extraction*	99.9	35.55	49.94	4.49	0.09	9.84	[46]

* Sequential extraction: Water, bicarbonate solution, NaOH solution, HCl

Table 2.7 Organic phosphorus determination by ^{31}P-NMR

Manure type	Total-P [g·kg$_{DM}^{-1}$]	Extractants	Organic P content [% total P$_{manure}$]					Ref
			P$_{inorg}$	P-Monoesters*	P-Diesters+	Poly-phosphates	Non-extractable P	
Pig	26.2	NaOH 0.5 M	20.0	65.0	14.0	-	1.0	[42]
	14.6	NaOH 0.5 M	83.2	8.56	0	0.28	7.96	[45]
Poultry	19.0	0.25 M NAOH + 0.05M EDTA	32.4	29.3 (21.6)	2.2 (1.0)	0.65	35.45	[48]
	1.6	NaOH 0.5 M	77.0	13.0	9.0	-	1.0	[42]
	15.9	NaOH 0.5 M	38.4	58.6	0	0	3.0	[45]
Cow	4.9	NaOH 0.5 M	58.9	20.97	3.96	6.12	10.05	[45]
	6.9	0.25 M NaOH + 0.05M EDTA	70.6	19.4 (5.4)	7.2 (0.8)	2.0	0.8#	[48]
	2.9	0.25 M NaOH + 0.05M EDTA	55.9	18.6 (7.5)	3.7 (1.2)	3.7	18.1	[47]
	5.8	0.25 M NaOH +0.05M EDTA	71.5	17.6 (8.8)	6.9(1.8)	2.65	1.35	[51]
	2.9	water	41.1	5.9	3.3 (0.8)	0	49.7	[47]

* The value in parenthesis is the phytic acid content
+The value in parenthesis is DNA content
Measured as phosphonate

ii.   Phosphorus utilization by farms animals

The composition of manure, regarding its phosphorus forms, depends primary on the type of animal and its alimentation. P from feedstuff is not completely utilized by animals because it can be in an unavailable form or its absorption is influenced by different factors.

As an example, the P utilization by dairy cattle is presented in Table 2.8. The authors calculated the P content for four different diets and assumed a daily milk yield of 30 Kg (containing 1g $P \cdot kg_{milk}^{-1}$). The theoretical feed intake is taken from the Dutch net energy system for lactation, which is 61 g $P \cdot day^{-1}$. The excreted P is calculated as the difference between the P intake and the P retained in milk [52].

Table 2.8 P balance for dairy cattle [52]

Dietary component	Proportion [%$_{DM}$]	P content [$g \cdot kg_{DM}^{-1}$]	P intake [$g \cdot day^{-1}$]	Excreted P* [$g \cdot day^{-1}$]
Diet 1		4.3	87	57 (66)
Grass silage	75	4.2	-	-
Concentrates	25	4.8	-	-
Diet 2		3.9	77	47 (61)
Grass silage	40	4.2	-	-
Maize silage	35	2.1	-	-
Standard concentrates	25	5.6	-	-
Diet 3		3.3	67	37 (55)
Maize silage	75	2.1		
Protein-rich concentrates	25	7.0		
Diet 4		2.5	50	20 (40)
Maize silage	75	2.1	-	-
By-products	25	3.5	-	-

* The numbers in parenthesis are the mass % respect to the P-intake

The results show that the actual intake differs from the recommended in a range 11-26 g $P \cdot day^{-1}$ in the four diet example presented. The P intake ranges from 40% to 66%, which means that the rest is lost in the excrements.

A further discussion about the P absorption by different farm animals is presented as follows:

i.   Ruminants (Cattle)

The P content in cattle feed is present as inorganic phosphates and organic P compounds, mainly phytate, phospholipids and phosphoproteins. In ruminants, phytate is hydrolyzed by action of microbial enzymes in the rumen. On the other hand, inorganic phosphates are solubilized by acid gastric juices in the small intestine [53].

The most common nutrient deficiency for cattle is from P, especially for animal raised in pasture. The P metabolization is a complex process and therefore the exact animal P needs are difficult to predict. For this reason, national recommendations for P requirements vary from region to region [53]. The P bioavailability from different common feedstuff for ruminants is shown in Table 2.9.

Table 2.9 P bioavailability of feedstuff for ruminant animals [54]

Feedstuff	P bioavailability [%] (mean value)
Lucerne, hay	84
Maize silage	81
Dicalcium phosphate	73
Monophosphates, Na and Ca	90
Phosphoric acid	90
Rock phosphate, soft	32
Urea ammonium polyphosphate	100
Rock phosphate, defluorinated	89

ii. Non-ruminants (pig and poultry)

P supplementation contributes to a faster and more efficient bone development and better growing of pig and poultry [55]. Normally dicalcium phosphate is used as a source of inorganic P because non-ruminants are not able to hydrolase the organic P, normally as phytate, present in seeds and grains. Phytase enzyme is normally added to the feedstuff to increase the P_i release from phytate, thereby reducing the need of inorganic supplements [53]. However, the P bioavailability only increased by maximum 54% by adding phytase enzyme [55, 56]. The bioavailability of different feedstuff for pigs and chickens are shown in Table 2.10.

The excess of phosphorus in the manure of non-ruminant and ruminant animal, can be caused by phosphorus overfeed by farmers. Knowlton *et al.* [57] suggest the following reasons for the overfeeding of farm animals:

1. The perception of farmers that high P diets will improve reproductive performance of animals (specifically dairy cows).

2. A safety margin is kept in case of variation in the P content of feeds and inconsistencies between the requirements and nutritional advice farmers received by different entities.

3. Inclusion of feeds in the diet with naturally high phosphorus content. For example, products of maize processing and ethanol production are increasingly popular feed

supplements cattle because of the protein and energy supply. However, inclusion of these feeds often increases dietary P content beyond animal requirements.

Table 2.10 P bioavailability of feedstuff for non-ruminant animals [54]

Feedstuff	P bioavailability [%]	
	Poultry	Pigs
Cereal grains and byproducts		
Barley	38	30
Maize, dry	21	18
Maize, gluten feed	95	59
Sorghum, dry	26	26
Sorgum, high moisture	47	43
Oats	42	29
Rice bran	10	-
Triticale	31	46
Wheat	39	50
Oilseed meals		
Canola meal	45	34
Soybean meal, low phytate	77	63
Sunflower meal	23	3
Animal based feedstuffs		
Meat and bonemeal	76	73
Fishmeal	103	93
Milk products	48	94
Inorganic supplements		
Dicalcium phosphate	91	97
Monophosphates, Na and Ca	96	100
Rock Phosphate, soft	47	53
Rock phosphate, defluorinated	89	90

2.2.1 Inorganic phosphorus compounds

Phosphorus can form oxoacids with the structure H_3PO_n (n = 2, 3, 4, 5 and 6), known as monophosphoracids and with the form $H_4P_2O_n$ (n = 4, 5, 6, 7 and 8), known as diacids [58]. The most common structure is phosphoric acid, H_3PO_4, The phosphoric acid molecule can form primary phosphate (dihydrogen phosphate, $H_2PO_4^-$), secondary phosphate (hydrogen phosphate, HPO_4^{2-}) and tertiary phosphate (ortho-phosphate, PO_4^{3-}), liberating up to three protons in the process [59]:

$$H_3PO_{4(s)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + H_2PO_4^-{}_{(aq)} \qquad pK_1 = 2.23$$

$$H_2PO_4^-{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + HPO_4^{2-}{}_{(aq)} \qquad pK_2 = 7.21$$

$$HPO_4^{2-}{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+{}_{(aq)} + PO_4^{3-}{}_{(aq)} \qquad pK_3 = 12.32$$
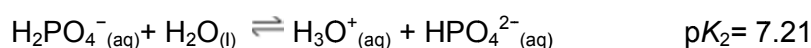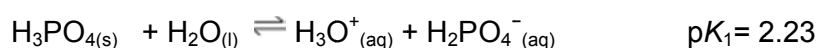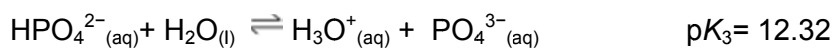
The formation of the different ions of phosphoric acid depends on the pH value. The dependency of the molar concentration of the different species with pH value is shown in Figure 2.3.
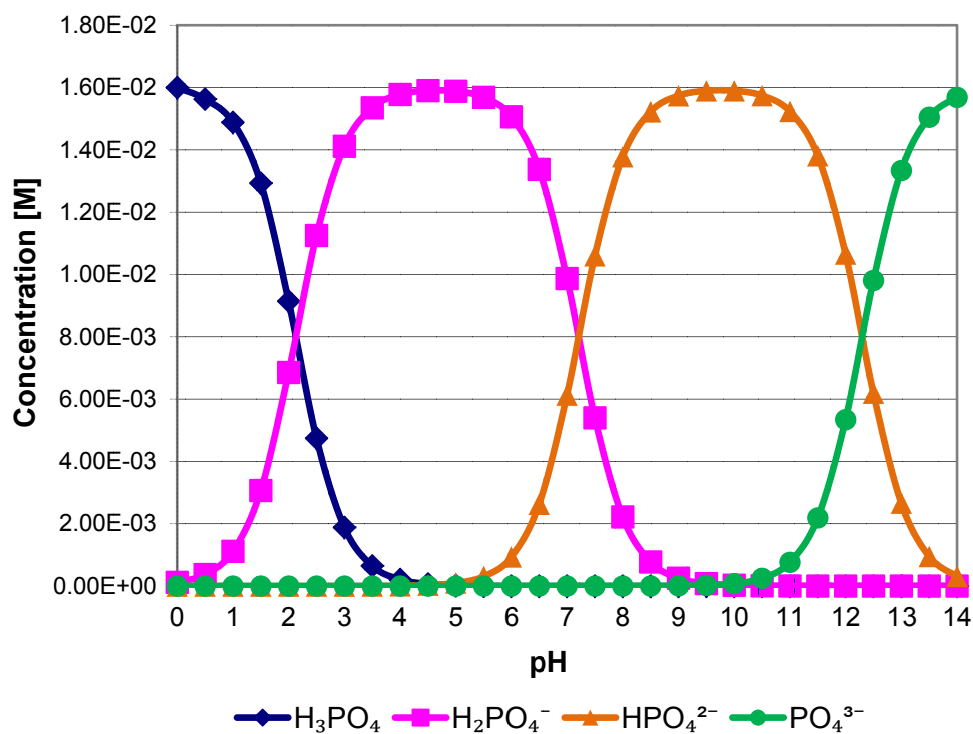
Figure 2.3 Dependency of ion concentration on pH value of a phosphoric acid solution

Condensed phosphates can be formed by the repeated condensation of different PO_4^{3-} groups. They characterized by an extremely slow rate of hydrolysis. These compounds are very numerous and exist both as crystalline and amorphous salts. Condensed phosphates are divided into cyclophosphates, polyphosphates and branched inorganic phosphates [60].

Almost most naturally occurring phosphorus compounds are salts of orthophosphoric acid. They can react with several different cations and anions forming different compounds, for example with alkali-metal and alkaline earth elements. Alkali-metal orthophosphates, such as sodium phosphate (Na_3PO_4), potassium dihydrogen phosphate (KH_2PO_4) and ammonium phosphate ($(NH_4)_3PO_4$), are commonly used in pH control, as food additives and in detergent compositions. Alkaline earth orthophosphates are much less soluble than alkali-metal orthophosphates [60]. Two very important types of these orthophosphates are calcium phosphates and magnesium ammonium phosphate. They are important in fertilizer production and in phosphorus recycling from wastewater. They are presented in more detailed as follows.

i.   Calcium phosphates

Calcium phosphates refer to a family of minerals containing calcium ions together with orthophosphates (PO_4^{3-}), metaphosphates ($P_3O_9^{3-}$) or pyrophosphates ($P_2O_7^{4-}$) [38]. In addition, the chemical composition of calcium phosphates may include hydrogen, fluoride, chloride or hydroxide ions as well as incorporated water [26].

Calcium orthophosphates exist in equilibrium with solutions more acid than the solid phase composition. Therefore, the salts dissolve incongruently to form phosphoric acid and more basic salts (surface hydrolysis). Reactions involving compounds in the calcium orthophosphate system are present difficulties in attaining equilibrium. These compounds are all insoluble and their reactions often involve the growth of crystals of one kind from those of another. If a large excess of water is present, the equilibrium will lean towards to the formation of the thermodynamically stable hydroxyapatite $Ca_5(PO_4)_3(OH)$ [60].

Calcium phosphates are very insoluble in water and their reactions are slow. Solubility of calcium phosphates is very sensitive. A slight variation of pH, temperature and rate of formation affects the surface compositions of precipitated phosphates [60].

Depending on the solution composition and pH, calcium orthophosphates crystallize in different forms, where Ca^{2+}/P molar ratio ranges between 0.5 and 2.0 (Table 2.11).

Table 2.11 Description of calcium orthophosphate compounds [61]

Ca^{2+}/P molar ratio	Compound	Formula	Solubility at $25^{o}C$, $-\log(K_{sp})$
0.5	Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$	1.14
0.5	Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	1.14
1.0	Brushite or dicalcium phosphate dihydrate (DCPD)	$CaHPO_4 \cdot 2H_2O$	6.59
1.0	Monetite or dicalcium phosphate anhydrous (DCPA)	$CaHPO_4$	6.90
1.33	Octacalcium phosphate (OCP)	$Ca_8H_2(PO_4)6 \cdot 6H_2O$	96.6
1.5	α-Tricalcium phosphate (α -TCP) β-Tricalcium phosphate (β -TCP)	$\alpha\text{-}Ca_3(PO_4)_2$ $\beta\text{-}Ca_3(PO_4)_2$	25.5 28.9
1.2-2.2	Amorphous Ca phosphate (ACP)	$Ca_3(HPO_4)_2$	ǂ
1.67	Hydroxylapatite (HAP)	$Ca_5(PO_4)_3(OH)$	116.8
2.0	Tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	38–44

The solubility is given as the logarithm of the ion product with concentrations in $mol \cdot l^{-1}$
ǂ Cannot be measured precisely

Based on the solubility products of the different calcium phosphates, solubility isotherms can be calculated for the different phases. The solubity phase diagrams (Figure 2.4) show that at high calcium concentrations, more than one calcium phosphate may be supersaturated, depending on solution pH value. Other forms of calcium phosphate such as amorphous calcium phosphate (ACP) will act as precursor phases and eventually will crystallize to form hydroxylapatite (HAP) [62].
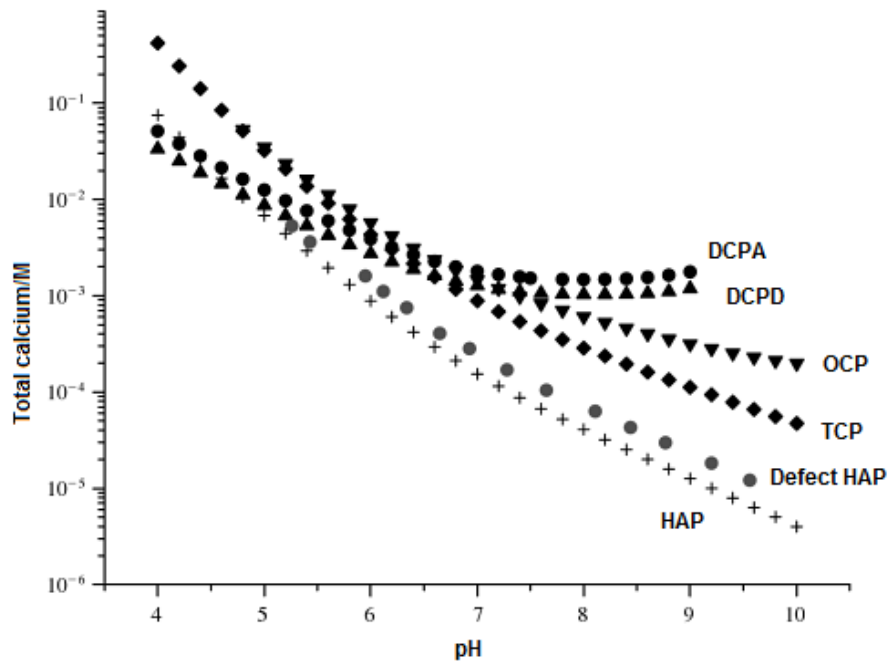
Figure 2.4 Formation of calcium phosphate compounds as a function of pH [62]

Inhibition of hydroxyapatite formation

Several ions and organic acids can inhibit the formation of hydroxyapatite. Such inhibitors include: Mg^{2+} and CO_3^{2-} ions [63].

The presence of carbonate may affect the solubility of calcium phosphate due to different reasons: First, CO_3^{2-} induces the precipitation of Ca^{2+} as calcium carbonate ($CaCO_3$) rather than phosphate. The formation of $CaCO_3$ is influenced by the pH value, normally occurs at a pH range of 8-11 [64] . As the pH value increases the solubility of $CaCO_3$ decreases.

The second effect of carbonate is the substitution of two sites in the hydroxylapatite structure, namely the phosphate (substitution type B) and hydroxyl (substitution type A) group site (Figure 2.5). This effect reduces the crystallinity of the apatite and increases considerably its solubility [65, 66].
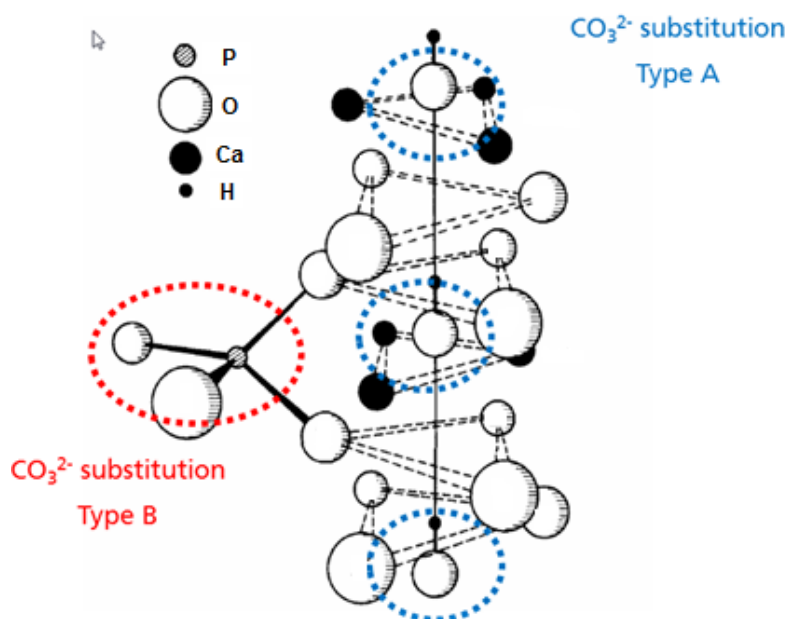
Figure 2.5 Types of CO_3^{2-} substitution in hydroxyapatite structure (modified from [67])

Cao and Harris [64] reported that at a pH value of 7.1 the influence of carbonate was mainly by substitution in the crystal structure. At pH values of 9.2, calcium was removed from the solution by calcium carbonate precipitation, increasing the solubility of the phosphates. Ferguson [65] reported that there is a pronounced phosphate solubility minimum near pH 8 and a maximum between pH 9 and 10.

Magnesium ions are also responsible for the inhibition of the calcium phosphate formation. This can be explained by to effects: First, magnesium could kinetically hinder the nucleation and growth of crystal because it competes with the chemically similar calcium ions. Alternately, magnesium could be included in the precipitated solids and could modify the solids by its smaller size and greater tendency to bond covalently [65].

ii.   Magnesium ammonium phosphate (struvite)

Struvite is a white crystalline substance composed of magnesium, ammonium, and phosphate in equal molar concentrations [68]. Its chemical formula is $NH_4MgPO_4 \cdot 6H_2O$ with a molecular weight of 245.41 $g \cdot mol^{-1}$. Struvite is formed according to the following chemical reaction [68]:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6\ H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O$$
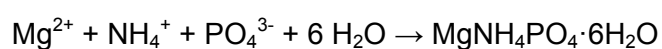
The precipitation of struvite is controlled by the pH value, temperature, the initial concentration of the reactants and the presence of competing ions in solution such as Ca^{2+} [68]. Calcium ions inhibits struvite precipitation by blocking its active crystal growth sites and

competing for PO_4^{3-} to form calcium phosphates [69]. The precipitation of calcium phosphate occurs at pH values above 9.5, whereas effective struvite precipitation occurs at pH values of higher than 8.0 [70]. However, as the Ca^{2+}/Mg^{2+} molar ratio in the solution increases, PO_4^{3-} is preferentially removed with Ca^{2+} instead of Mg^{2+}. According to Huchzermeier *et al.* [69] struvite precipitation is hindered when the Ca^{2+}/Mg^{2+} molar ratio is higher than 0.2:1, and no longer dominant when the ratio is higher than approximately 0.5:1[68].

2.2.2 Organic phosphorus compounds

The most common organic phosphorus compounds found in organic residues includes phosphate monoester (especially inositol phosphates), nucleic acids and phospholipids:

i.   Inositol phosphates

Inositol phosphates are a group of mono to polyphosphorylated inositols. They play a crucial role in diverse cellular functions, such as cell growth, cell migration, apoptosis, and cell differentiation [71]. *Myo*-inositol hexakisphosphate is a compound in which all six hydroxyl groups of *myo*-inositol (one of the nine possible stereoisomers of inositol) are esterified as phosphates. This compound is commonly known as phytic acid. The salts from phytic acid are called phytates and can be present as insoluble or soluble compounds. Normally the insoluble salts involve polyvalent cations (e.g. Fe^{3+}) and the soluble salts monovalent cations (e.g. Na^+) [72].

Phytic acid (Figure 2.6) is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule [71] .
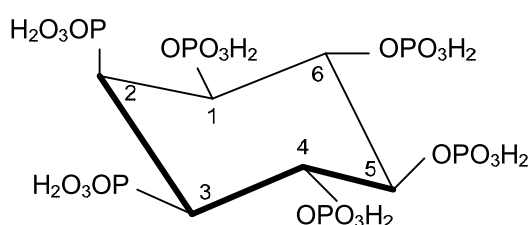
Figure 2.6 Structure of phytic acid [40]

Phytic acid is present in grains and seeds as mixed salt, phytate, mainly involving Mg, Ca, Na and K. As a result of its chelating capacity, phytate may form complexes with minerals, starch and proteins. Phytic acid is a moderately strong acid with five to six H^+ dissociating with pK greater than 8. The large negative charge may be counterbalance by positively charged molecules including mineral cations, low molecular weight carbo-cations and proteins at pH values less than their isoelectric points. The complexing is possible within a phosphate group or between two phosphates groups on either the same or different phytic acid molecules. The interaction of the phytate anion with the counter-cation may result in precipitation of the cation-phytate complex [73].

ii.   Nucleic acids

The term "nucleic acids" refers to DNA and RNA, members of a family of biopolymers. Nucleic acids were named in this way for their discovery in cell nucleus and for the presence of phosphate groups, related to phosphoric acid [74]. The basic component of biological nucleic acids is the nucleotide, each of which contains a pentose sugar (ribose or deoxyribose), a phosphate group, and a nucleobase [75]. The formula of a polynucletide is represented in Figure 2.7.
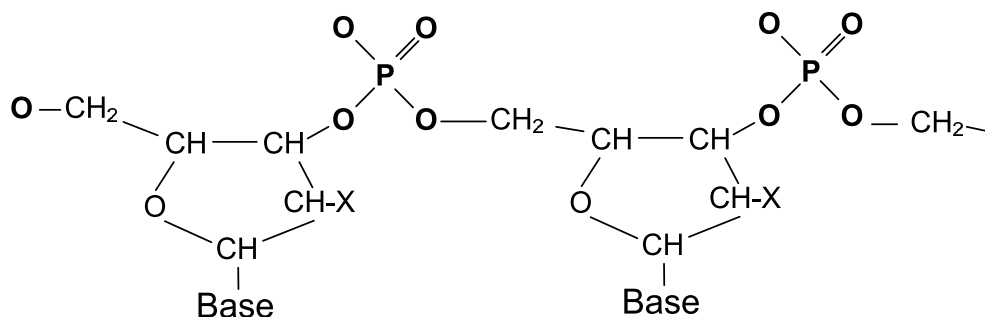
Figure 2.7 General structure of a polynucleotide (modified from [75])

Where for

RNA$\rightarrow$ X = OH; Base= Uracil, cytosine, guanine or adenine
DNA$\rightarrow$ X = H; Base= Thymine, cytosine, guanine or adenine

iii.   Phospholipids

Phospholipids are lipids that contain one or more polar phosphate group. They are major components of cell membranes and occur widely in bacteria, animal and plant tissues. They

are involved in enzyme action and transport of triglycerides through the liver, and they have a role in electron transport and oxidative phosphorylation [60]. The structure of most abundant naturally occurring phospholipids is showed in Figure 2.8.

$$CH_2 - O - C(O) - R$$
$$|$$
$$CH - O - C(O) - R'$$
$$|$$
$$CH_2 \text{———} O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O-X$$
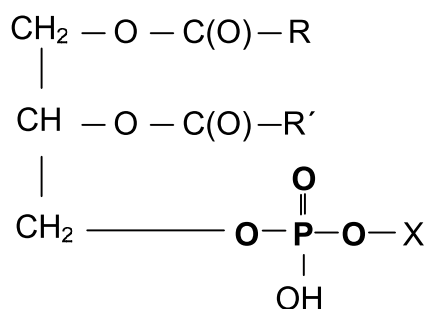
Figure 2.8 Structure of most abundant phospholipids (modified from [75])

Where,

R = Long hydrocarbon chains derived from the fatty acids $HOOC(CH_2)_nCH_3$
X = H, choline, ethanolamine, L-serine or inositol

2.2.3 Adsorbed phosphate

Absorption/desorption is a surface process that occurs at the interfaces of a solid-liquid system [76, 77]. Adsorption influences the distribution of the substance between the aqueous phase and the particulate matter. Moreover, it affects the electrostatic properties of suspended particles and colloids, which, at the same time, influences their tendency to aggregate and attach (coagulation, settling, filtration). It has been shown that the rates of processes such as precipitation (heterogeneous nucleation and surface precipitation), dissolution of minerals, and catalysis and photocatalysis of redox processes are critically dependent on the properties of the surfaces [77].

Not only dissolved ions are able to adsorb to surfaces, but also uncharged inorganic species and neutral molecules, such as organic compounds. It has been reported that proteins, carbohydrates and their hydrolysis products are adsorbed on clay surfaces and this increases their resistance to enzymatic hydrolysis. The extent and nature of this adsorption depends on the specific surface and the net electric charges of the clay, the equilibrium pH of the adsorbing medium and the nature of the adsorbed molecules [39].

Orthophosphate may be adsorbed in the organic matrix by ion-exchange adsorption or by precipitation of simple and complex phosphates of magnesium, calcium, aluminum, iron and manganese, when their solubility products are exceed in the solution [39].

i.   Ion exchange process

Charge ions in solution can interact by electrostatic forces with opposite charged surfaces. This type of binding is relatively weak and adsorption/desorption kinetic is very fast. Therefore, ions adsorbed by electrostatic forces on surfaces can be easily exchanged and being dissolved again. Ion exchange processes play an important role in the storage of nutrients in soils, especially of Ca^{2+}, Mg^{2+}, K^+ and NH_4^+, but also trace metals (e.g. Cd^{2+}, Zn^{2+}) and anions (e.g. Cl^-, NO_3^-)[76].

ii.   Surface complexation reactions

Ions can associate with the reactive groups of a surface by coordinative bonds. These types of bonds are much stronger and the kinetics of adsorption/desorption is significantly slowly. This process is called specific adsorption or chemisorption [76].

The association can take place as inner-sphere or outer-sphere complex, depending on whether a chemical bond between the metal and the electron-donating oxygen is formed (as in an inner-sphere type solute complex) or if a cation of opposite charge approaches the surface groups to a critical distance, as with solute ions pairs, the cation and the base are separated by one or more water molecules. Furthermore, ions may be in the diffuse swarm of the double layer [77].

In inner-sphere complexes the surface oxide ions act as σ-donor ligands, which increase the electron density of the coordinated metal ion. For example, Cu (II) bound inner-spherically is a different chemical entity than if it were bound outer-spherically or presenting the diffuse part of the double layer. They have different chemical properties [77]. The bounds of out-spherical complexes are weaker than inner sphere complexes

Different types of surface complexes represented schematically in Figure 2.9. Highly hydrated monovalent ions, such as Cl^-, NO_3^- and Na^+, form weak non-spherical complexes at oppositely charged surfaces. Many oxyanions (for example, $H_2PO_4^-/HPO_4^{2-}$; $H_2AsO_4^-/HAsO_4^{2-}$) form, on the other side, much more stable inner-sphere surface complexes with reactive hydroxyl groups at oxide. Depending on, whether the ion is coordinated with one or two surface groups, the complexes are referred to as monodentate or bidentate [76].
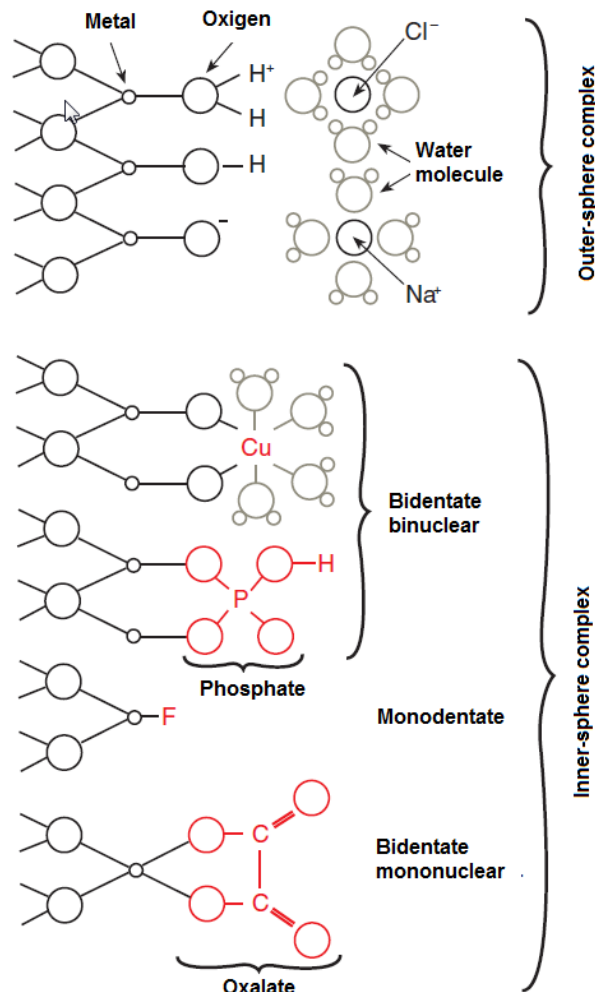
Figure 2.9 Different types of surface complexes. Phosphate forms bidentate binuclear inner-sphere complexes (modified from [76])

The adsorption of phosphate (HPO_4^{2-}) to a surface can be represented by the following equation:

$$\equiv SOH^{0.5-} + H^+ + HPO_4^{2-} \leftrightarrow \equiv SHPO_4^{1.5-} + H_2O$$
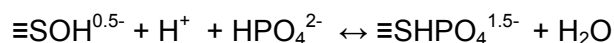
The surface hydroxyl group $\equiv SOH^{0.5-}$ is thereby protonated and subsequently replaced as H_2O by the ligand HPO_4^{2-}. The specific adsorption of anions on oxide surfaces is therefore referred as ligand-exchange reaction [77].

The surface complex $\equiv SOH^{0.5-}$ is a monodentate mononuclear complex. Phosphate may be also absorbed as bidentate, binuclear or bidentate mononuclear complex. The equation shows that in the specific adsorption of anions on an oxide surface, protons are consumed and the surface is more negatively charged. The equilibrium the reaction is shifted to the left when the pH of the solution increases. The adsorption of phosphate and other oxyanions on oxide surfaces thus decreases with increasing pH [77].

The pH dependency of sorption is influenced also by the type of ions species. Wherein the slope of the adsorption curves have most changes in the pH values of the pKa values correspond to the respective acid (e.g. for phosphate: H_3PO_4 with pK_1 = 2.15, pK_2 = 7.20 and pK_3 = 12.38). At pH values near or below the pK_1 value of the acid is often a maximum reached the sorption, because below this pH value the undissociated (and thus uncharged) acid dominates more and more.

2.3 Enzymatic hydrolysis of organic phosphorus

2.3.1 General description of phosphatase enzymes

Enzymes are biological catalysts that increase the rate of biochemical reactions without undergoing any overall net change. In the natural environment, most enzymes are found within living cells, either freely dissolved or as a component of membranes [78].

The widely used classification system for enzymes was the created by the enzyme commission (EC). This consists in the assignation of code numbers with four digits to each individual enzyme. The first digit shows the main class of the enzyme, namely: 1) Oxidoreductases, 2) Transferases, 3) Hydrolases, 4) Lyases, 5) Isomerases and 6) Ligases [79]. The second digit indicates the subclass, for example for hydrolyses, it shows the type of bond hydrolyzed. The third and fourth number represents the sub-subclass and the serial number, respectively [79].

Phosphatases are hydrolases that catalyze the removal of phosphate groups form organic compounds (Table 2.12). Acid and alkaline phosphatases (EC 3.1.3.1-2) are a group of widely distributed enzymes of very broad specificity. This means, they act on a wide range of monoesters of orthophosphoric acid, both aliphatic (e.g. glycerol-1-phosphate and glycerol-2-phosphate) and aromatic (e.g. 4-nitrophenyl phosphate). However, they do not act on phosphoric diesters or triesters [79-81]. Their optimum pH value depends on their source and can vary between 2.5 and 9 [82]. Alkaline phosphatase from different sources is able to hydrolize creatine phosphate, inorganic pyrophosphate and a number of polyphosphates including ATP and metaphosphate of average chain length [82].

Phytase is a phosphatase that catalyzes the stepwise hydrolysis of phytic acid (*myo*-inositol hexakisphosphate) to a series of low phosphate ester of *myo*-inositol phosphate and orthophosphate [71]. In general, there are two types of phytases, plant phytases are 6-phytases (EC 3.1.3.26), which means that the dephosphorylation start in the phosphate group located in the carbon six. Microbial phytases are 3-phytases (EC 3.1.3.8) and they act in the phosphate located in position three [83] . Most commercially phytases are produced by

the filamentous fungus *Aspergiullus Niger*. They contain a variety of other enzymes as impurities, mainly acid phosphatases. This results in activities toward a wide range of organic phosphorus compounds including other orthophosphates monoester, inorganic and organic polyphosphates even nucleic acids [71].

Table 2.12 Description of different phosphatases [78]

Name	Reaction
Alkaline Phosphatase (EC3.1.3.1) Orthophosphoric-monoester phosphohydrolase (alkaline optimum)	Orthophosphoric monoester+ water → alcohol + orthophosphate
Acid Phosphatase (EC 3.1.3.2) Orthophosphoric-monoester phosphohydrolase (acid optimum)	Orthophosphoric monoester+ water → alcohol + orthophosphate
3-Phytase (EC 3.1.3.8) *Myo*-Inositol hexakisphosphate 3-phosphohydrolase	*Myo*-Inositol hexakisphosphate + water→ 1-D-*myo*-inositol 1,2,4,5,6-pentakisphosphate + orthophosphate
6-Phytase (EC 3.1.3.26) *Myo*-Inositol hexakisphosphate 6-phosphohydrolase	*Myo*-Inositol hexakisphosphate + water→ 1-L-*myo*-inositol 1,2,4,5,6-pentakisphosphate + orthophosphate
Phosphordiesterase I (EC 3.1.4.1) Oligonucleate 5'-nucletidohydrolase	Hydrolytically removes 5'-nucleotides successively from the 3'-hydroxy termini of 3'-hydroxy-terminated oligonucleotides

Industrial phytase is used currently as a feed additive for poultry, swine and other simple-stomach animals to increase the phosphorus assimilation from phytate (phytic acid salt) and reduce the phosphorus concentration in manure. Phytate is the main phosphorus form in the seeds used for animal feed. In this way, organic phosphorus is converted to phosphate and is available for the animal nutrition. Phytase produced for feed additive must have special characteristics: Thermal stability during processing (e.g. pelletisation), high efficiency at the very low stomach pH values and high resistance against gastrointestinal proteinases [56].

The phosphomono- (and di-) estereases (EC 3.13-4) are often enzymes of comparatively low specificity [79].

2.3.2 Inhibition of the enzymatic hydrolysis

There are different factors that can influence the enzymatic hydrolysis of organic phosphorus compounds by phosphatases. Besides the pH value and temperature, which are essential for the optimal performance of an enzyme, the factors that have an influence are: Presence of inhibitors and activators and interaction of the enzyme with the residue matrix (i.e. adsorption).

i.   Inhibitors and activators

Most acid phosphatases and phytases are inhibited by fluoride, which is a strong non-competitive inhibitor. Polyvalent ions (e.g. phosphate, molybdate, arsenate) metal ions (e.g. silver, zinc, mercury III, copper II , iron II, manganese II) and chelating agents (e.g. ethylenediaminetetraacetic acid EDTA, tartrate, oxalate) [82]. The inhibition can be caused by competitive reaction with an activator, precipitation of substrates, alteration of the active conformation of the enzyme or steric hindrance of substrate active site [84].

Activators are substances that increase the catalytic activity of enzymes. In the absence of activator, some enzymes can only function to a limited extent, whereas others require the activation before showing any activity [78]. Different alkaline phosphatases and phytases are activated by divalent metal ions, such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} [82]. However, metals ions can act as an activator or inhibitor depending on its concentration.

ii.   Interaction of phosphatases with residue matrix constituents

When phosphatases are in contact with organic residues, they have to endure many factors to keep their functionality [84] :

- Deactivation and inhibition by adsorption and immobilization on solid particles
- Degradation of the enzyme protein by microorganism
- Inhibition by metal ions, anions and metabolites
- Denaturation by environmental factors (e.g. temperature, pH, light)

Enzymes like other proteins have a strong affinity for solid surfaces. The extent and nature of the adsorption depends on the specific surface, the net electric charges of the minerals, the equilibrium pH and the nature of the molecules [39]. The adsorption of phosphatases restricts their mobility by diffusion and can modify the conformation of the whole proteic structure and lead to an unfavorable orientation of its catalytic site [82]. However, adsorption can increase the resistance of the enzymes against degradation. In Figure 2.10 a schematic representation of the enzyme adsorption is shown.

The enzyme adsorption phenomena can be explained results of enthalpic (intermolecular) and entropic (intramolecular) forces. According to the second law of thermodynamics, the spontaneous adsorption of a protein at constant temperature and pressure decreases the Gibbs energy of the system. The Gibbs energy (G) depends on enthalpy (H), which is a measure of the potential energy (energy that has to be supplied to separate the molecular constituents from one another) and entropy (S), which is related to the disorder of the system [82]:

$$\Delta_{Ads} G = \Delta_{ads} H - T \Delta_{ads} S < 0$$

Where T is the absolute temperature and Δ_{ads} is the change in the thermodynamic functions resulting from adsorption.
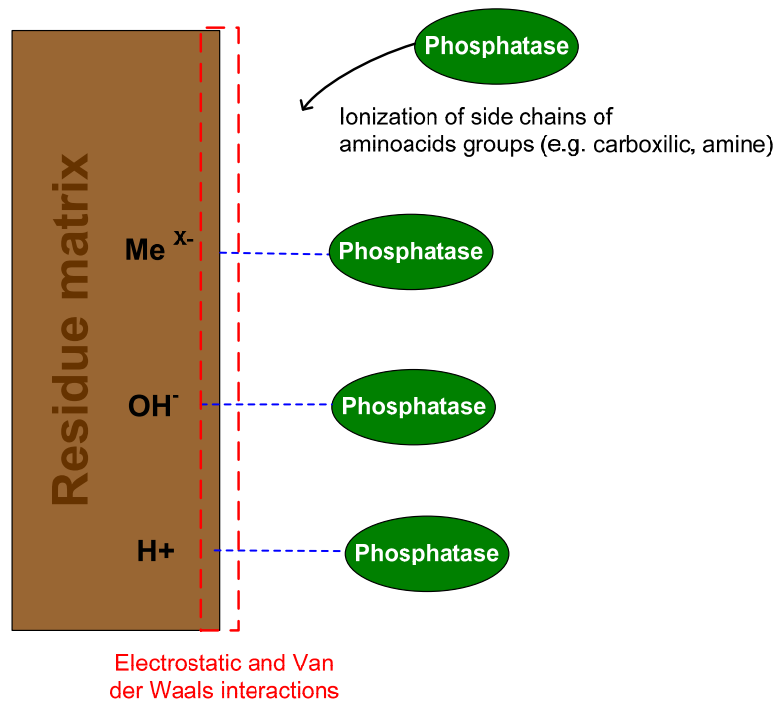
Figure 2.10 Representation of the adsorption of phosphatases to the residues matrix

The intermolecular forces that take place in adsorption are electrostatic interactions or van der Waals interactions. Electrostatic forces are long-range and strong intermolecular forces originating from the overlap of the electrical diffuse double layers of the enzymes and the interacting surface. For the enzyme proteins, the electrical charge originates from the ionization of carboxylic, tyrosyl, imidazole and amine groups of the side chains of amino acids. For mineral surfaces, the electrical charge originates from pH-independent isomorphic substitutions in the crystal lattice or from pH-dependent ionization of surface hydroxyls [82]. The van der Waals interactions are short-range and weaker but they act on all molecules, even if they do not bear electrical charge. Since they are independent of the pH and the ionic strength of the soil solution, the influence of this type of force is more difficult to demonstrate experimentally [82].

Regarding the entropic effect of the enzyme adsorption phenomena, two types of interactions are important: hydrophobic interaction and modifications in the enzyme protein molecular structure. Any perturbation in the hydrophobic interaction of the systems results in an increased disorder in the surrounding water of the protein, which increases the entropy of the systems. On the other hand, the main effect of the chain in the protein structure results from changes in the secondary structure of proteins from ordered arrangements such as helices or sheet to more disorder structures [82].

2.4 State of the art of agricultural residues treatment and phosphorus recovery from agricultural residues

In the first part of this section, the most common manure and digestate management practices (i.e. storage, transport and land application) are presented. In the second part, more specific treatments (i.e. solid-liquid separation, treatments of solids and liquid fractions) are presented with emphasis in phosphorus recovery technologies.

2.4.1 Common residues management practices

Storage, transport and land application are the most widespread management practices for agricultural residues. They are described in more detail as follows:

i. Storage

The residues have to be stored to wait for the most suitable spreading time, depending on the crop growing season. In Europe the storage time is about six months in many countries but can vary between 2 and 12 months depending on the region. Liquid manure or slurry is stored in one of different vessel designs, for example above-ground bolted panel tanks, concrete stores below slatted floor of animal houses, lagoons, lined ponds, etc. [12]. The capital investment for slurry storage vessels are in the range of 30-150 €/m^3, depending on the material and coverage [13]. The facilities for storage require large area availability this results in a high investment cost for the storage facilities, which increase the production costs considerably. Burton and Turner [12] give an approximation of the quantity of liquid manure produced per animal, to estimate the storage volume necessary, as shown in Table 2.13.

Table 2.13 Estimation of the amount of liquid manure produced per animal [12]

Animal	Manure produced per animal over 6 months [m^3]
Dairy cow	9.7
Beef cattle (>2 years)	5.8
Sow plus litter	2.0
Pig (dry ration)	0.8
100 laying hens	2.1
100 broilers	1.1

In many pig farmers lagoon systems are employed. They can be constructed either by a flush or a "pull-plug" system for the transport of the slurry. These effluents normally contain

less than 2% total solids and can be handled by conventional pumps, pipes and irrigation systems [22]. The difference between the storage in tanks and lagoons is that the latest are operated to encourage anaerobic digestion. For this operation to be optimal, the lagoon has to be properly designed. As a result, lagoons can be more expensive than other storage systems [21]. An example of a lagoon design is shown in Figure 2.11.
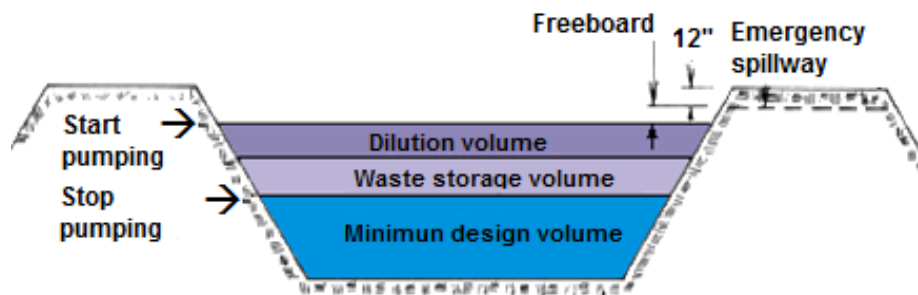
Figure 2.11 Lagoon system for the collection of animal manure [21]

A "minimum volume" without pumping is necessary to provide the system with the anaerobic microorganisms, which will treat the input in the system. In spite of a proper operation, an "over turning" of the lagoon can occur when the temperature decreases. This means that due to difference in density, partially digested manure can go to the top of the lagoon, causing odor problems. To avoid this, multiple lagoons in series can be installed [21].

ii.   Transport and land application

If no cropland is available close to the livestock farms, the residues have to be transported to arable farms where the nutrients can be recycled. The appropriate technique for transport and application depends on different conditions, for example, the structure of the farm, types of crops and soils, available mechanization in the farm, manure composition, etc. [13]. Seven scenarios for manure transport and application are presented in Figure 2.12. In the scenarios 1 and 2, store tanks are located close to the farm and manure is spread by a slurry tank or underground pipelines, respectively. In scenarios 3 to 7, the store tankers are located in the croplands and the fields can be spread by slurry tankers or pipelines. Both, transport with road transporter or pipelines have high costs for the farms.
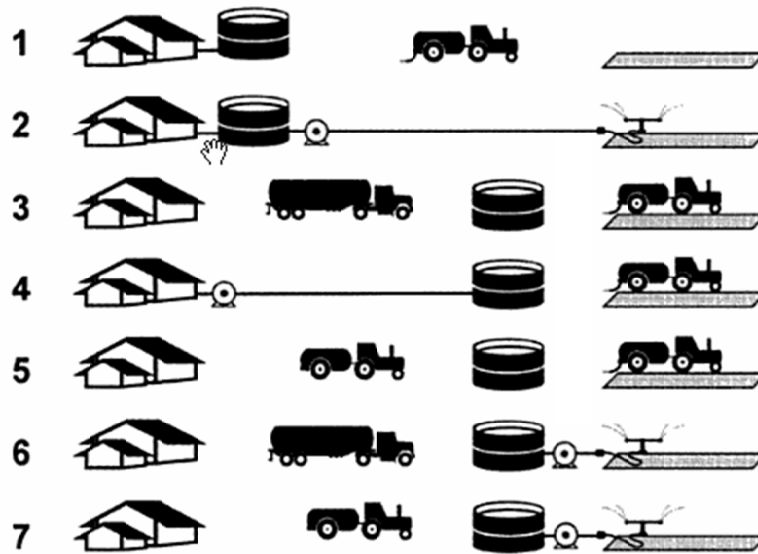
Figure 2.12 Manure transport and application [12]

2.4.2 Treatment technologies

Manure treatment are carried out to address specific management problems or to meet special requirements. For example, solid-liquid separation can used to obtain solid fraction with higher solids content which is easier and more economically to transport. Treatment can also help to sanitize manure and reduce environmental impact, for example by reduction of NH_3 emissions [13].

Foged *et al.* [14] carried out a compilation and analysis of data about manure processing technologies in Europe. In this section, selected data from this study are presented. The authors include information about the quantities of phosphorus treated, but it is not clear if phosphorus is effectively recycled or not.

i.   Separation

One conventional procedure for the treatment of agricultural residues is a solid-liquid separation. This can be carried out mechanically by centrifugation, sedimentation, drainage, pressurized filtration, etc. The separation efficiency of these mechanical separators may vary widely because it is affected by the variable physical and chemical composition of the residues [72]. The purpose of the separation is to separate the residues into two flows: a concentrate (solid fraction) and a diluted fraction (liquid fraction) [14]. The liquid fraction is pumped more easily and the risk of pipe blockage is reduced. Moreover, the application on

fields can be carried out in a more controlled way. The solids removed have a total solid content of more than 80% [13].

The simplest way to separate the solid and liquid fraction is by natural settling or sedimentation. However, this process can be inefficient and very time and space consuming [71, 85]. A faster separation can be obtained using mechanical screening. There is a wide range of mechanical separation available in the market for this purpose. A selection of this is presented in Figure 2.13. F denotes the feed stream, L the screened liquid stream produced and S the solids output. The devices are: I. rotating screen; II. brush-roller; III. vibrating screen; IV. screw press; V. belt press; VI. run-down screen [86].
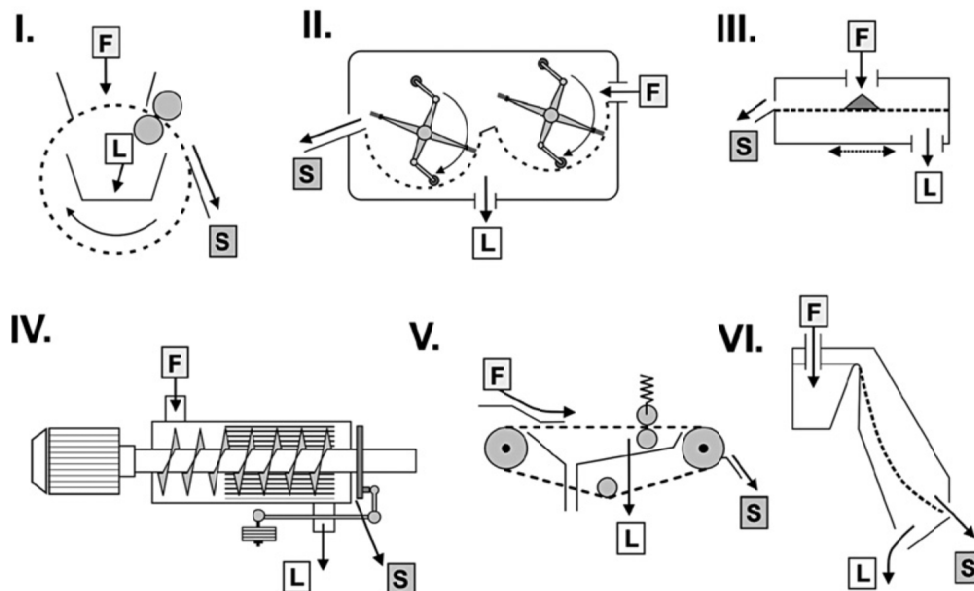
Figure 2.13 Common separation and screening technologies [86]

Depending on the residue characteristics, sometimes mechanical screening is not enough for separation the suspended solids for the liquid phase. The disperse particles can be charged negatively and stay floating due to electrostatic repulsion. In this case, the use of flocculants is necessary for the stabilization of the charged particles. The most common flocculants used are Fe^{3+} and Al^{3+} salts and $Ca(OH)_2$ [11].

Table 2.14 presents information about the separation technologies used in Europe. The processing of about 49 million tonnes of residues is carried out on 11,130 installations. This is equal to 3.1% of the entire livestock manure production in EU[14]. Regarding phosphorus, around 53,000 tonnes are treated annually.

Table 2.14 Residues treated in Europe by separation technologies [14]

Separation technology	Raw manure* [10^3 tonnes·a^{-1}]	Other residues‡ [10^3 tonnes·a^{-1}]	Phosphorus [10^3 tonnes P·a^{-1}]
Grate	780	0	2,748
Screw pressing	11,549	547	13,280
Sieves	7,205	294	8,771
Filter Pressing	1,287	224	1,244
Centrifuge	3,388	1,194	4,893
Air flotation	0	120	n.a
Drum filters	12,434	7	14,771
Natural settling	6,578	756	7,060
Coagulation-Floculation	162	1,719	156
TOTAL	43,383	5,164	52,923

* "Raw manure" means that no previous process/treatment is carried out
‡"Other residues" means processed manure or other organic wastes

ii.   Primary treatments

Among the primary technologies for residues treatment are acidification, liming and temperature and pressure technologies. Residues acidification is carried out with the main purpose of reducing the ammonia and methane emissions from animal manures[87]. Different mineral (e.g. nitric acid, sulfuric acid) or organic minerals (e.g. citric acid, lactic acid) can be used. An annual dosage of 70 kg acid per animal unit is reported. However, for organic acid the consumption can be higher, because the acid is metabolized by microorganism [12].

Another procedure for the treatment of manure is liming. Lime (CaO) is added to animal manure to inactive the pathogen and reduced the risks when handling manure. When mixing lime, the temperatures of the residues rises exothermically until about 70°C, this increased temperature together with high pH values (10-11) reduces the pathogen population [12].

Thermal treatments for the reduction of pathogen from manure include pasteurization and sterilization. In pasteurization, the residues are heated between 55°C and 70°C until a predefined period of time. With sterilization higher temperatures are apply, usually above the water boiling point, over extended periods of time. The main advantage of these thermal methods is that they have a high level of confidence regarding inactivation of pathogens. However, they have high energy costs, handling and maintenance of the equipment is complicated and also have the problem of ammonia and odors emissions [12].

Table 2.15 presents information about the primary technologies used in Europe. The processing of about 7.5 million tonnes of residues is carried out on 668 installations. This is equal to 0.5 % of the entire livestock manure production in EU [14]. Regarding phosphorus, around 5,600 tonnes are treated annually.

Table 2.15 Residues treated in Europe by primary technologies [14]

Primary technology	Raw manure* [10^3 tonnes·a^{-1}]	Other residuesⱡ [10^3 tonnes·a^{-1}]	Phosphorus [10^3 tonnes P·a^{-1}]
Acidification of liquid manures	1,476	1,546	1,377
pH increasing (liming)	100	0	209
Temperature and pressure	502	0	665
Other additives	3,799	50	3,355
TOTAL	5,877	1,596	5,606

* "Raw manure" means that no previous process/treatment is carried out
ⱡ"Other residues" means processed manure or other organic wastes

iii.   Solid fraction treatment

A well-established process for the treatment of the solid residue fractions is composting. This is an aerobic process for stabilization, volume decrease, odor removal and pathogen reduction [12, 85]. The important parameters for composting are oxygen availability, moisture content, temperature, C/N ratio and biodegradable part of the organics. Sufficient oxygen must be available to avoid the production of nuisance odors. On the other hand, if oxygen supply is too high it will decrease the temperature and moisture content of the pile and can affect the performance of the microorganisms. The residues suitable for composting includes solid dung, separated solids, settle sludge, garden green residues, food residues, among others [12, 13]. The quality of the product is affected significantly by the compost technology and management of the process. The most common types of composting systems are: passive aeration, windrow/mechanical agitation, forced aeration/ aerated static pile and in-vessel systems [12].

Drying of agricultural residues is an alternative to reduce surplus volume and weight in a short period of time, significantly decreasing storage requirements and transportation cost. In addition, it helps to stabilize the solid fraction after solid liquid separation resulting in a product that is biologically stable [88]. For the drying of agricultural wastes, there are in the market different types of driers, for example rotary belt, feed-and-turn and fluidized bed dryers [11]. Some limitations of these dryers are inefficient energy use, high emissions of volatile compounds, which cannot be recovered and dust production. Solar drying can also

be used for agricultural residues. For this process, lightweight halls similar to greenhouse constructions are used. The disadvantage of solar drying is that an effective drying can only been achieved during months with enough radiation, which is not the case in most of the European countries. Besides, it is necessary a large superficial area to spread the residues. The drying process is more attractive for residues with dry matter higher than 20%. For residues with high moisture content a first evaporation step can be carried out in a conventional heat exchanger where the liquid mover over heated surfaces. When the residues reached an specific total solids a different mechanical equipment is necessary to provide agitation [12].

The incineration of solid residues is a complicated process with high plant cost. Moreover, the operation of an incineration plant only for agricultural wastes is not economically feasible due to the large content of water [12].

In Table 2.16 the information about the solid processing technologies used in Europe is presented. The processing of about 10.4 million tonnes of residues is carried out on 1,486 installations. This is equal to 0.8 % of the entire livestock manure production in EU [14]. Regarding phosphorus, around 23,000 tonnes are treated annually.

Table 2.16 Residues treated in Europe by solid fraction technologies [14]

Processing technology	Raw manure* [10^3 tonnes·a^{-1}]	Other residuesⱡ [10^3 tonnes·a^{-1}]	Phosphorus [tonnes P·a^{-1}]
Composting or fiber fractions	3459	1803	10341
Vermicomposting	21	5	36
Bio-drying	1,145	73	1,843
Thermal drying	1,395	951	7,344
Pelletizing	280	177	355
Combustion	1,122	4	3,128
TOTAL	7,422	6,379	23,047

* "Raw manure" means that no previous process/treatment is carried out
ⱡ"Other residues" means processed manure or other organic wastes

iv.   Liquid fraction treatment

Among the technologies for the treatment of liquid manure are membrane processes. These are physical processes for the separation of the substrate in treated liquid (filtrate) and a concentrated phase (concentrate). According to the size of the membrane pore, there are different membrane processes, such as reverse osmosis (around 0.0001 micron) and ultrafiltration (0.002-0.1 microns) [11]. An important consideration is that membrane

processes work by cross-flow rather than direct flow as in the case of filter presses for example. Consequently this technology is most applicable to vey dilute effluents [12]. It is reported technologies to treat pig slurry by reverse osmosis. The final permeate was largely free of salts but the cost was high and practical problems such as membrane cleaning remains [12].

Among the advanced oxidation processes to treat liquid agricultural resides is ozonation. This technology can effectively kill microorganisms and for this reason is used for disinfection. The process is economically attractive only if moderate dosages rates are applied. If the organic content is too high the dosage needed to the inactivation of microorganisms would increase its costs [85].

Regarding phosphorus recovery, agricultural residues with high water content or the liquid fractions after solid-liquid separation can be treated using similar technologies to the ones developed for phosphorus recovery from municipal wastewater. There are a number of researchers investigating phosphorus recovery as insoluble salts, mainly as struvite (magnesium ammonium phosphate) and calcium phosphates [15, 16, 89-92]. To precipitate phosphate salts high pH values (between 9 and 10) are required. Thus, chemicals like sodium hydroxide have to be added in the process. In the case of struvite, magnesium compounds like $MgCl_2$ or $Mg(OH)_2$ are added to promote the precipitation. There are different reactor designs for the crystallization of the salts. The most common are fluidized bed reactors. An overview of some of the technologies available for chemical precipitation of phosphorus is shown in Table 2.17.

Table 2.17 Technologies for the recovery of phosphorus liquid residues fractions

Technology	Description
Fraunhofer ICT Process [93]	After solid liquid separation, the liquid manure fraction is passed through an ultrafiltration unit and an inverse osmosis unit. The final step is chemical precipitation of the soluble phosphate as struvite.
DHV Crystalactor® [94]	Fluidized bed reactor developed for wastewater treatment. Phosphate can be recovered as struvite, calcium phosphate, magnesium phosphate and potassium magnesium phosphate. The technology has been used in industrial application.
REM NUT ion Exchange [95]	Process developed to remove phosphate, ammonium and potassium from dilute streams through selective ion exchange followed by struvite precipitation.

In Table 2.18 further information about the liquid processing technologies used in Europe is presented. The processing of about 9.4 million tonnes of residues is carried out on 1,486 installations. This is equal to 0.7 % of the entire livestock manure production in EU [14]. Regarding phosphorus, around 1,300 tonnes are treated annually.

Table 2.18 Residues treated in Europe by liquid fraction technologies [14]

Processing technology	Raw manure* [10^3 tonnes·a^{-1}]	Other residuesł [10^3 tonnes·a^{-1}]	Phosphorus [tonnes P·a^{-1}]
Ultrafiltration	0	55	n.a
Reverse osmosis	0	250	n.a
Concentration by vacuum evaporation	180	1,482	173
Concentration by atmospheric evaporation	0	584	n.a
Ammonia stripping and absorption	0	32	n.a
Electro-oxidation	1	0	n.a
Ozonizing	838	0	n.a
Anaerobic digestion (aeration)	850	357	792
Nitrification-denitrification (conventional)	3,171	1,118	364
Constructed wetlands	0	485	n.a
TOTAL	7,254	2,149	1,329

* "Raw manure" means that no previous process/treatment is carried out
ł"Other residues" means processed manure or other organic wastes

3 Material and Methods

This section is divided into three main parts:

 I. Characterization of agricultural residues and determination of P fractions

 II. Enzymatic process for the mineralization of organic P compounds

 III. Chemical processes for the release of adsorbed P and dissolution of phosphate minerals

3.1 Characterization of agricultural residues and determination of P fractions

The objective of this section was to characterize different types of agricultural residues in regard to their nutrient content. In the first subsection, the total nutrient content of pig manure, anaerobic digestate and poultry manure from three locations was determined. For the following two sections the investigation focused mainly in pig manure and anaerobic digestate from commercial farms.

3.1.1 Total nutrient determination

Materials

Different types of animal manure and anaerobic digestate were collected from three locations: First, the Research Station for Livestock Farming and Animal Breeding "Unterer Lindenhof" of the University Hohenheim located in Enningen, Baden Württemberg. This research station serves as field laboratory in the areas of animal husbandry, breeding, nutrition and hygiene; veterinary medicine, agricultural engineering, bioenergy, among others [96]. Additionally, samples were collected from two neighboring commercial farms located in the region of Hohenlohe, Baden Württemberg. These farms operate under conventional production practices. The overview of the samples collected is presented in Table 3.1.

The pig slurry samples (Pig-1a and Pig-1b) were collected from manure storage pits below the slatted floor in the pig stables. The animal stock of farm A and farm B are about 700 and 2,000 heads, respectively. In the research farm, the total pig inventory is about 1,000 animal heads [96, 97]. The total capacity of the stable, where the samples were taken, was about 600 animals [96, 97].

The research biogas plant in Unterer Lindenhof is feed with the manure produced in the farm (liquid and solid manure from approximately 300 livestock units) and silage of renewable raw materials [98, 99].

In the laying hens stable the total animal inventory varies during the year between 1,100 to 4,000 animal heads [96, 97]. The production of broiler chicken does not take place regularly during the year. For this reason, the sample (Ch-2) was only collected once.

Table 3.1 Agricultural residues collected

Sample	Description	Location	Number of collections
i. Fattening pig manure			
Pig-1a	Liquid mixture of excrement and urine (slurry)	Commercial farm A	5
Pig-1b	Liquid mixture of excrement and urine (slurry)	Commercial farm B	3
Pig-2	Solid mixture of excrement, urine and rests of bedding material	Research farm	3
ii. Anaerobic digestate			
Dig-1	Substrate to biogas plant: • 35% manure (15% poultry, 50% cow, 35% pig) • 35% vegetables rest • 20% maize- and rye silage • 10% other plant residues (e.g. wine grape, cereals)	Commercial farm A Biogas plant: • 450 kW_{elec} • Mesophilic conditions	3
Dig-2	Substrate to biogas plant: • 36.5% - 62.7% liquid manure • 7.5% - 12.2% solid manure • 11.0% - 24.3% grass silage • 12.7% - 16.7% maize silage • 0.0% - 5.0% cereal grain • 0.0% - 4.0% maize grain	Research farm Biogas plant: • 186 kW_{elec} • Mesophilic conditions	3
iii. Chicken manure			
Ch-1	Solid manure from laying hen	Research farm	2
Ch-2	Solid manure from barn hen	Research farm	1

After collection of the samples, they were kept under refrigeration at 4°C until use.

Analytical methods

The parameters measured were total solids and total nutrient content. For the total nutrient analysis, the samples were digested with aqua regia (DIN EN 13650) or nitric acid (DIN EN ISO 15587-2) to degrade all organic compounds into inorganic. The total solid content and the nutrients were measured according to standard DIN methods (Table 3.2).

Table 3.2 Analytical methods used to characterize the residues samples

Parameter	Method
Total solids	DIN EN 1288
$P-PO_4^{3-}$, K^+, Ca^{2+}, Mg^{2+}	DIN EN ISO 11885
$N-NH_4^+$	DIN 38406-E5
N_{total}	DIN ISO 13878

3.1.2 Sequential and parallel extraction for determination of P fractions

The aim of the second characterization step was to determine the proportion of available P (mainly soluble inorganic phosphates) and refractory P (e.g. organic phosphorus, condensed phosphates, adsorbed phosphorus) forms in agricultural residues. For this purpose, a modification of the Hedley extraction method for soil characterization was carried out [100, 101]. This method was developed for P soil characterization and involves the sequential extraction with increasing strong chemical solutions to quantify pools of P with varying degrees of solubility [41]. According to Hedley *et al*. [100], the sequential extraction with water and a solution of 0.5 M $NaHCO_3$ results in the extraction of the readily soluble P.

The water extractable fraction included soluble inorganic phosphates that are only lightly bounded with the organic matter. The bicarbonate extractable fraction included, besides soluble inorganic P, slightly insoluble calcium phosphates and weakly adsorbed P[41].

Materials

The experiments were carried out using pretreated and fresh residues samples. According to soil science literature, the pretreatment was necessary to assure the homogeneity of the material. It consisted in air drying the samples using an oven (Binder E115, Germany) at maximum 60°C to avoid degradation of organic compounds. Afterwards, samples were milled, sieved to pass 0.5 mm and kept refrigerated under -20°C until use. The pretreatment was performed to the samples listed in Table 3.2, except for Pig-1a and Pig-1b. The drying of these two samples was not practical because of their high water content (>96%).

Since the pretreatment could modify the composition of the samples and have an effect in the solubility of the P compounds, further experiments with fresh samples were carried out. The samples selected for this purpose were Pig-1a, Pig-2, Dig-1 and Dig-2.

i.   Sequential and parallel extraction using pretreated samples

The sequential extraction procedure (Figure 3.1) consisted in mixing 0.5 g of pretreated sample with 50 ml distilled water, to have a final solution with 1% total solids (TS). The solution was mixed in an orbital shaker (IKA, Germany) at 170 rpm and 30°C during 16 hours. After this time the solution was centrifuged for 20 min at 60,500 g (Beckman Coulter, Germany) and filtered using a filter paper with porosity <15 µm. The solid fraction was used for the following extraction step with a solution 0.5 M $NaHCO_3$ and was mixed, centrifuged and filtered under the same conditions as the ones extracted with distilled water. Both liquid fractions were analyzed for inorganic P content. All experiments were carried out in triplicates.
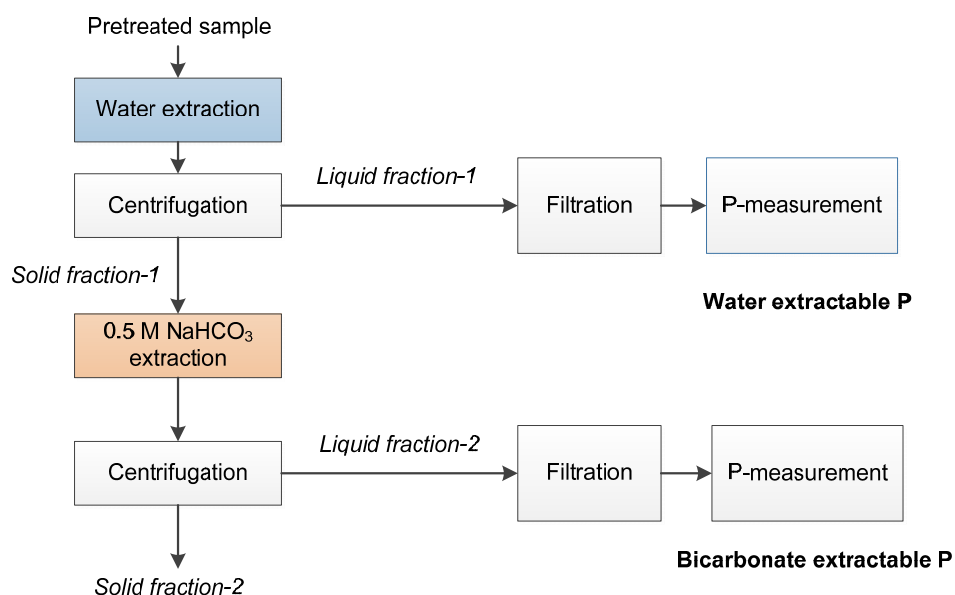
Figure 3.1 Sequential extraction from pretreated samples

The methodology of the parallel extraction (Figure 3.2) was performed under the same conditions than the sequential one, with the difference that the water and 0.5 M $NaHCO_3$ extractions were carried out in a parallel way. The purpose of this experiment was to determine, whether the methodology could be simplified to only one step to reduce the time needed for the procedure.

ii.   One step extraction using fresh samples

The P extraction from the fresh samples was performed in one step using 0.5 M $NaHCO_3$ (Figure 3.2 b). The procedure was similar to the one described for pretreated samples. The

only difference in this case was that the quantity of sample added was calculated based on the total solid content of the fresh sample, in order to have a final solution with 1% TS.
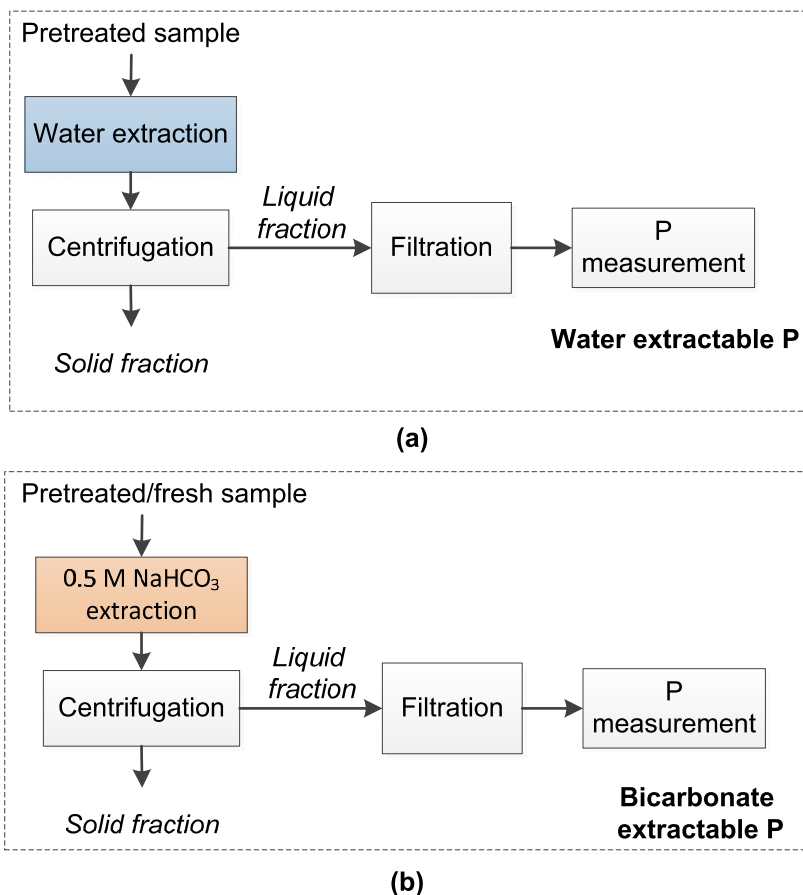
Figure 3.2 Parallel extraction of pretreated and fresh samples
(a) Water extraction (b) Bicarbonate extraction

Analytical methods

Inorganic phosphorus (P-PO$_4^{3-}$) was measured from the liquid fractions by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E). This colorimetric determination was carried out by means of a photometer analyzer (Merck, Germany) or by a flow injection analysis (FIA) device (Lachat Instruments, USA).

3.1.3 Mass balances and element distribution after solid-liquid separation of pig manure and anaerobic digestate

The aim of this section was to determine the mass balances of macronutrients, micronutrients and possible pollutants after solid-liquid separation of the residues. For this detailed characterization, pig manure from commercial farms was selected. The reasons of focusing the investigation on pig manure is that the quantities of pig manure produced in

Europe are considerable, namely about 177 million tonnes per year (Table 2.2). They are only exceed by cattle manure, however since pigs are non-ruminant animals, it was expected that the quantity of refractory P in this residue was higher than in cattle manure.

The macronutrients determination was also carried out for anaerobic digestate for a commercial biogas plant. The treatment of these residues is also of increasing importance due to the growing number of biogas plants in Europe (only in Germany, more than 7,000 biogas plants are already operating) [102].

Materials

Three samples of pig slurry were selected for the detailed characterization. They were collected from commercial farm A (n=1) and commercial farm B (n=2). Since both farms have similar management conditions, the total number of samples (n=3) was considered as one type of sample, referred as Pig-1. After collection, the samples were separated into a liquid and a solid fraction using a laboratory decanter (Lemitec MD 80, Germany). With these samples a mass balance was carried out to determine the macronutrients, micronutrients and possible pollutants balances.

For the anaerobic digestate (Dig-1), original raw digestate, solid fraction and liquid fraction were collected directly in the biogas plant, because a decanter was already available in the farm. For this sample (n=1) only the macronutrients balances were carried out.

Analytical methods

For the elemental analysis, the samples were digested with aqua regia (DIN EN 13650) or nitric acid (DIN EN ISO 15587-2) to degrade all organic compounds into inorganic. Total solid content, nutrients, heavy metals and other possible pollutants were measured according to standard DIN methods (Table 3.3).

Table 3.3 Analytical methods for the characterization the residues

Parameter	Method
Total solids	DIN 38414-S2
Macro and micro nutrients	
P, K, Ca, Mg, S, Na, B, Co, Mn, Mo, Fe	DIN EN ISO 11885
NH_4^+	DIN 38406-E5
N_{total}	DIN ISO 13878
Heavy metals and other possible pollutants	
Pb, Cd, Cr, Cu, Ni, Zn, As, Al	DIN EN ISO 11885
Tl	DIN EN ISO 17294-2
Hg	DIN EN 1483-E12-4

3.2 Enzymatic process for the mineralization of organic P compounds

The aim of this section was to investigate the mineralization of organic P compounds into phosphate using enzymes. In the first part, seven different phosphatases were selected and analyzed regarding its activity. Subsequently, the enzyme selectivity was determined using organic P model compounds and the process parameters defined. Finally, the enzymatic treatment was carried out with real residues in batch and continuous operation.

3.2.1 Enzyme selection and determination of the enzyme activity

Seven different phosphatases (six analytical-grade and one industrial-grade) were selected (Table 3.4), based on literature research (Section 2.3), to determine their effect in hydrolyzing organic P from model compounds and real residues samples

Table 3.4 Description of the enzymes selected

Enzyme name	Short name	EC number	Supplier	Enzyme activity (supplier specification)	
1. Analytical grade					
Wheat phytase	WPhy	EC 3.1.3.26	Sigma Aldrich	0.01-0.04	$U \cdot mg_{solid}^{-1}$
Aspergillus niger phytase	AsPhy	EC 3.2.1.8	ASA Spezial-enzyme	1.315	$U \cdot mg_{solid}^{-1}$
Acid phosphatase from potato	AcPhos	EC 3.1.3.2	Megazyme	85	$U \cdot mg_{protein}^{-1}$
Alkaline phosphatase from *E.coli*	Alkphos-1	EC 3.1.3.1	Megazyme	200	$U \cdot ml^{-1}$
Alkaline phosphatase from *E.coli*	Alkphos-2	EC 3.1.3.1	Sigma Aldrich	30-90	$U \cdot mg_{protein}^{-1}$
Phosphodiesterase from *Crotalus atrox*	Phosdie	EC 3.1.4.1	Sigma Aldrich	≥0.01	$U \cdot mg_{solid}^{-1}$
2. Industrial grade					
Aspergillus niger phytase (Natuphos® 10 000)	Natuphos	EC 3.2.1.8	BASF	10	$U \cdot mg_{solid}^{-1}$

Methodology

The catalytic activity of the enzymes could vary from the values reported by the suppliers due to the natural denaturalization of proteins that occurs with time. Therefore, the activity had to

be controlled on a regular basis. The different suppliers suggest analytical assays under different incubation conditions. Based on this information, a methodology was developed to measure the activity of each type of enzyme, maintaining similar conditions in the assays of the different enzymes. The parameters for all enzyme assays are presented in Table 3.5.

Table 3.5 Incubation conditions for the activity assay of the selected enzymes

Enzyme	Substrate	Buffer	Time and temperature
WPhy AsPhy Natuphos	2 mM phytic acid sodium salt hydrate	10 mM sodium acetate + 1 mM $MgSO_4 \cdot 7H_2O$ (pH 5.0)	30 min 35°C
AcPhos	2 mM p-nitrophenyl phosphate	10 mM sodium acetate + 1 mM $MgSO_4 \cdot 7H_2O$ (pH 5.0)	10 min 37°C
Alkphos-1 Alkphos-2	2 mM p-nitrophenyl phosphate	10 mM Tris HCl + 1 mM $MgSO_4 \cdot 7H_2O$ (pH 9)	10 min 37°C
Phosdie	2mM bis-p-nitrophenyl phosphate	10 mM Tris HCl + 1 mM $MgSO_4 \cdot 7H_2O$ (pH 9)	10 min 37°C

The assay mixture consisted in 20 µl of enzyme stock solution, 100 µl buffer stock solution and 60 µl distilled water. To initiate the reaction 20 µl of the appropriate substrate stock solution was added. The substrate concentration in the incubation mixture was 2mM. Incubation was carried out in a thermocycler (Biometra,Germany) at the selected time and temperature according to Table 3.5.

Analytical methods

The quantity of organic P hydrolyzed by the phytases (WPhy, APhy and Natuphos) was determined by an adaptation of the Heinonen and Lahti method [103]: In a microplate well, 25 µl of the incubation solution were mixed with 200 µl acetone-acid-molybdate solution (AAM solution) and 20 µl of 1M citric acid. Inorganic orthophosphate (PO_4^{3-}) reacted with the molybdate ions to form a bright yellow phosphomolybdate complex, which was measured at 400 nm using a spectrophotometer (Biotek, United States). Besides developing the color for the P determination, the AAM solution was also used as a stop solution for finishing the enzymatic reaction. The activity of the enzymes is expressed in units (U). For the phytases, one unit was defined as the release of 1 µmol of orthophosphate per minute at appropriate incubation conditions.

In the case of the phosphatases (AcPhos, Alkphos-1, Alkphos-2) and diphosphatase (Phosdie), the hydrolysis of p-nitrophenyl phosphate and bis-p-nitrophenyl phosphate,

respectively, results in the release of inorganic phosphate and *p*-nitrophenyl. In a microplate well, 100 µl of the incubation solution was mixed with 100 µl of NaOH 1M to stop the enzymatic reaction. The resulting bright yellow product, *p*-nitrophenyl, was measured at 400 using a spectophotometer (Biotek, United States). For the phosphatases and phosphodiesterase, one unit was defined as the hydrolysis of 1 µmol of substrate (*p*-nitrophenyl phosphate or bis-*p*-nitrophenyl phosphate) per minute.

3.2.2 Enzyme specificity assay and determination of process parameters using model P compounds

The aim of this experiment was to determine the specificity of the different selected enzymes. This means, the range and type of substrates that each enzyme was able to hydrolyze and the extension of this hydrolyzing activity.

Materials

The degree of specificity of each enzyme in Table 3.4 was determined by incubating them with different organic P compounds (Table 3.6). These model compounds represented the organic P types found in organic residues, such as animal manure and anaerobic digestate (Section 2.2.2). The compound tripolyphosphate is not organic but it is an important refractory type of P found in nature, which can also be hydrolyzed by enzymes.

  i.   **Assay with individual model compounds and analytical enzymes**

Methodology

A solution of 1 mmol $P_{total} \cdot l^{-1}$ of each of the model compounds was incubated with each enzyme. The exception was the RNA solution, which had a P concentration of 0.2 mmol $P_{total} \cdot l^{-1}$ because of its low water solubility. The enzyme-substrate ratio used was 20 $U \cdot mmol\ P_{total}^{-1}$.

The assay consisted in mixing 20 µl of enzyme stock solution, 100 µl of appropriate buffer (as shown in Table 3.5) and 60 µl distilled water. To start the reaction 20 µl of the model compound stock solution was added. This mixture was incubated at 37°C during 1, 6 and 16 hours. After this time, the released inorganic P was measured by the Heinonen and Lahti method [103]. Two types of blanks were used in these experiments. The first type of blank (blank-1) consisted in a sample with enzyme but without substrate (model compound). With this value it was determined whether the enzymes contained P traces that could increase the P concentration in the solutions. The second type of blank (blank-2) was a sample with substrate but without enzyme. This was used to determine whether mineralization of the

organic P compounds occurred due to the incubation conditions (i.e. temperature, pH value). The experiments were carried out in triplicates and the results are shown as the average with standard deviations. The values presented were corrected with both blanks; this means if P was present in the blanks, this was subtracted from the P released in the samples.

Table 3.6 Model compounds used in the enzyme specificity assay [40, 104]

Model compound	Functional group	Type of bond	Structure
Myo-Inositol hexakisphosphate sodium salt $C_6H_{18}O_{24}P_6 \cdot xNa^+ \cdot yH_2O$	Phosphate monoester	Refractory C-O-P	
α-D-Glucose-1-phosphate disodium salt $C_6H_{11}O_9PNa_2 \cdot xH_2O$	Phosphate monoester	Labile C-O-P	
D-Glucose 6-phosphate sodium salt $C_6H_{12}O_9PNa$	Phosphate monoester	Labile C-O-P	
Adenosine 5′ monophosphate monohydrate (AMP) $C_{10}H_{14}N_5O_7P \cdot H_2O$	Phosphate monoester	C-O-P	
Adenosine 5′-triphosphate (ATP) disodium salt hydrate $C_{10}H_{14}N_5O_{13}P_3Na_2 \cdot xH_2O$	Phosphate monoester Condensed polyphosphate	C-O-P P-O-P	
Ribonucleic acid (RNA) Type IV from torula yeast	Phosphate diester	Labile C-O-P	n/a
2-Aminoethylphosphonic acid $H_2NCH_2CH_2P(O)(OH)_2$	Phosphonate	Refractory C-P	
Pentasodium tripolyphosphate $Na_5P_3O_{10}$	Inorganic condensed polyphosphate	P-O-P	

ii.   Assay with mixture of model compounds and analytical enzymes

The specificity of a mixture 1:1 (respect to units content) of two selected enzymes, wheat phytase (Wphy) and alkaline phosphatase 2 (AlkPhos-2), was determined using a solution with equimolar concentration of all the model compounds listed in Table 3.5. Since these two enzymes had different optimal pH conditions, the assay was carried out at three different pH values: pH 5 (optimal for Wphy), pH 7(intermediate pH) and pH 9 (optimal for AlkPhos). The incubation time was 6 h.

In addition, the Wphy-AlkPhos mixture was tested with a model compound mixture with composition shown in Table 3.7 with the selected pH value of 5. This mixture solution simulated the organic P composition in animal manure.

The methodology followed in this section was analogous to the one described for the individual model compounds and enzymes in Section 3.2.2-i.

Table 3.7 Composition of the substrate mixture

Substrate mixture	$\%_{mol}$ of P_{total}
Inositol phosphate	30
α-D-Glucose 1-phosphate	15
D-Glucose 6- phosphate	15
AMP	10
ATP	10
RNA	15
Tripolyphosphate	5

iii.   Assay with individual and mixture of model compounds and industrial enzymes

Analytical grade enzymes were used in the enzyme specificity assay to assure the repeatability of the method and minimize the variability of the results. However, for a pilot and industrial scale, the use analytical grade enzymes was not economically feasible. For this reason, experiments were carried out using the industrial phytase Natuphos 10 000 from the company BASF. This enzyme is commercialized as a feed additive to increase the P intake from pigs and poultry. The P_i release using this phytase was investigated for the individual model compounds and with the mixture shown in Table 3.7.

The methodology followed in this section was analogous to assay described for the individual model compounds and enzymes in Section 3.2.2-i. However, in this case the enzyme-substrate ratio used was increased to 60 U·mmol P_{total}^{-1} to be able to measure a difference in the P_i concentration after incubation. The samples were incubated 16 h.

3.2.3 Batch enzymatic process using real residues

The objective of these experiments was to determine the effect of analytical-grade and industrial phytase on the P_i release from real substrates. In the first section, pretreated residues samples were used to assure homogeneity and the experiments were carried out in a small scale, this means with total volume of 2 ml. In the second and third section, the enzymatic treatment was carried out with fresh samples and analytical and industrial enzymes, respectively, with a total volume of 50 ml. The last section is an approach to explain the effect of interferences in the enzymatic process.

i.   Enzymatic treatment of real residues using analytical-grade enzymes

Pretreated residues samples Pig-2, Dig-1 and Ch-1 (Table 3.1) were incubated with wheat phytase (WPhy) to determine the effect in the release of inorganic P. The pretreatment consisted in air drying the samples using an oven (Binder E115, Germany) at maximum 60°C to avoid degradation of organic compounds. Afterwards, samples were milled, sieved to pass 0.5 mm and kept refrigerated under -20°C until use. The enzyme-substrate ratio used was 25 $U \cdot mmol \ P_{total}^{-1}$. The assay consisted in mixing 20 mg of pretreated sample in three different solutions: i) Buffer pH 5 (100 mM sodium acetate +10 mM $MgSO_4.7H_2O$) ii) Buffer pH 7 (100 mM Tris HCl + 10 mM $MgSO_4 \cdot 7H_2O$) ii) Distilled water (without pH adjustment). The appropriate quantity of enzyme was added to achieve the desired enzyme-substrate ratio from a WPhy stock solution of 20 $mg \cdot ml^{-1}$. The total volume of the incubation mixture was 2,000 µl.

The assay solutions were incubated in an orbital shaker (IKA, Germany) at 37°C and 170 rpm. After the completion of 16 h of incubation time, the samples were placed immediately in ice to decrease the temperature and stop the enzymatic reaction. An incubation time of 16 h was selected to assure the maximal degradation of all organic P compounds. Subsequently, the samples were centrifuged (Biofuge, Germany) for 5 min at 13,000 rpm and the liquid fraction was used for inorganic P determination.

The inorganic P content was determined by an adaptation of the Murphy and Riley method [105]: In a microplate well, 200 µl of the liquid fraction after incubation were mixed with 10 µl of distilled water and 40 µl of a mixed reagent (acidic solution of ammonium molybdate, ascorbic acid and potassium antimonyl tartrate). The inorganic orthophosphate (PO_4^{3-}) in the sample reacted with the molybdenum ions of the mixed reagent to form a blue colored complex, which concentration was measured at 800 nm using a spectrophotometer (Biotek, United States).

The quantity of P measured in the solutions was compared with the total phosphorus available in the residue sample. The inorganic P (P_i) released is expressed as a mol fraction respect to the total P content (mol P_i released·mol P_{total}^{-1}).

Two types of blanks were used during the experiments. The first type of blank (blank-1) consisted in a sample with enzyme but without real residue substrate. With this value it was determined whether the enzymes contained P traces that could increase the P concentration in the solutions. The second type of blank (blank-2) was a sample with real residues sample but without enzyme. This was used to determine if P, organic or inorganic, were mineralized or released due to the incubation conditions (i.e. temperature, pH value). The values presented were corrected with blank-1; this means if P was present in the enzyme added, this P content was subtracted from the P concentration of the samples. The experiments were carried out in triplicates and the results are shown as the average with standard deviations. When using real residues, the quantity of P_i released due to the incubation conditions is considerable, for this reason, the values of blank-2 are shown in the results.

ii.   Enzymatic treatment of fresh residues using analytical grade enzymes. Parameter variation experiment

A response surface methodology central composite (RSM-CC) was used to statistically investigate the effect of different parameters in the release of inorganic P from fresh pig manure Pig-2. This methodology is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data. It is used to quantify the relationship between the controllable input factors and the obtained responses [106].

The design was carried out with help of the software Design Expert ®. It consisted of 50 experimental runs including center and axial points for building a model equation. The general description of the experiments is shown in Table 3.8. The detailed experimental plan is present in Appendix II.

Table 3.8 Parameters used in the RSM-CC design

Parameters	Unit	Range studied
1. Factors		
Total solids	%	1.0-9.3
Time	h	4-16
Enzyme-substrate ratio	U·(mmol P_{total})$^{-1}$	0-12
2. Response		
P_i released	mol P_i·(mol P_{total})$^{-1}$	0-1

Methodology

Fresh pig manure Pig-2 was mixed with 50 ml distilled water to obtain a mixture with specific total solid content. The mixture was first adjusted to a pH value of 5 with 3 M H_2SO_4 and then incubated with wheat phytase (WPhy) in an orbital shaker (IKA, Germany) at 37°C and 170 rpm. After the incubation time was completed the samples were centrifuged for 20 min at 60,500 x g (Beckman Coulter, Germany) and filtered (<15 μm).

Analytical methods

Inorganic P (P-PO_4^{3-}) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA). Additionally, calcium concentration was measured from selected samples by inductively coupled plasma spectroscopy (DIN EN ISO 11885).

iii.   Enzymatic treatment of fresh residues using industrial enzymes

Materials

Based on the results of the parameter variation experiment (Section 4.2.3 ii), it was decided to carry out the experiments using a manure mixture with 1% TS. For preparing this mixture the solid fraction after solid-liquid separation of Pig-1a was used. The reason of using the solid fraction was that it contained a lower concentration, compared to raw manure, of buffering substances like carbonates and ammonium and other soluble ions such as potassium (Section 4.1.3). In this way, the quantity of acid used for pH adjustment was minimized.

The solid fraction of Pig-1a was mixed with distilled water to obtain 50 g of a mixture with 1% TS. The pH value was adjusted to 5 with 6 M H_2SO_4. The industrial enzymes were added individually in the concentrations shown in Table 3.9.

Table 3.9 Industrial enzyme types and concentration used

Industrial enzyme	Concentration
Fungal phytase (Natuphos ® 10000)	$60 \ U \cdot (mmol \ P_{total})^{-1}$
Fungal phytase + xylanase + glucanase (Natuphos ® 5000 Combi)	$60 \ U_{phytase} \cdot (mmol \ P_{total})^{-1}$
Cellulase (Celluclast ® 1.5 L)	$4 \ ml_{cellulase} \cdot (g_{TS})^{-1}$

Methodology

The samples were incubated in an orbital shaker (IKA, Germany) at 37°C during 16 h. Subsequently, they were centrifuged 20 min at 60,500 x g (Beckman Coulter, Germany) and filtered (<15 μm). Inorganic phosphorus was measured from the liquid fraction.

A further experiment with fungal phytase was carried out by adding inositol phosphate spike in the 1% TS manure mixture. The spike was added in an equal concentration to the total manure P. This means, from the final P_{total} concentration 50% P was from the spike. This procedure was carried out to assure that enough organic phosphorus was available in the sample to be able to measure a difference in the P_i released before and after incubation.

The experiments were carried out in triplicate and the results are presented as the molar fraction respect to the total P of the sample.

The quantity of P_i released only from the inositol phosphate spike was calculated using Equation 3.2.1:

$$P_{i\ released,\ spike} = \frac{P_{i,treated} - P_{no\ enzyme} - P_{no\ spike}}{P_{total\ spike}} \quad \text{(Equation 3.2.1)}$$

Where,

$P_{i,treated}$ P concentration of spiked sample with enzyme

$P_{no\ enzyme}$ P concentration of spiked sample without enzyme (blank)

$P_{no\ spike}$ P concentration of sample with enzyme without spike (release from manure)

$P_{total\ spike}$ Total P concentration from the spike

Analytical methods

Inorganic phosphorus (P-PO_4^{3-}) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA).

iv.   Interferences in the enzymatic treatment by the residue matrix components

The purpose of this experiment was to determine if the released phosphate was adsorbed by the biomass matrix or reacts with other ions (e.g. Mg^{2+}, Ca^{2+}) present in the samples to form insoluble phosphates. If this occurred, an increase in the P_i concentration in the liquid fraction after incubation cannot be measured.

Materials

The solid fraction after solid-liquid separation of pig slurry Pig-1a was mixed with distilled water to obtain 50 g of a solution with 1% total solids. The pH value was adjusted to 5 with 6M H_2SO_4. A stock solution of KH_2PO_4 with a concentration of 100 $g \cdot l^{-1}$ was used for the experiment.

Methodology

Phosphate stock solution was added to the 1% TS manure sample in increasing volumes to obtain the final P concentrations shown in Table 3.10. The samples were mixed in an orbital shaker (IKA, Germany) at 37°C and 170 rpm during 16 h. After this time, they were centrifuged during 20 min at 60,500 x g (Beckman Coulter, Germany) and filtered (<15 µm).The liquid fraction was measured for inorganic P concentration. The experiments were carried out in triplicates.

Table 3.10 Solutions used in the experiment

Added P concentration from stock solution [mg P·l⁻¹]	Final P_{total} concentration in the 50 g sample [mg P·l⁻¹]
0	340
25	365
50	390
100	440
250	590
300	640

Analytical methods

Inorganic P (P-PO_4^{3-}) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA).

3.2.4 Continuous enzymatic process using real residues spiked with inositol phosphate

In this section, the enzymatic process using real residues was performanced under continuous operation conditions. To assure that the organic P content in the sample was high enough to obtain a measurable P release, the sample were spiked with an inositol phosphate model compound.

Experimental Setup

The experimental setup for the enzymatic process is shown in Figure 3.3. The experiment was performed in a double wall 2-L glass reactor. The manure, acid and enzyme solutions were continuously added to the glass reactor using peristaltic pumps (Ismatec, Germany). The temperature in the reactor was maintained constant at 37°C by a water heating unit (Eppendorf, Germany). The pH value was fixed at 5 and monitored by a pH meter (Metler Toledo, Switzerland). The output was collected in a container for sedimentation. Subsequently, it was centrifuged and separated into a liquid and solid fraction. The liquid fraction was used for P_i determination.
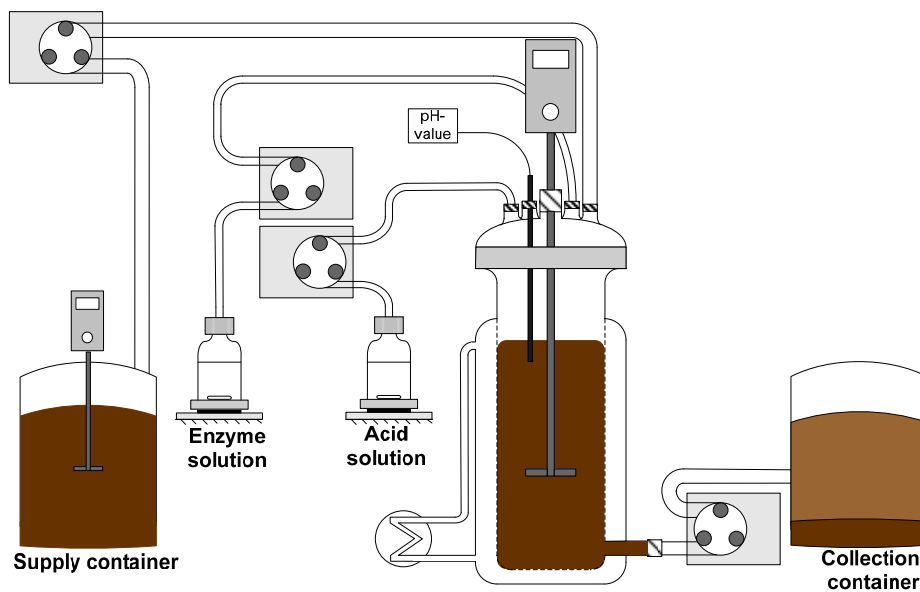
Figure 3.3 Set up of the continuous enzymatic process

Materials

The solid fraction after solid-liquid separation of pig manure (Pig-1a) was mixed with deionized water to obtain a mixture with 1% TS. Inositol phosphate salt was added as spike in an equal concentration to the total manure P. As a result, the final P_{total} concentration of the solution was 625.3 mg·l^{-1} (this means, 50% from the spike).

The pH value in the reactor was kept constant by the continuous addition of 6M H_2SO_4. An enzyme solution was prepared with the industrial phytase Natuphos®10000 and distilled water. This enzyme solution was continuously pumped into the reactor to have a final enzyme-substrate ratio of 60 U·(mmol P_{total})$^{-1}$.

Methodology

The retention time in the reactor was 16 h. The incubation parameters were set at 37°C and pH value of 5. These parameters were selected accordingly to the results of the batch experiments (Section 4.2.3). Samples were collected from the reactor before acidification, before enzyme addition, and after 24 h, 30 h and 40 h incubation time. Subsequently, they were centrifuged during 20 min at 60,500 g (Beckman Coulter, Germany) and filtered (<15 µm). Inorganic P was measured from the liquid fraction.

Analytical methods

Inorganic P (P-PO$_4^{3-}$) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA).

3.3 Chemical processes for the release of adsorbed P and dissolution of phosphate minerals

3.3.1 Acidification process using real residues

The objective of the following experiments was to determine the effect of acidification of the residues in the solubility of P compounds. Moreover, to determine the quantity of acid needed to adjust the pH value and to test the process in continuous operation.

   i.   **Effect of the pH value in the release of phosphate from fresh residues**

Materials

The solid fraction after solid-liquid separation of pig manure Pig-1 was mixed with deionized water to obtain a mixture with 1% TS, which was used for the experiments. To adjust the pH value 6 M H$_2$SO$_4$ was used.

Methodology

The 6 M H$_2$SO$_4$ solution was added stepwise to 50 g of the 1% TS manure mixture until a constant pH value was reached. Then, the sample was mixed for 1 h more at room temperature (21°C) using a magnetic stirrer (VWR, Germany). The pH values investigated were 3, 4, 5, 6 and 7. Afterwards, the samples were centrifuged and filtered. The liquid fraction was used for inorganic P determination. The experiment was carried out in triplicates.

Analytical methods

Inorganic P ($P-PO_4^{3-}$) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA). The pH value was determined by means of a pH meter (Metler Toledo, Switzerland).

ii.   Quantification of the acid consumption

The objective of this experiment was to determine the acid neutralizing capacity of pig manure samples to be able to predict the acid consumption of the process.

Materials

The solid fraction after solid-liquid separation of pig manure Pig-1 was mixed with deionized water to obtain a solution with 1%, 2% or 5% TS.

To adjust the pH value 0.1 M HCl or 6 M H_2SO_4 was used.

Methodology: Determination of the acid neutralizing capacity to pH 4.3 (Ks$_{4.3}$)

The methodology for the determination of the acid neutralizing capacity was based on the norm DIN 38409-7, which is used for the analysis of water, wastewaters and sludge. It consisted in the acidification of 100 ml sample by 0.1 M HCl until a constant pH value of 4.3 was reached. The endpoint at pH 4.3 is determined by the equilibrium system of carbonate acid in water. At this pH value, only dissolved carbon dioxide is present.

The neutralizing capacity is then calculated from the acid consumption by Equation 3.2.

$$K_{S4.3} = \frac{c(HCl) \cdot V_1 \cdot 1000}{V_2} \quad \text{(Equation 3.2)}$$

Where,

c(HCl)   Hydrochloric acid concentration [M]
V1   Hydrochloric acid volume in to modify the pH to 4.3 [ml]
V2   Sample volume [ml]

The Ks$_{4.3}$ was calculated for manure samples with increasing total solids TS content of 1%, 2% and 5%. The experiments were carried out in triplicates.

Methodology: Determination of the acid neutralizing capacity to pH 5

The methodology followed was similar than the procedure for $Ks_{4.3}$ with the difference than the pH was adjusted to pH 5 using 6 M H_2SO_4. This was decided because in other sections of this study, it was determined that pH 5 was the optimal for the Prelease and for the phytase activity. Moreover, the addition of chloride ions in the process is not desired due to environmental concerns. In contrast, sulfuric acid was preferred because the sulfur ions have no negative effect for plants and because sulfur is a plant nutrient.

The neutralizing capacity to pH 5 was calculated for manure samples with increasing total solids TS content of 1%, 2% and 5%. The experiments were carried out in triplicates.

Analytical methods

The pH value was determined by means of a pH meter (Metler Toledo, Switzerland).

iii.   Continuous operation acidification process and phosphate salts precipitation

In this section the acidification process of the residues was carried out in continuous operation. After this process, the acidified liquid fraction containing higher concentration of nutrients was used to precipitate the phosphate salts by increasing the pH value to 9.

Experimental Setup

For the experiments a double wall 2-L glass reactor was used (Figure 3.4). The manure sample and acid solution were continuously added to the glass reactor using peristaltic pumps (Ismatec, Germany). The temperature in the reactor was maintained constant by a water heating unit (Eppendorf, Germany). The pH was fixed at 5 and monitored by a pH meter (Metler Toledo, Switzerland).
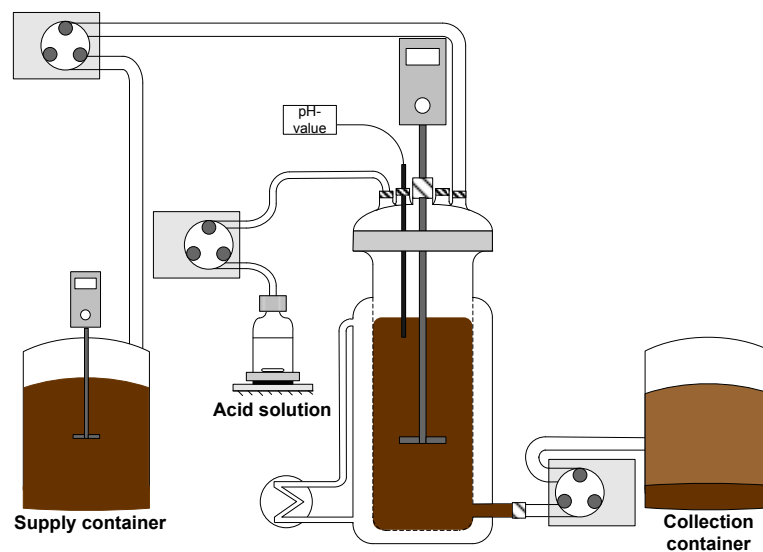
Figure 3.4 Experimental setup for the continuous operation process

Materials

The solid fraction after solid-liquid separation of Pig-1 was mixed with deionized water to obtain a mixture with 1% TS. The pH value of the sample in the reactor was kept constant to 5by the continuous addition of 6 M H_2SO_4. The output was collected in a container for sedimentation. Subsequently, it was centrifuged and separated into a liquid and solid fraction. For the precipitation step, a solution of NaOH 8 M was used for pH adjustment.

Methodology

Although phosphorus release by acidification occurs immediately, the retention time in the reactor was 1 h to assure homogenization of the sample. The process parameters were set at 21°C and 37°C and pH value of 5. After 4 hours operation time the sample was centrifuged to separate it into a solid and a liquid fraction. From the liquid fraction, an aliquot was filtrated and used for inorganic phosphate, magnesium and calcium analysis.

For the precipitation experiment, 500 g of the liquid fraction was mixed at room temperature (21°C) using a magnetic stirrer (VWR, Germany). A solution of NaOH 8M was added stepwise until a pH value of 9.4 was reached. The precipitated salts were separated by decanting and dried at room temperature during 48 h under an extraction fume.

 Analytical methods

In the acidified liquid fraction, inorganic P (P-PO_4^{3-}) was measured using a flow injection analyzer (Lachat Instruments, USA). For the determination of calcium and magnesium concentrations inductively coupled plasma spectroscopy (DIN EN ISO 11885) was used. The pH value was measured by means of a pH meter (Metler Toledo, Germany).

A sample of the precipitated salt was digested with aqua regia (DIN EN 13650) to complete dissolution. Nutrients and possible pollutants were determined with the same methods used for the residues characterization (Table 3.3).

3.3.2 Carbonate addition process using real residues

The objective of this section was to investigate the effect of carbonate ions in the dissolution of P ions from biomass matrix. Carbonate acts as a competitive ion in the adsorption sites of the biomass and also reacts with calcium to form insoluble calcium carbonate. In this way, the phosphate ions are solubilized in the liquid fraction.

Materials and setup

The solid fraction after solid-liquid separation of Pig-1 was mixed with distilled water to obtain a sample with 1%TS. As a carbonate source, $NaHCO_3$ analytical grade was used. The pH value was adjusted with 1M NaOH.

Methodology

The experiments were conducted in a 100 ml Erlenmeyer flask containing 50 g of the manure sample. The appropriate amount of $NaHCO_3$ was added based on the Ca^{2+} molar concentration, this parameter is referred as CO_3^{2-}/Ca^{2+} ratio. After the addition of $NaHCO_3$, the pH of the sample was adjusted using 1 M NaOH. The samples were stirred at constant temperature in an orbital incubator (IKA, Germany) and covered with parafilm to minimize atmospheric CO_2 entering the solution. After the mixing period, the samples were centrifuged and filtered. The supernatant was analyzed for P, Ca and Mg concentrations. The experimental conditions of the different experiments are shown in Table 3.11.

Table 3.11 Conditions of the experiments with carbonate addition

CO_3^{2-}/Ca^{2+} molar ratio	Time [h]	Temperature [°C]	pH-value
i. Effect of the carbonate ratio and pH value			
0; 60; 110; 160	6	37°C	8.5; 9.2
ii. Effect of time and temperature			
0; 60	1; 3; 6	21°C, 37°C	8.5

In the first part of the experiments, the effect of the carbonate concentration and pH value of the solution was investigated and time and temperature were kept constant. In the second part, the CO_3^{2-}/Ca^{2+} molar ratio and pH value were fixed and the time and temperature varied. For the two sets of experiments blank solutions were prepared with no addition of bicarbonate ions, this means the CO_3^-/Ca^{2+} molar ratio is zero. The experiments were carried out by triplicate.

Analytical methods

Inorganic P ($P-PO_4^{3-}$) was measured from the liquid fraction by the molybdenum blue-ascorbic acid method (Standard Method APHA 4500-P E) using a flow injection analyzer (Lachat Instruments, USA).

Calcium and Magnesium concentrations were determined by inductively coupled plasma spectroscopy (DIN EN ISO 11885).

The pH value was determined by means of a pH meter (Metler Toledo, Switzerland)

3.3.3 Thermodynamic modeling

The interaction of the different species present in the pig manure samples determines the composition and distribution of the ions in the solid and liquid phases. In this section, the species distribution was calculated by solving a set of equations resulting from equilibrium constants and mass balances in the system.

The dissolution and precipitation of the species can be described by the mass-action law as reversible and heterogeneous reactions [107]. For instance, the dissolution of a mineral AB into its components can be described as follow [108]:

$$AB \leftrightarrow A + B$$
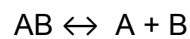
$$K_{sp} = a_A \cdot a_B$$

Where,

a_A, a_B Activity or effective concentration of the ions

K_{sp} Solubility product constant

The state of the saturation of the sample with respect to the different ions, called saturation index (SI), was calculated by comparing the solubility product with the activities calculated from analytically determined concentrations. The latter term is called ion activity product (IAP) [107]. For the modeling, the IAP was calculated by considering ionic strength and complex formation in the simulation:

$$SI = log \frac{IAP}{K_{sp}}$$

Where,

SI   Saturation index

IAP   Ion activity product

K_{sp} Solubility product

Input for the simulation

The macronutrient molar concentration of the 1% TS pig manure sample was the input values for the simulations (Table 3.12). These concentrations were the average value of the different sample analysis carried during the investigation. In the case of the acidification

process, the sulfur content from the sulfuric acid addition was also considered. Additionally, it was assumed that the macronutrients dissolved completely in the liquid fraction at pH 5. Subsequently, the pH value is increased to 9 for the precipitation of the P minerals from the acidified liquid. For the carbonate addition process, the carbonate molar concentration was included for a carbonate-calcium ration of 60. The pH value was fixed at 9.2.

For the input of the simulation it was assumed that initially the macronutrients were complete available as free ions. This means, that in the case of P, the organic P content was not considered.

Table 3.12 Input concentration values for the simulation

Element	Precipitation from acidified liquid fraction	Carbonate addition
	Concentration [mmol·l^{-1}]	
P	18.73	18.73
N-NH$_4$	28.56	28.56
K	4.35	0.43
Ca	13.47	13.47
Mg	15.22	15.22
S	9.37	0.61
CO$_3$$^{2-}$	0	789.87
pH value	9.0	9.2

Methodology

The simulations were carried out by means of the program EES (Engineering Equation Solver). The model consists of a system of linear equations that describe the equilibrium and non-equilibrium conditions of the different ions present in the solution.

The original model was developed in the investigation of Frank [109], which considered the equilibrium of the different ions that are present in pig slurry (Appendix III). The system was complemented with the equilibrium of calcium sulfate and calcite (the most stable form of CaCO$_3$) [110]:

$$CaSO_4 \rightarrow Ca^{2+} + SO_4^{2-} \quad (\log K_{sp} = -5.04)$$

$$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-} \quad (\log K_{sp} = -8.04)$$
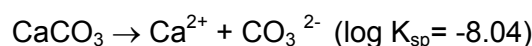
These two equilibrium equations were added to the equation system to considerer the addition of sulfur ions from sulfuric acid in the acidification process and of carbonate ions in the carbonate additions process.

The assessment consisted of two simulations: Non-equilibrium and equilibrium conditions. In the non-equilibrium simulations the saturation index (SI) was determined from activities that were calculated from the measured concentrations by considering ionic strength and complex formation and temperature was kept constant at 25°C. This index indicates if a solution is in equilibrium, undersaturated or supersaturated respect to a solid phase at a given set of conditions [107]. If the value is positive (saturated), the phase may precipitate to an equilibrium condition, subject to nucleation and crystal growth kinetics. Conversely, if the value is negative (undersaturated), the phase can never precipitate because no thermodynamic driving force for phase formation exist.

The simulation under non-equilibrium conditions provided information about the final precipitate composition. For this, the thermodynamic model was solved to equilibrium conditions, by forcing SI=0 and freeing the molar amount of the relevant constituent be a dependent value.

3.4 Statistical Methods

The data from all experiments were statistically evaluated using an analysis of variance test (ANOVA). The basis for every statistical test is to assume the null hypothesis that the average values of two different conditions compared are equal. If the null hypothesis is rejected, it is then concluded that statistically significant differences between the results with a probability <0.05 exist.

4   Results

4.1   Characterization of agricultural residues and determination of P fractions

4.1.1 Total nutrient determination

In this section, the total solids and macronutrient concentration of different types of agricultural residues was determined and compared with the purpose to define the phosphorus and other nutrients recovery potential from these residues.

i.   Pig manure

The total macronutrient content in pig manure varied between 1% and 13% on a dry-matter basis (Figure 4.1). In both pig manure samples, the nutrient with the highest concentration was nitrogen (N). In the pig slurry sample (Pig-1a), ammonium N (N-NH_4^+) comprises 80.7% of the total N. In contrast, in the solid pig manure Pig-2 only 26.8% of the nitrogen was present as N-NH_4^+.

When comparing the concentration of total P with the anions, in Pig-1a the K^+ and Ca^{2+} concentrations were 2.2 and 1.2 higher than P, respectively. Whereas the Mg^{2+} concentration was 1.9 lower. Opposing to this, in Pig-2 the concentrations of total P, K^+, Mg^{2+} and Ca^{2+} were comparable. On the dry matter basis, Pig-1a had a higher P concentration than Pig-2. However, Pig-1a had around 5 times lower solid content. This means, that the actual average P concentrations for the fresh Pig-1a and Pig-2 were 0.11 ± 0.01% and 0.23 ± 0.05%, respectively.

	% TS
Pig-1a	3.6 ±0.5
Pig-2	16.8±5.5
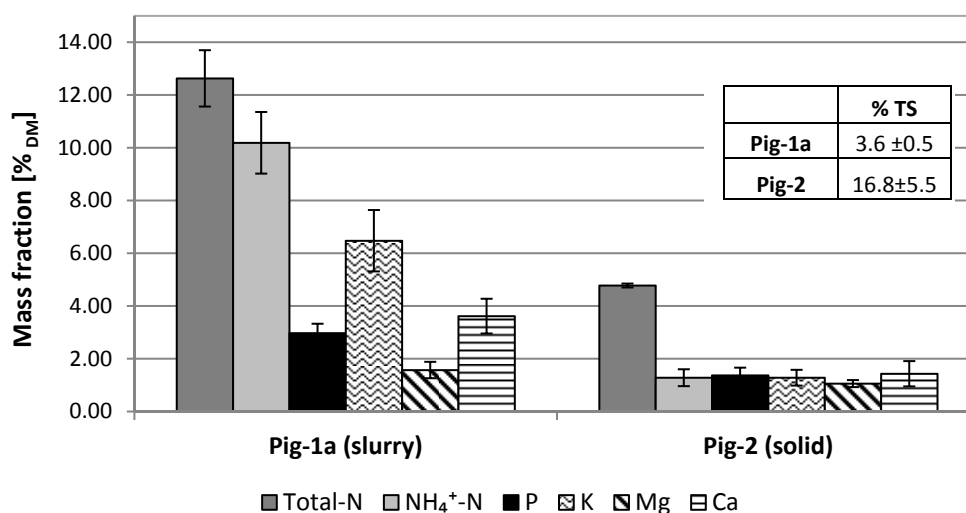
Figure 4.1 Total macronutrient content of pig manure (DM basis)

ii.   Anaerobic digestate

The total solids and macro nutrient content of the digestate samples Dig-1 and Dig-2 were comparable (Figure 4.2). The nutrients present in higher concentrations were total N (4.3% - 5.3%) and K^+ (4.0 - 4.4%). The P content, in both samples ranged from 1.4% to 1.2%. The Ca^{2+} content in the digestate samples was about 2 times higher than that of P, whereas the Mg^{2+} content was up to 1.6 times lower.

	% TS
Dig-1	9.2±0.3
Dig-2	8.7±0.7

Total-N   NH$_4^+$-N   P   K   Mg   Ca
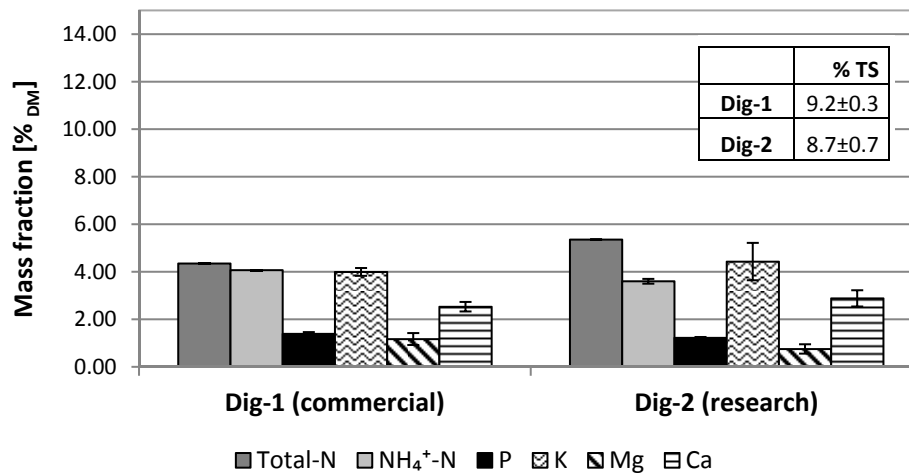
Figure 4.2 Total macronutrient content of anaerobic digestate (DM basis)

iii.   Chicken manure

Chicken manure presented a very high total solid content (Figure 4.3). The samples also contained considerable Mg^{2+} and Ca^{2+} amounts. In the case of Ch-1, the Mg^{2+} and Ca^{2+} content were almost 3 and 2 times higher than P, respectively. For the sample Ch-2 the Mg^{2+} concentrations were more than 6 times higher than P. The N-NH$_4$ content respect to the N$_{total}$ is lower than in the case of pig manure and digestate samples, namely 37.6% for Ch-1 and 14% for Ch-2.

	% TS
Ch-1	27.3±1.0
Ch-2	80.0± n.a

Total-N   NH$_4^+$-N   P   K   Mg   Ca
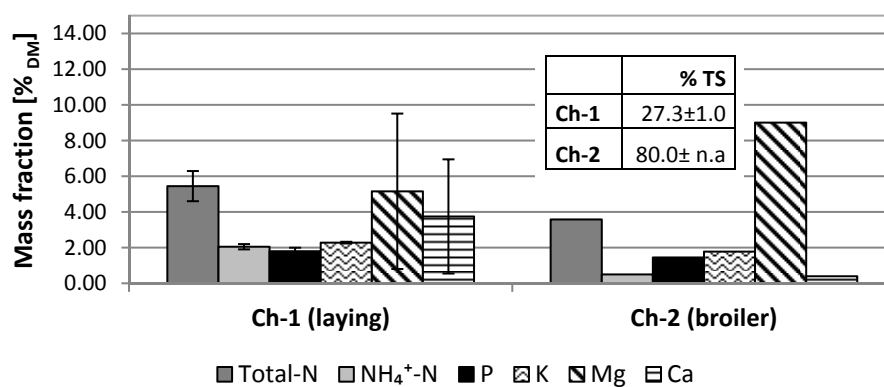
Figure 4.3 Total macronutrient content of chicken manure (DM basis)

4.1.2 Sequential and parallel extraction for determination of P fractions

The readily available P in pig manure and digestate samples was determined by means of extraction procedures. As a first step, sequential and parallel water and 0.5 M $NaHCO_3$ extractions were carried out using pretreated (i.e. dry and milled) samples. Subsequently, a one-step 0.5 M $NaHCO_3$ extraction was carried out using fresh residues samples.

i.   Sequential and parallel extraction using pretreated samples

The available P_i content was defined as the sum of the P released with the water and bicarbonate extractions. The samples with a higher available P_i were Pig-2 and Dig-2, with 67% and 65% respect to P_{total}, respectively (Figure 4.4).

For most of the samples, the higher amount of P_i was extracted during the first step using water, namely, between 67% and 73%. The exception was in sample Dig-2 from which only 35% of P_i was extracted during the water step. This suggests that sample Dig-2 had a higher content of slightly insoluble calcium phosphates or weakly adsorbed P.
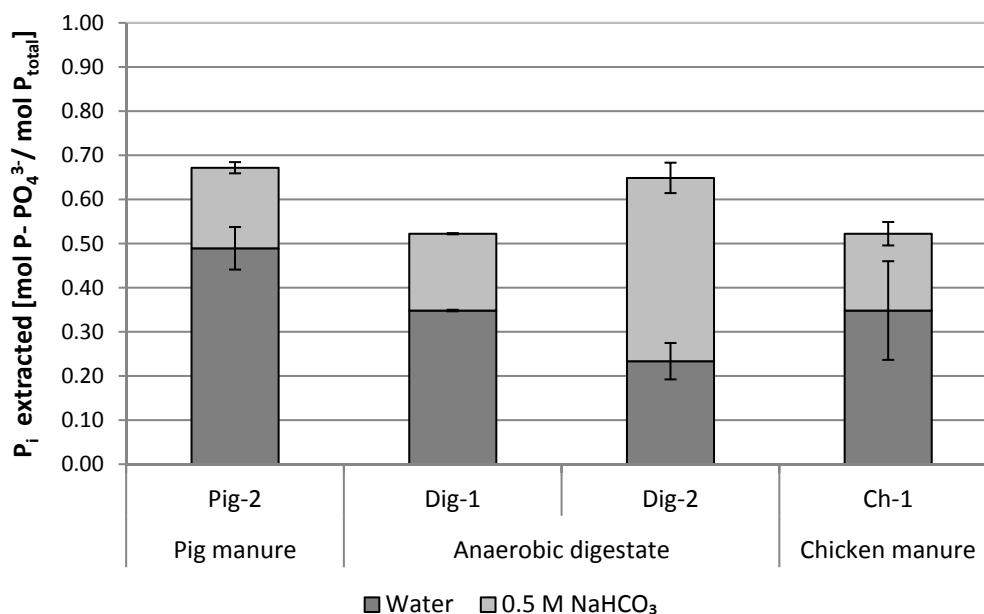
Figure 4.4 Sequential extraction of pretreated samples

The results of the parallel extraction of the pretreated samples (Figure 4.5) showed that, for most of the samples, around 12% more phosphorus could be extracted by the sodium bicarbonate solution than by water. The exception was sample Dig-2, where considerable lower P_i was extracted by water than by sodium bicarbonate (59% lower).

Similarly to the sequential extraction, the samples with a higher available phosphorus concentration after the bicarbonate step were Pig-2 and Dig-2, with an average P extraction of 77% and 84%, respectively. This means, that most of the P was present in inorganic form.
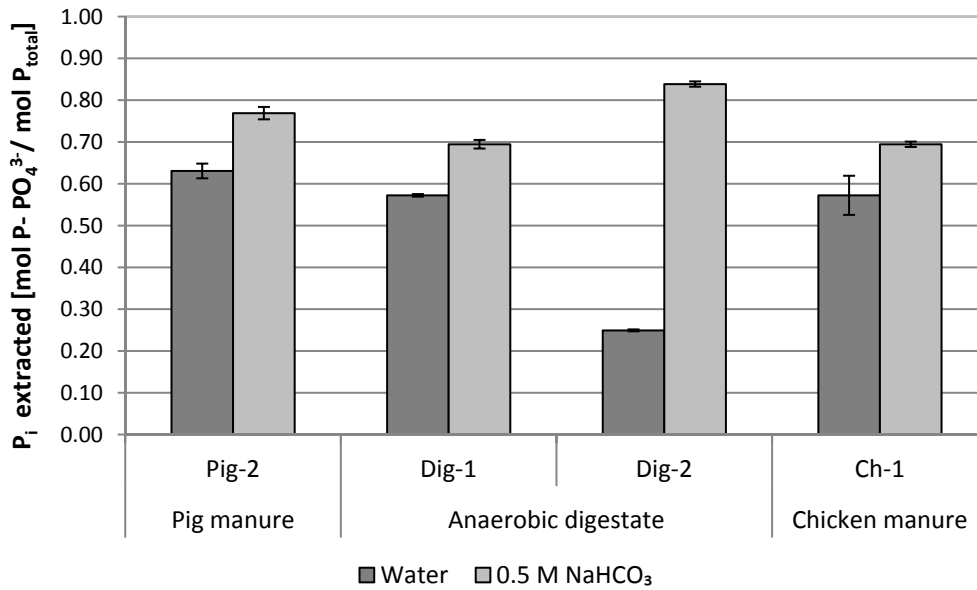
Figure 4.5 Parallel extraction of pretreated samples

From the comparison of the sequential and parallel extractions it was determined that the method could be simplified to a one step extraction process using 0.5 M NaHCO$_3$. This simplify method was used for the analysis of fresh residues samples.

ii.   **One step extraction using fresh samples**

The results of the one step extraction using 0.5 M NaHCO$_3$ (Figure 4.6) shows that for both pig manure samples, Pig-1a and Pig-2, about 20% of the phosphorus could be extracted as P$_i$. In the case of anaerobic digestate the value was about 40% for Dig-1 and Dig-2.
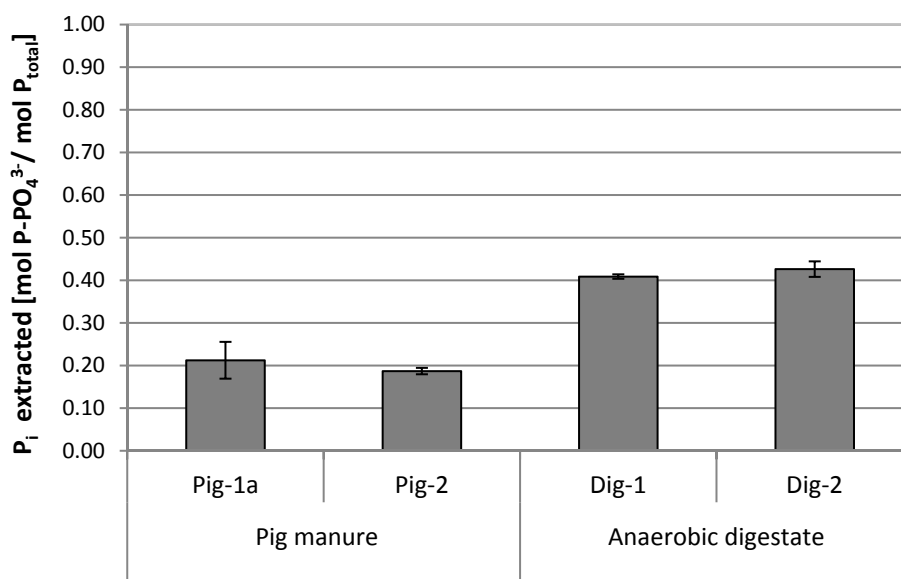
Figure 4.6 Bicarbonate extraction of fresh samples

4.1.3 Mass balances and element distribution after solid-liquid separation of pig manure and anaerobic digestate

The mass balances and element distribution after a mechanical solid-liquid separation of pig manure and anaerobic digestate were determined. This provided further information about the nutrient recovery potential from the separated liquid and solid fraction.

i.   **Pig manure**

The macronutrients mass balance after solid-liquid separation of 1,000 kg of raw pig slurry (Pig-1) is represented in Figure 4.7. The tables with the detailed concentrations and the calculations are shown in Appendix I. The original raw manure had a total solid content of 3.6 ± 0.9%. After decanting the sample, a solid fraction with 19.2 ± 0.4%TS and a liquid fraction with 2.4 ± 0.4 %TS was obtained. Regarding the total mass, 90.7% remained in the liquid fraction and 9.3% in the solid fraction after separation. The elements that were more soluble and therefore mostly remained in the liquid fraction (>60%) were nitrogen, potassium and sulfur.
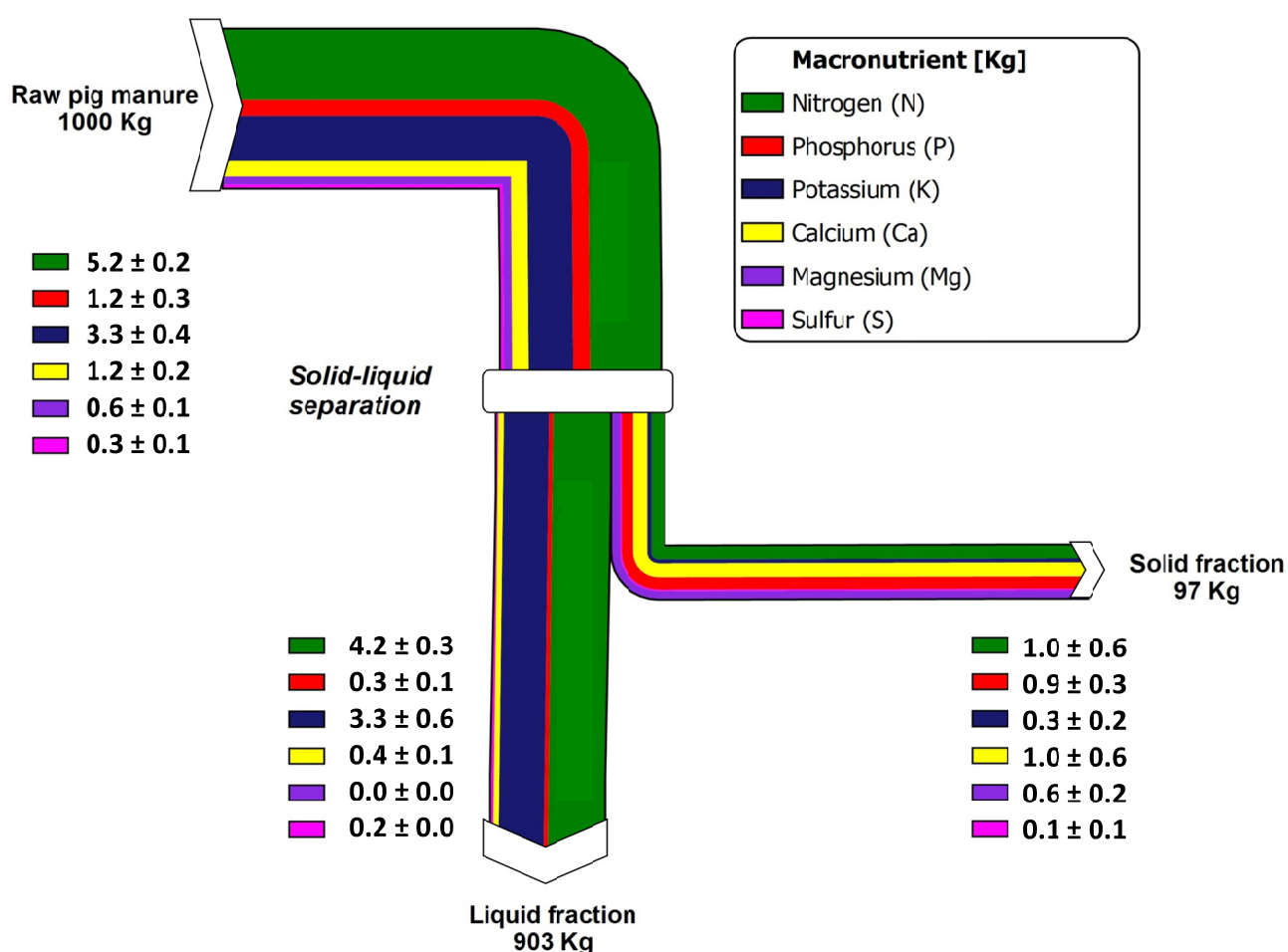
Figure 4.7 Macronutrient mass balance after solid-liquid separation of 1,000 kg pig manure

The nutrients present in higher proportion in the solid faction were phosphorus, calcium and magnesium (Figure 4.8).The graphic shows that the P recovery potential if all P_i were released from this solid fraction into the liquid fraction would be 75%.
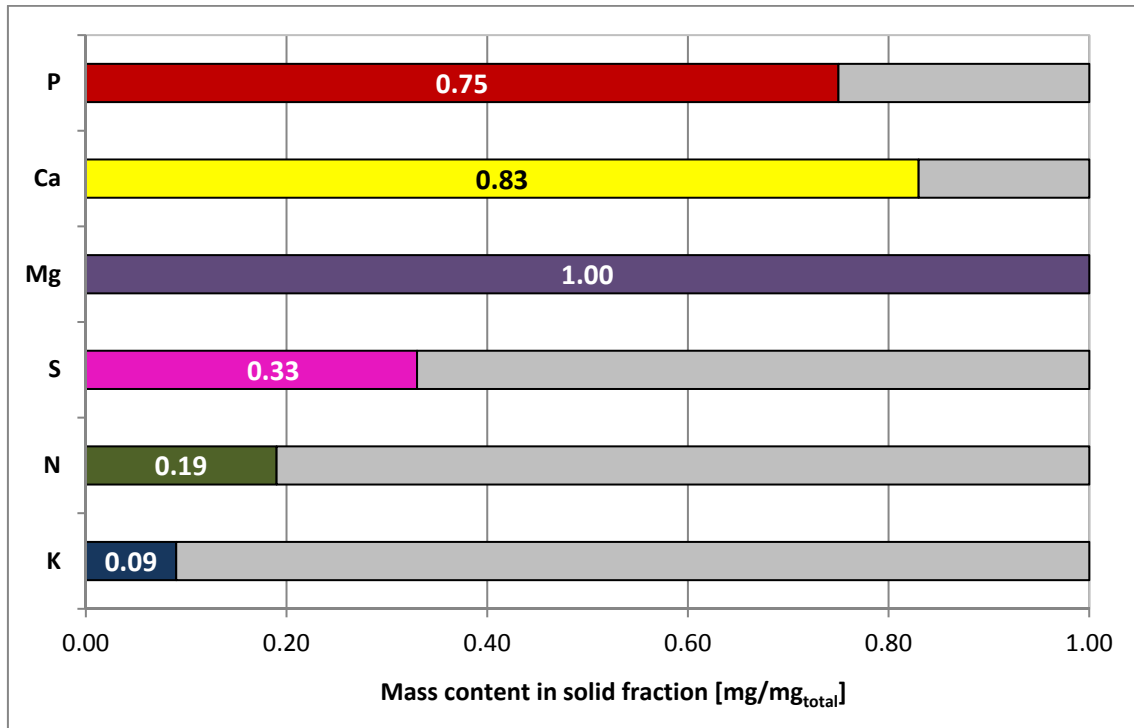
Figure 4.8 Macronutrients content in the solid fraction

The micronutrients balance after solid-liquid separation of 1,000 kg raw manure is present in Figure 4.9. The tables with the concentrations and detailed calculations are in Appendix I.

The results showed that boron remained mainly in the liquid fraction (76%) and manganese in the solid fraction (73%). Zinc and iron were equally distributed in the solid and liquid fraction after separation. Nevertheless, due to the high standard deviation of the micronutrient concentrations measured in the different fractions, it was not possible to make a clear statement about the distribution of the elements.

A mass balance was also carried out for heavy metals and other possible pollutants (Appendix I). For most of the elements the concentration was below the detection limit in the different fractions: Pb(< 5 mg·kg^{-1}); Cd(< 0.5 mg·kg^{-1}); Hg(< 0.05 mg·kg^{-1}); As(< 4 mg·kg^{-1}) and Tl(< 0.2 mg·kg^{-1}).
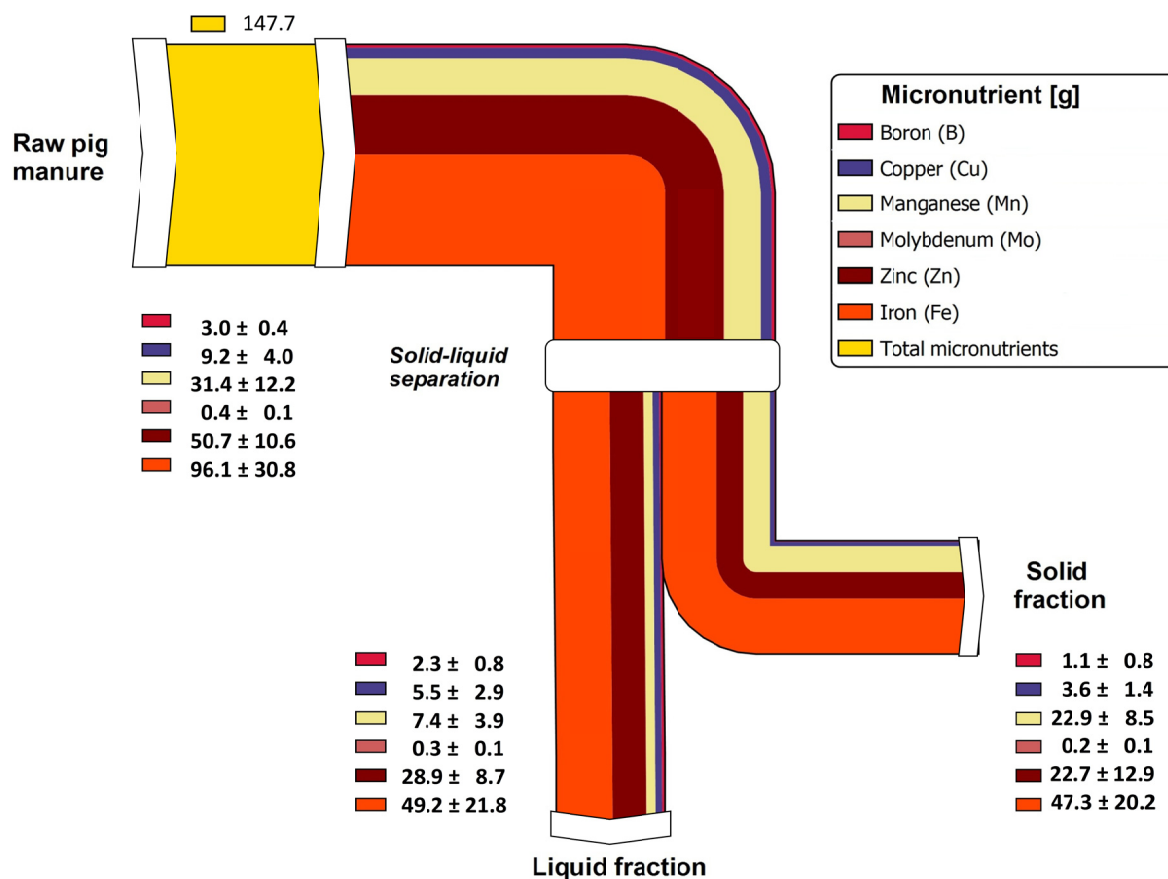
Figure 4.9 Micronutrients mass balance after solid-liquid separation of 1,000 kg pig manure

The mass balances of chromium, nickel and aluminum are present in Table 4.1 . Due to the high standard deviation, no distinctive trend could be determined.

Table 4.1 Pollutant mass balance after solid-liquid separation of 1,000 kg pig slurry manure

Element	Mass [g]		
	Raw sample	Liquid fraction	Solid fraction
Cr	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
Ni	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Al	9.8 ± 1.0	8.3 ± 1.5	8.1 ± 5.0

ii.   **Anaerobic digestate**

The mass balance of the solid-liquid separation of 1000 kg of raw anaerobic digestate (Dig-1) is represented in Figure 4.10. In this case, only the macronutrients were determined. The original raw digestate had a total solid content of 9.4%. After decanting the sample, a solid fraction with 27.9%TS and a liquid fraction with 3.9 %TS was obtained. From the total mass,

77% remains in the liquid fraction and 23% in the solid fraction after decanting. The elements which mainly remained in the liquid fraction (>60%) were nitrogen and potassium.
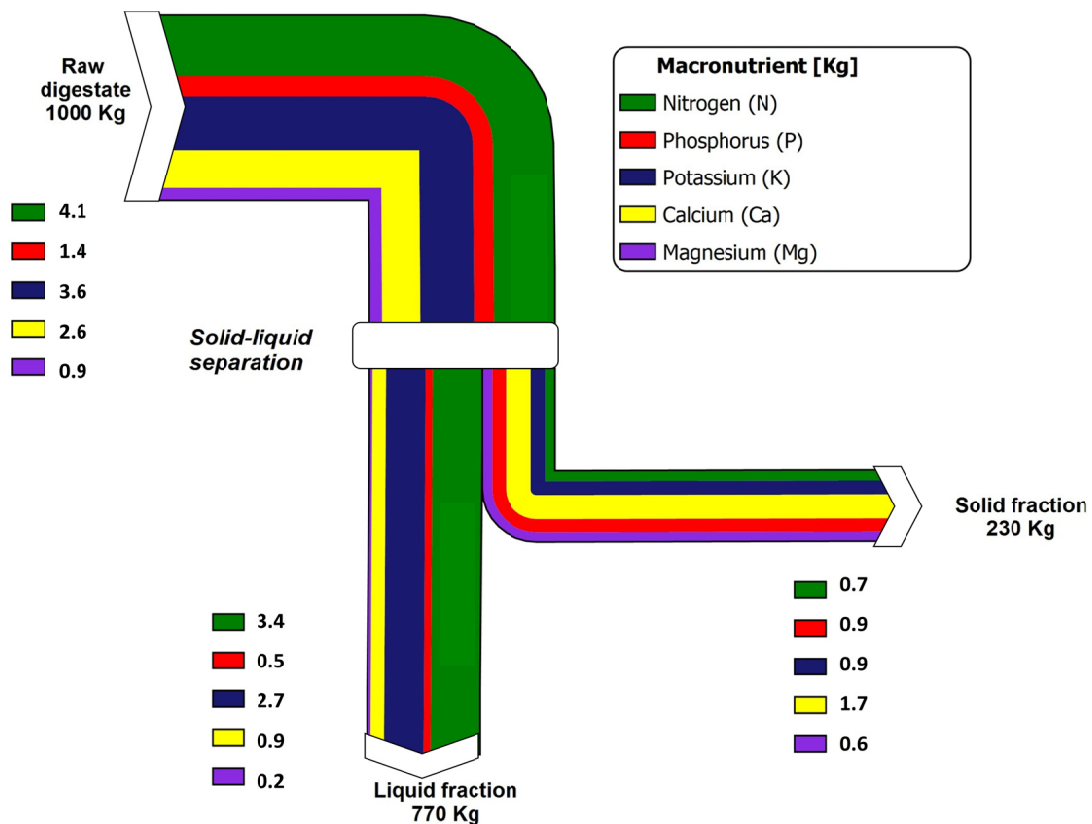
Figure 4.10 Mass balance of anaerobic digestate

Similar to pig manure, P, Ca^{2+} and Mg^{2+} were present in higher concentration in the solid fraction, this suggests that these elements were mainly present as insoluble components in anaerobic digestate. Ammonia nitrogen was equally distributed between the solid and liquid fraction whereas potassium mainly remained in the liquid fraction (Figure 4.11).
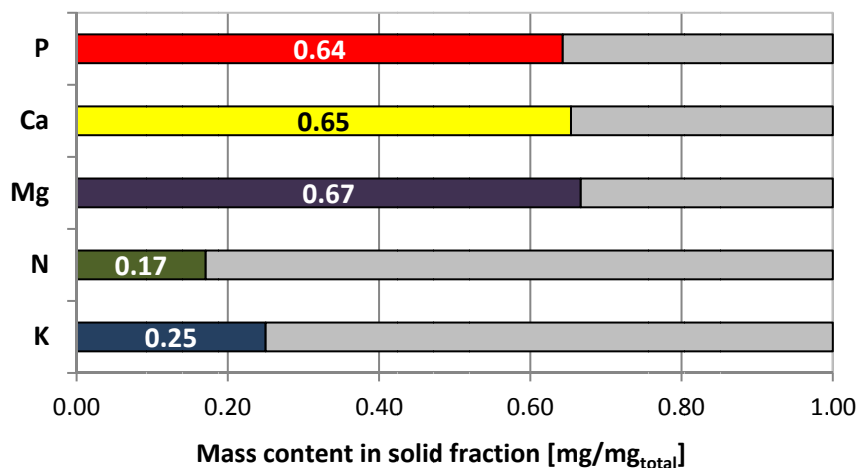
Figure 4.11 Macronutrients distribution after solid-liquid separation of digestate

4.2 Enzymatic process for the mineralization of organic P compounds

In this section the mineralization of organic P compounds by means of phosphatases was investigated. As a first approach, the enzymatic process was carried out using phosphorus model compounds to determine the performance of the enzymes toward the different model compounds and to define the process parameters. Subsequently, the enzymatic treatment was carried out using pig manure residues in batch and continuous operation.

4.2.1 Enzyme selection and determination of the enzyme activity

The activity of the selected enzymes was determined using the developed method described in Section 3.2.1. The results showed that the measured activity was comparable to the one reported by the enzyme suppliers (Table 4.2).The determination of the enzyme activity was important to calculate the quantity of enzyme necessary to achieve the enzyme-substrate ratio in the following parts of the investigation.

Table 4.2 Activity of the different enzymes

Enzyme	Enzyme activity [$U \cdot mg_{solid}^{-1}$]	
	Supplier specification	**Measured**
WPhy	0.01-0.04	0.05
AsPhy	1.32	1.13
AcPhos	85.00*	17.67
Alkphos-1	200.00‡	165.30 ‡
Alkphos-2	30-90*	29.00 ‡
Phosdie	≥0.01	0.068
Natuphos	10.00	10.00

Enzyme activity in: (*) $U \cdot mg_{protein}^{-1}$, (‡) $U \cdot ml^{-1}$

4.2.2 Enzyme specificity assay and determination of process parameters using model P compounds

i.   Assay with individual model compound and analytical enzyme

The enzyme specificity assay was carried out for the different enzymes using all model compounds (Figure 4.12). Regarding the model compound 2-aminoethylphosphonic, no P

released was measured; this means this compound could not be mineralized by any of the enzymes. In the case of the phosphate monoesters, the enzymes with higher activity were wheat phytase and the alkaline phosphatases. Inositol phosphate has a very stable bond, therefore only 60% of the P was mineralized by the wheat phytase and around 30% by the fungal phytase after 16 h incubation.

Tripolyphosphate is an inorganic condensed compound; however it was hydrolyzed up to 87% by the wheat phytase and 52% by the alkaline phosphatase-2. This result confirmed that condensed polyphosphates can be hydrolyzed enzymatically. In the case of the AMP, phosphodiesterase and alkaline phosphatase hydrolyzed more than 70% of the organic P. For ATP, the wheat phytase has a higher activity, with a P recovery of 82%. The P_i release from the RNA molecule was higher with phosphodiesterase, up to 84% and with wheat phytase, up to 100%.

The results show that wheat phytase (Wphy) and alkaline phosphatase (Alk-Phos2) present a higher activity over a wider range of P model compounds. By comparing the P_i released from the different model compounds between 6 h and 16 h incubation it could be determined whether the reaction reached its maximum or a longer incubation time was necessary. In the case of Wphy and Alk-Phos2, the reaction with inositol phosphate, α-D-glucose-1-phosphate, AMP and RNA was completed after 6h incubation. For ATP, the reaction was completed with Alk-Phos2 and increased about 20% between 6h and 16 h incubation with Wphy.

In the case of tripolyphosphate, there was a decreased in the P_i released by WPhy between 1h and 6 h incubation. This behavior could only been explained by an error in the analytics. However, when comparing the results between 1 h and 16 h it could be determined that the reaction reached its maximum. The P release by Alk-Phos2 increased around 15% between 6h and 16h.

The results showed that an incubation time of 16 h was sufficient to reach the maximum P release from most of the model P compounds. For this reason, 16h incubation time was selected for the experiments with real residues substrate.
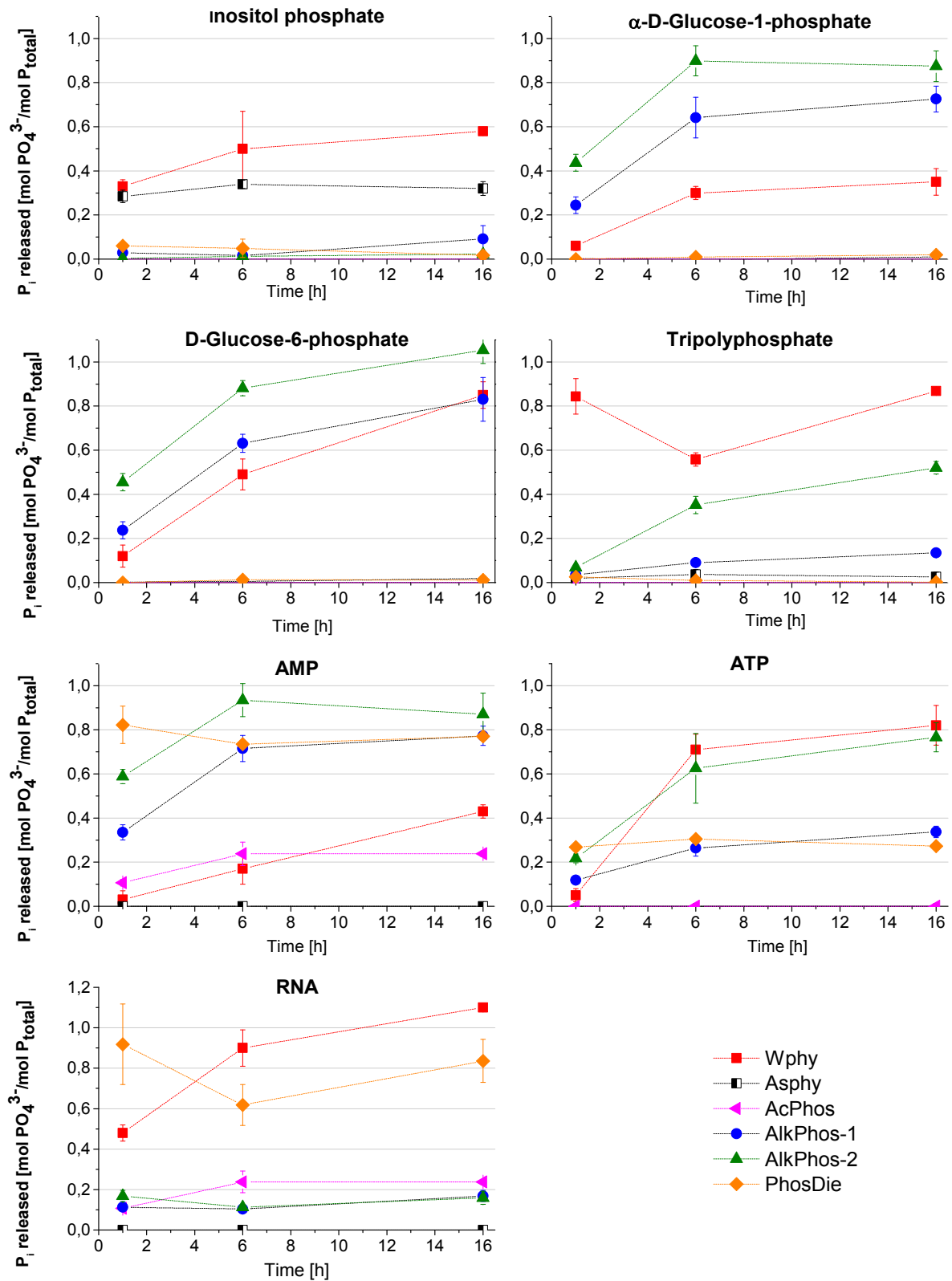
Figure 4.12 Enzyme specificity of analytic enzymes on model compounds

ii.   Assay with mixture of model compounds and analytic enzymes

The results of the pH variation experiment for an equimolar model compound solution are shown in Figure 4.13. The alkaline phosphatase had a constant activity over a wider range of pH values. Regarding wheat phytase, its activity is reduced by 50% when the pH is increased to 9. At the intermediate pH of 7, both enzymes have similar activities than at their optimal pH value.
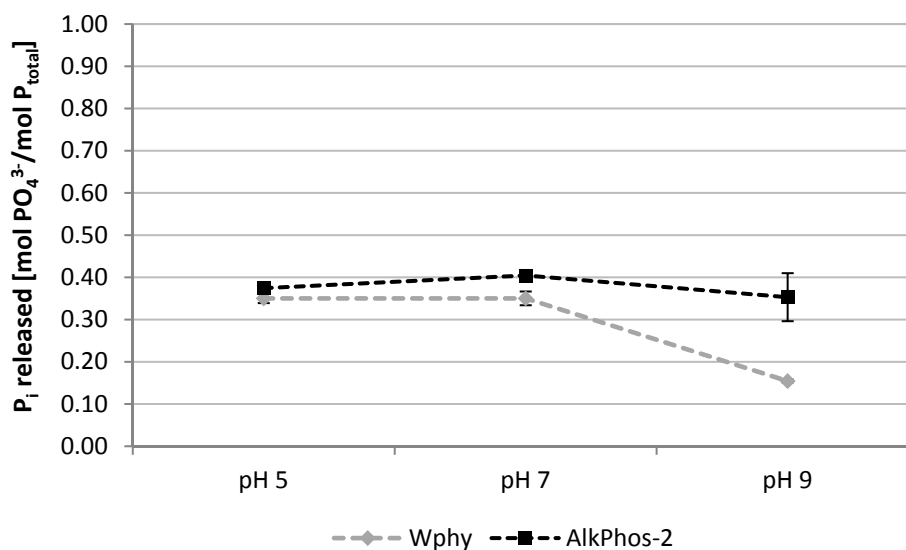
Figure 4.13 Effect of pH variation in P_i released by WPhy and AlkPhos-2

Based on the previous results, the parameters selected for a combination of wheat phytase and alkaline phosphatase were pH 5 and temperature 37°C. This parameter combination was investigated for all the individual phosphorus compounds and a mixture of them. The results are shown in Figure 4.14.

The P release from the model compounds reached its maximum value for inositol phosphate, tripolyphosphate and ATP, this could be determined by comparing the release between 6 h and 16 h incubation time. The P release from D-glucose-6-phosphate, α-D-glucose-1-phosphate and RNA increased 36%, 43% and 46% between 1h and 6 h, respectively. In the case of ATP the P released increased in 25% between 1h and 16 h. The decreased measured after 6 h could be explained by an error in the analytics. For the compounds RNA and tripolyphosphate a higher P release than 100% was measured. This indicated a possible error in the preparation of the solutions, probably due to the low solubility in water of these compounds.
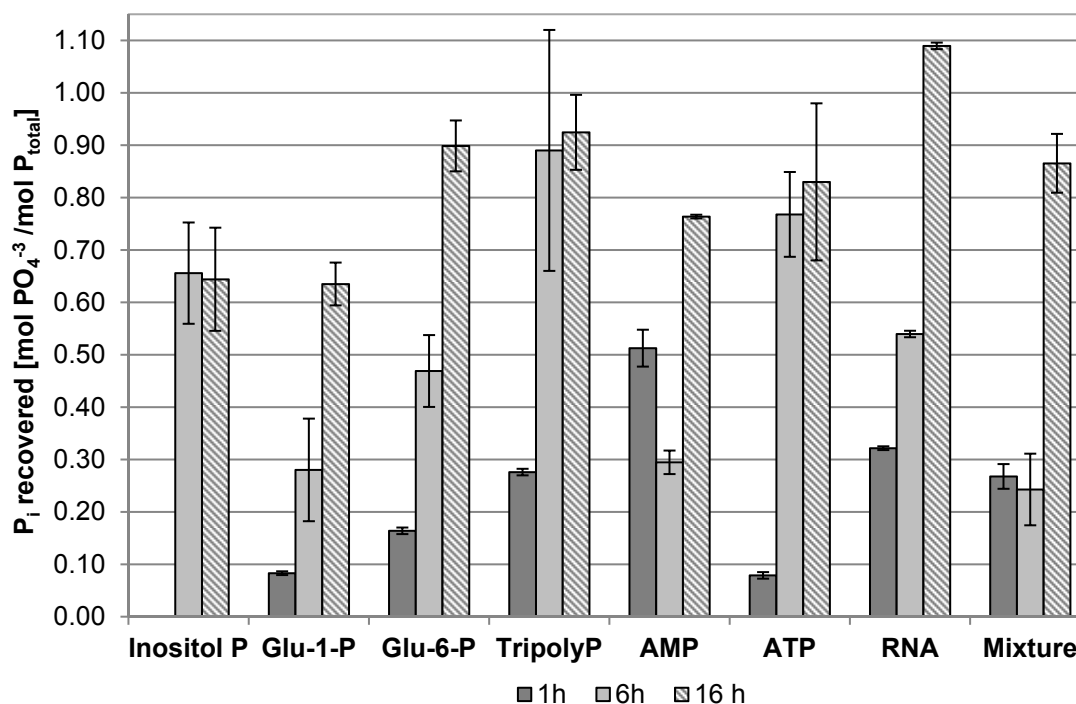
Figure 4.14. P$_i$ released by enzyme mixture from model compounds

iii.   Assay with individual and mixture of model compounds and industrial enzyme

The enzyme specificity assay was carried out using the industrial enzyme Natuphos 10 000 with an incubation time of 16 h. The results (Figure 4.15) showed that the industrial phytase had higher activity towards inositol phosphate and tripolyphosphate (60% and 38% P$_i$ release, respectively) than with the other model compounds. From the rest of individual model compounds and the mixture, less than 27% P$_i$ could be released, which demonstrate a low activity of the industrial enzyme toward these P monoesters and nucleotides.
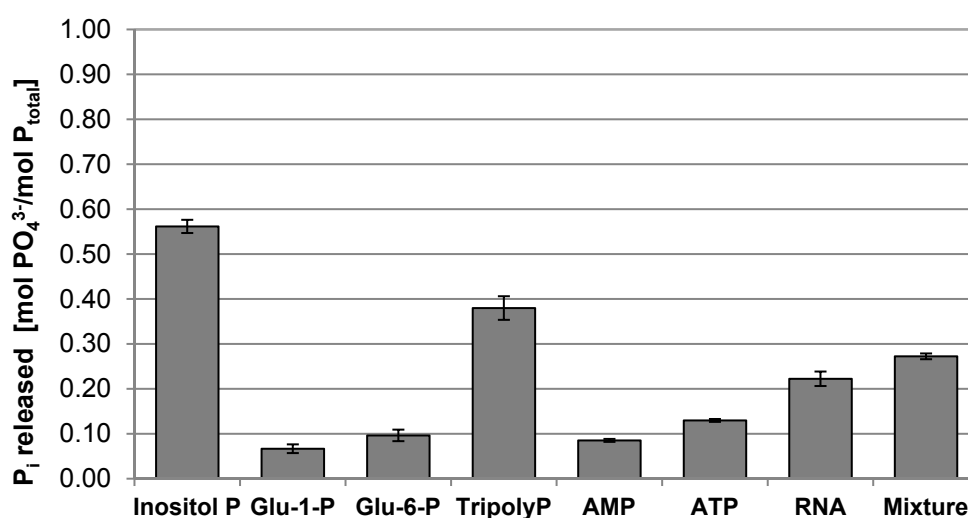
Figure 4.15 P$_i$ released by industrial phytase from model compounds (16 h incubation)

4.2.3 Batch enzymatic process using real residues

In this section, the performance of analytical-grade and industrial phytases was determined in real manure and digestate residues on batch scale. The enzymatic treatment was carried out using first pretreated and subsequently fresh residues samples. In the last section, it was determined whether the released inorganic P could interact with the residues matrix.

i.   Enzymatic treatment of pretreated residues using analytical-grade enzymes

For most of the samples, no significant difference was determined between the samples treated with enzymes and the blanks at different pH values (Figure 4.16). The exception was for samples Pig-2 and Ch-1 with no pH adjustment (no buffer). In the first case, the P_i concentration of the samples decreased in 13.1% when the enzyme was added. Conversely, in sample Ch-2 the P_i concentration increased in 16.5% respect to the blank.

Regarding the effect of the pH value, more than 70% of the P_i was released for all samples at pH 5.
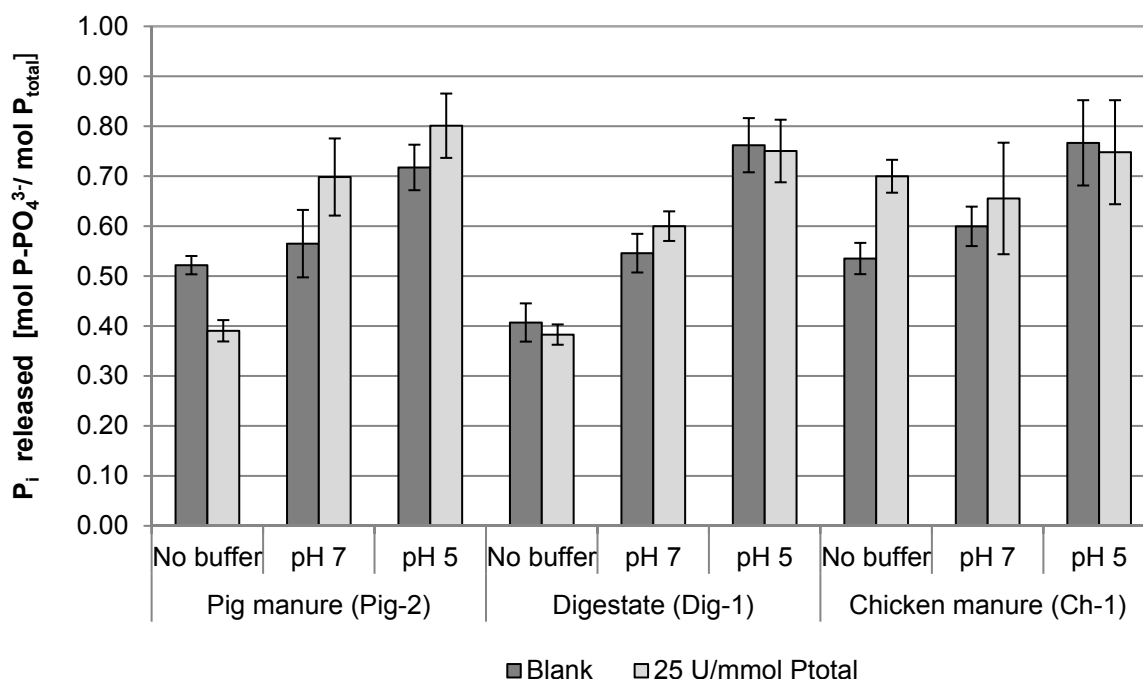
Figure 4.16 P_i released after enzymatic treatment of pretreated samples

ii.   Enzymatic treatment of fresh residues using analytical grade enzymes. Parameter variation experiment

The overview of the results of the parameter variation experiment is presented in Table 4.3. The table shows the mean value of triplicates with their standard deviation, the exception was sample number 4 and 20 that were measured only one because of the high amount of

analytical enzyme needed. With these data a model was generated by the program Design Expert® 8. The obtained p-values ("Prob> F") < 0.05 indicated that the model was significant. According to the analysis generated, the input parameters that had a significant effect on the P_i release were total solid content and enzyme-substrate ratio. In Figure 4.17 the contour diagram showing the effect of these parameters at 16 h incubation time is shown.

Table 4.3 Parameters and response variables from the experiment design

Run	Enzyme-substrate ratio [U·mmol P_{total}^{-1}]	Total solids [%]	Incubation time [h]	P released [mol P_i·mol P_{total}^{-1}]
1-3	0	9.3	16	0.65 ± 0.05
4	12	9.3	16	0.82 ± n.a
5-7	12	1.0	16	0.99 ± 0.03
8-10	0	1.0	16	0.85 ± 0.02
11-13	6	1.7	21.5	0.94 ± 0.00
14-16	6	1.7	1	0.84 ± 0.01
17-19	0	9.3	4	0.64 ± 0.02
20	12	9.3	4	0.76 ± n.a
21-23	0	1.0	4	0.90 ± 0.03
24-26	12	1.0	4	0.98 ± 0.03
27-35	6	1.7	8	0.78 ± 0.08
36-38	16.4	1.7	8	0.97 ± 0.01
39-41	6	0.7	8	0.73 ± 0.02
42-44	0	1.7	8	0.79 ± 0.01
45-47	0	1.0	8	0.88 ± 0.04
48-50	12	1.0	8	0.91 ± 0.10
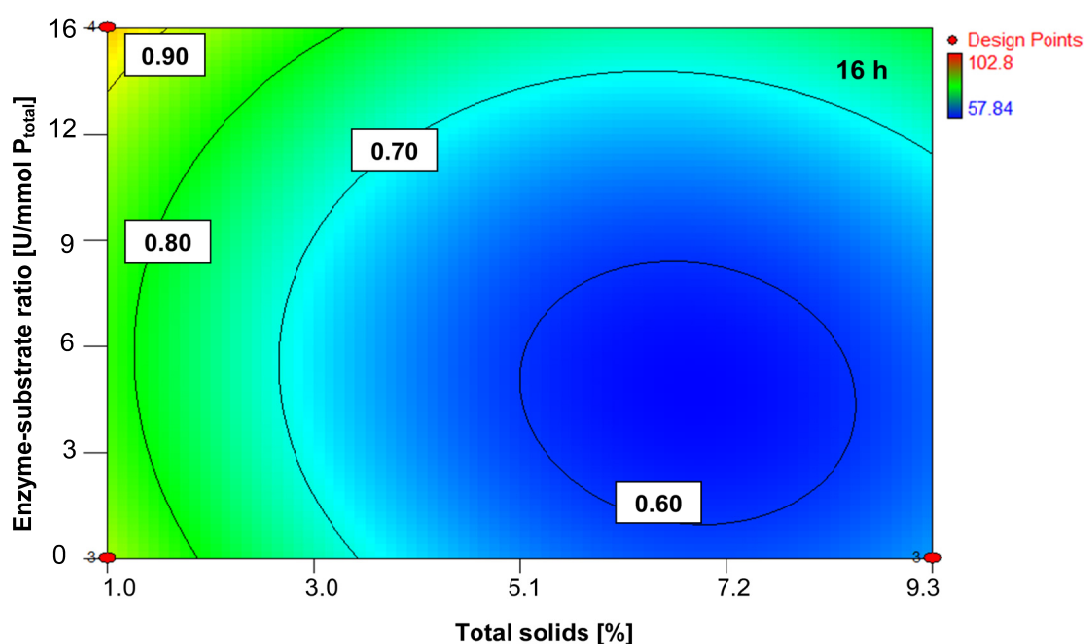
Figure 4.17 Effect of the total solid content and enzyme-substrate ratio on the P_i release [mol P_i·mol P_{total}^{-1}]

The total solids of the sample had a considerable effect on the P_i release. The data show an increase of about 25% in the P_i release when the total solids of the samples decreased from 9.3% to 1%. Regarding the effect of the enzyme, it was only evident when the total solids of the sample were low and the enzyme concentration high. For instance, for the case of 1% TS there was a difference of less than 10% in the P_i release. An important observation is that, due to the pH adjustment to 5, without enzyme addition the P_i released varied from about 60% to 85%.

iii.   Enzymatic treatment of fresh residues using industrial enzymes

The results of the enzymatic treatment of pig manure Pig-1a with industrial enzymes are shown in Table 4.4. No significant difference between the blanks and the samples treated with the different enzymes were determined.

Table 4.4 Enzymatic treatment of pig manure using industrial enzymes at pH 5

Enzyme	P_i released [mol P-PO$_4^{3-}$ · mol P$_{total}^{-1}$]
Phytase	0.73 ± 0.003
Phytase+Xylanase +Glucanase	0.69 ± 0.006
Cellulase	0.68 ± 0.003
Blank (no enzyme)	*0.67 ± 0.02*

A further experiment was carried out by adding an inositol phosphate spike in the manure mixture (Table 4.5). In this case, the P_i released by adding industrial enzyme significantly increased respect to the blank. The proportion of organic phosphorus hydrolyzed from the spike was 24.2%. For this calculation, it was considered, as confirmed in the previous experiment, that the enzymatic treatment had no effect in the manure P and only in the spiked phytic acid.

Table 4.5 Enzymatic treatment of spiked pig manure using industrial phytase at pH5

Sample	P_i released[*ǂ] [mol P-PO$_4^{3-}$ · mol P$_{total}^{-1}$]
Blank (no enzyme)	*0.38 ± 0.003* (11.24 ± 0.07)
60 U·mmol P$_{total}^{-1}$	*0.51 ± 0.01* (15.23 ± 0.33)

*The values in parenthesis are the molar mass of P-PO$_4^{3-}$ in the 50 g sample
ǂ The total P content in 50 g sample is 29.52 mg. From this, 16.49 mg P from the inositol phosphate spike

The effect of the incubation time and enzyme concentration in the released of P_i from the spiked manure solution was also investigated (Table 4.6). Opposite to expected, the P_i released decreased with time between 16 h and 24 h incubation time but only in 3%. Regarding the enzyme-substrate ratio, no significant different was measured between 60 $U \cdot mmolP^{-1}$ and 120 $U \cdot mmolP^{-1}$.

Table 4.6 Effect of incubation time and enzyme concentration

Enzyme-substrate ratio [$U \cdot mmol\ P_{total}^{-1}$]	Incubation time [h]	P_i released [$100\ mol\ P\text{-}PO_4^{3-} \cdot mol\ P_{total}^{-1}$]
60	16	0.51 ± 0.01
60	24	0.48 ± 0.004
120	24	0.51 ± 0.03

iv.   Interferences in the enzymatic treatment by the residue matrix components

The purpose of this experiment was to determine if the released phosphate into solution (e.g. by enzymatic mineralization) was adsorbed by the biomass matrix or precipitated with other ions. For this purpose inorganic P ions from a stock solution were added in increasing concentrations. The P fraction that was bounded (this means, adsorbed or precipitated) was calculated as the difference between the total P in the sample and the P_i measured in the liquid fraction. The relation between P bounded and sample total P is shown in Figure 4.18.
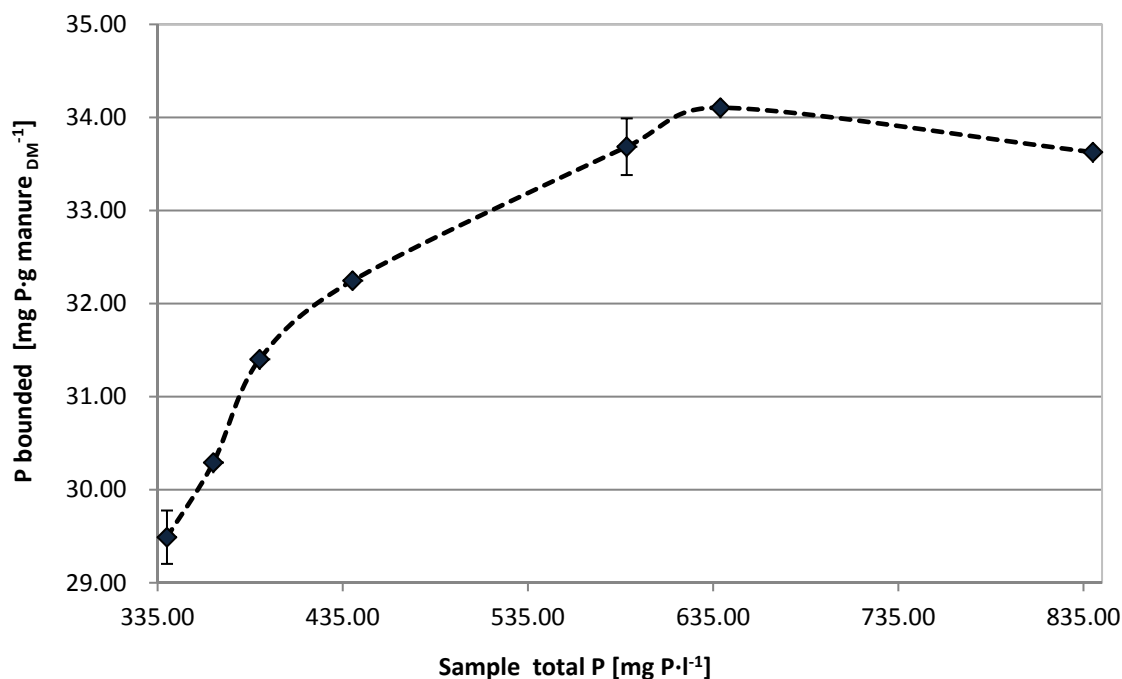
Figure 4.18 Bounded P with increasing sample total P concentration

The results show that the added phosphate ions reacted with the cations in the solution or were adsorbed until a maximum value of 34 mg $P_{bound} \cdot g_{manureDM}^{-1}$. This was a measure of the capacity of the manure sample to bound phosphate ions. After this saturation point was reached, the added phosphate was not adsorbed anymore. This is shown in the graphic as a decrease in the P bounded when the sample P concentration increase.

4.2.4 Continuous enzymatic process using real residues spiked with inositol phosphate

In the continuous enzymatic process with real residues and a spike of inositol phosphate, 68% of P was released to the solution after 48 h of operation, which is three times the retention time (Figure 4.19).
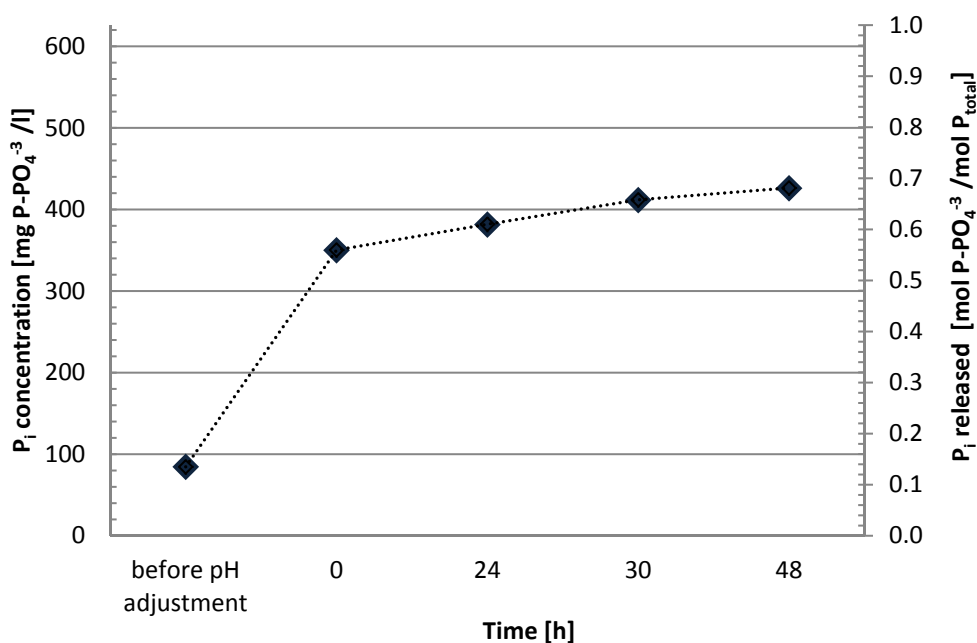
Figure 4.19 Continuous enzymatic process of spiked pig manure

For the calculation of the P mineralization from inositol phosphate, it was considered, based on the results of the batch experiments (Section 4.2.3 iii), that the enzymatic treatment had no effect in P from the manure and only in the inositol phosphate spiked. The results showed that 29% of the spike P was released (Table 4.7).

Table 4.7 Calculation of the P mineralization from the inositol phosphate spiked

	P-PO$_4^{3-}$ concentration [mg·l^{-1}]
Total P in spiked manure sample	625.34
-P only from spiked inositol phosphate	261.70
After 48 h incubation	
Blank (no enzyme)	350.00
60 U·mmol P$_{total}^{-1}$	426.00
P$_i$ released from spiked	***29.04%***

4.3 Chemical processes for the release of adsorbed P and dissolution of phosphate minerals

In this section two chemical process, acidification and bicarbonate addition, were investigated for the release of P that is adsorbed to the pig manure matrix or precipitated as insoluble P minerals. Furthermore, a thermodynamic modeling was carried out for the two chemical processes by solving a set of equations resulting from equilibrium constants and mass balances in the system.

4.3.1 Acidification process using real residues

i. Effect of the pH value in the release of phosphate from fresh residues

The change in the pH value had an influence in the Pi release from manure: The lower the pH, the highest was the P$_i$ release (Figure 4.20). The higher increase in the P released occurred when the pH value was adjusted from 7 to 6, with an increase of 54%. A further increase in almost 14% occurred when the pH is further decreased to pH 5. After this value, the difference when decreasing the pH of the solution is 4%.
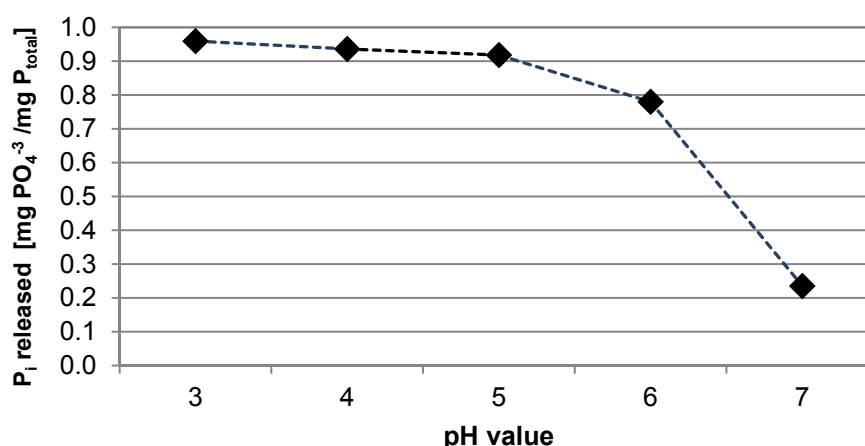
Figure 4.20 Effect of pH value in P$_i$ release

ii.   Quantification of the acid consumption for the process

In the first part of this experiment, the $Ks_{4,3}$ was determined for pig manure solution with 1%, 2% and 5% TS. Secondly, a modified procedure was carried out with the conditions used in the previous acidification experiments, this means, adjustment to pH 5 with H_2SO_4. The results showed a linear behavior between the acid consumption and the total solids of the samples (Figure 4.21). This is because, the total solids was an indirect value of the concentration of buffer substances.

The plot shows data with fit line $y = 46.314x$ and $R^2 = 0.9817$, with x-axis "Total solids [%]" and y-axis "$Ks_{4.3}$ [mmol H^+/l]".
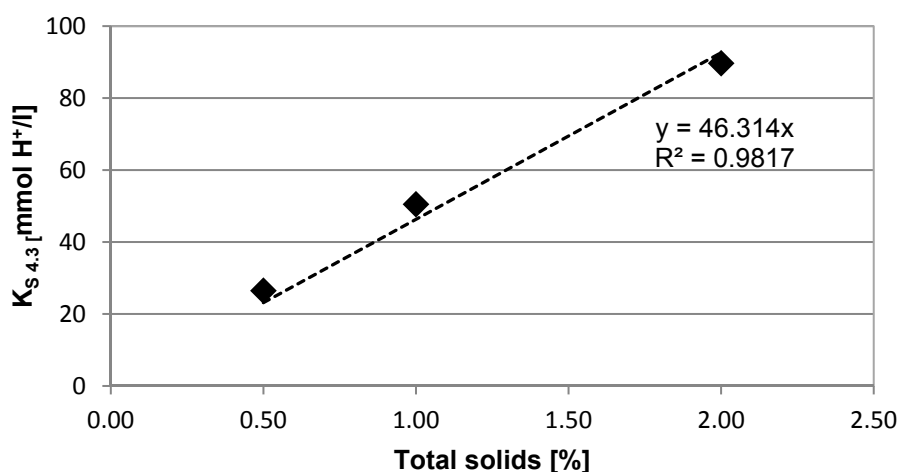
Figure 4.21 Acid neutralizing capacity to pH 4.3

In Table 4.8 a comparison between the standard analytical method $Ks_{4.3}$ and acidification procedure carried out in this investigation at pH 5 using H_2SO_4 is showed. The results of acid consumption under the standard conditions (according to the norm) and the experimental conditions in this investigation are comparable.

Table 4.8 Comparison of $Ks_{4.3}$ and adjustment at pH 5

Total Solids [%]	$Ks_{4.3}$ [mmol $H^+ \cdot l_{sample}^{-1}$]	H_2SO_4 quantity to increase pH value to 5 [mmol $H^+ \cdot l_{sample}^{-1}$]
1	52.5 ±4.4	53.6 ± 0.6
2	111.8 ±0.6	102.4 ±0.6
5	276.7 ±2.4	256.0 ±1.5

iii.   Continuous operation process using fresh residues and phosphate precipitation from the liquid fraction after solid-liquid separation

The quantity of P_i, Ca and Mg released at 21°C and 37°C was comparable, this means that temperature did not have a significant effect on the nutrient release (Figure 4.22). Therefore, no heating would be necessary for the process.
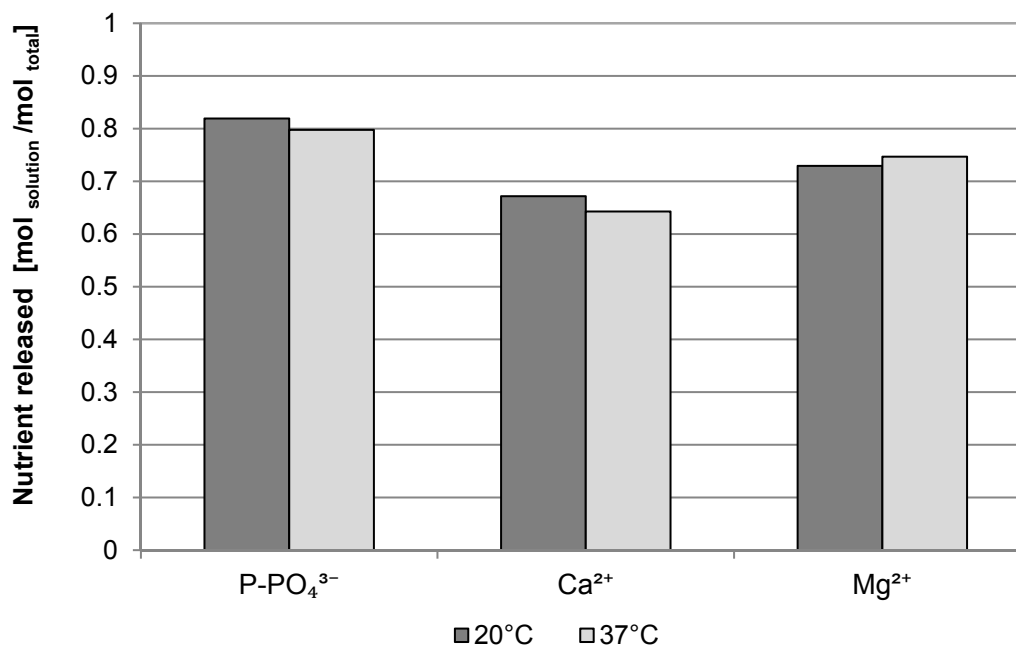
Figure 4.22 P, Ca^{2+} and Mg^{2+} released after acidification process

The molar concentrations of the nutrients in the acidified liquid fraction are shown in Table 4.9. The quantity of Mg and Ca are almost equimolar compared to phosphorus. For this reason, it was decided to carry out a precipitation process only by pH increase by base addition.

Table 4.9 Molar concentration of the nutrients in the liquid fraction

Nutrient	Concentration [mmol·l^{-1}]	
	20°C	37°C
P-PO$_4^{3-}$	11.62	11.32
Ca^{2+}	10.43	9.98
Mg^{2+}	8.64	8.84
N-NH$_4^+$	21.43	20.71

After pH increase by base addition, a rapid reduction of the P concentration was observed during the first five minutes (Figure 4.23). After 30 minutes the phosphorus concentration

was reduced in 99.4% to a final concentration of 2.25 mg·P·l^{-1}. The calcium concentration decreased in 68.9% to a final concentration of 130 mg·L^{-1}.
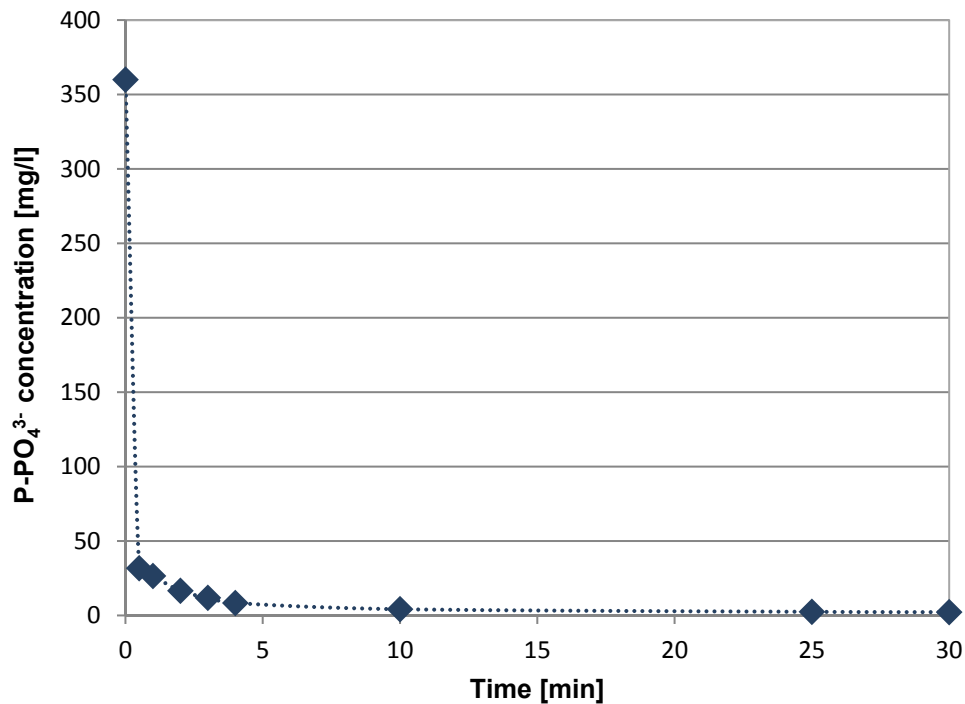
Figure 4.23 P$_i$ concentration in the liquid fraction after alkali addition

The precipitated salts (Figure 4.24) were analyzed to determine their composition. The results are shown in Table 4.10. The macronutrients in higher concentration were phosphorus, calcium and magnesium. Regarding the micronutrients, the precipitated salt had higher sodium, manganese and iron content.
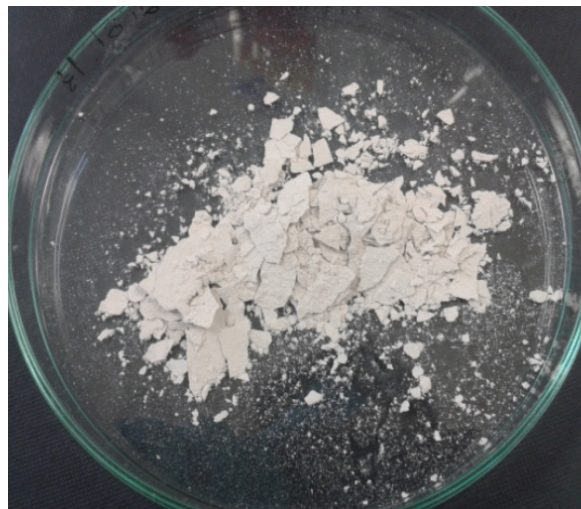
Figure 4.24 Precipitated phosphate salts

Table 4.10 Element composition of the precipitated salt

Element	Content
Macronutrients [%$_{weight}$]	
P_{total}	13.8
Ca	11.2
Mg	5.8
K	0.1
$N-NH_4^+$	2.6
S	1.1
Micronutrients [mg·kg$_{salt}^{-1}$]	
B	25
Cu	28
Mn	1600
Mo	1.2
Zn	280
Fe	910
Na	4500
Heavy metals / possible pollutants [mg·kg$_{salt}^{-1}$]	
As	0.15
Pb	0.23
Cd	0.13
Cr (total)	2.3
Cr (VI)	<0.1
Ni	2.9
Hg	<0.05
Zn	280
Tl	<0.25

4.3.2 Carbonate addition process using real residues

i.   Effect of carbonate concentration and pH value

The addition of carbonate ions to the residues samples significantly increased the P_i released (Figure 4.25) from the absorbed matrix into the solution compared with the blanks. The maximum release of 65.5% occurred at a carbonate-calcium ratio of 60 and pH value of 9.2. This condition was selected as the optimal for the further experiments. For higher carbonate-calcium ratios, 110 and 160, no significant difference between pH 8.5 and 9.2 was

determined. Moreover, the P_i release at pH 9.2 did not further increase significantly compared to the carbonate-calcium ratio of 60.
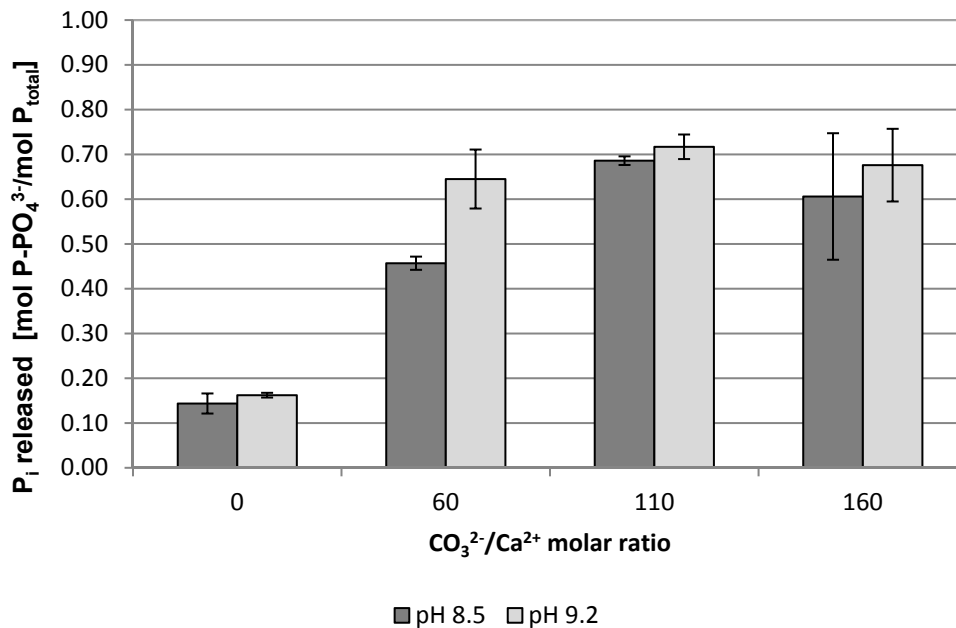
Figure 4.25 Effect of carbonate concentration and pH value on P_i release

The effect of the carbonate concentration and the pH value on the solubility of calcium is shown in Figure 4.26. The calcium release in the solution remains lower than 10% for all the conditions.
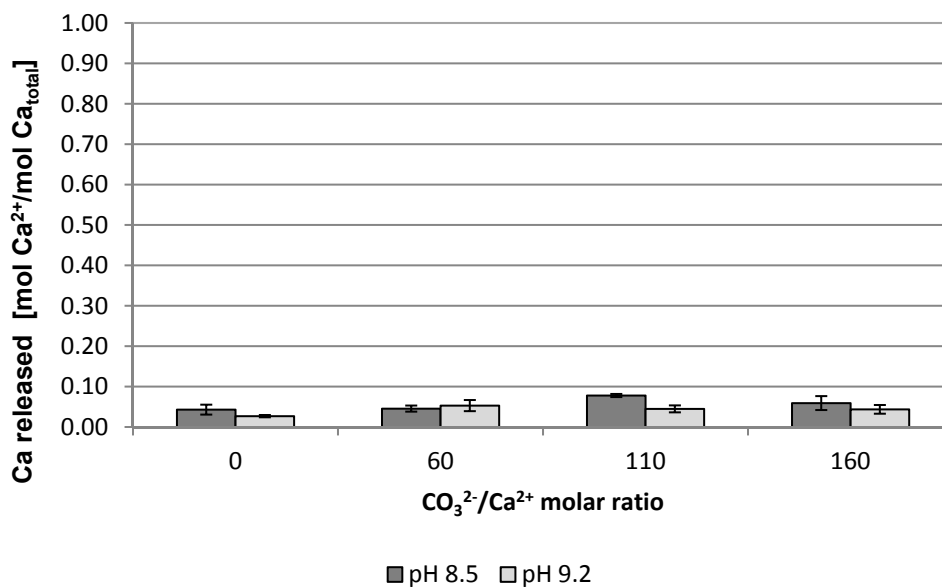
Figure 4.26 Effect of carbonate concentration and pH value on Ca release

The effect of the carbonate concentration and the pH value on the solubility of magnesium is shown in Figure 4.28. In general, a significant different of the samples with carbonate addition with respect of the blanks was determined. The magnesium release had a maximum of 72% at a pH value of 8.5 and carbonate-calcium ration of 110.
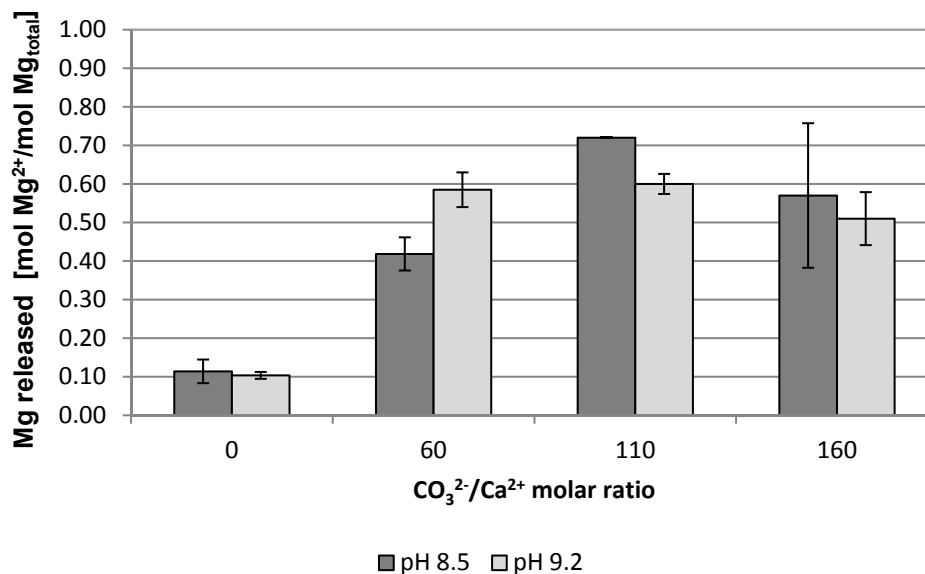
Figure 4.28 Effect of carbonate concentration and pH value on Mg release

ii.   Effect of time and temperature

The effect of time and temperature on P release was determined for the optimal conditions carbonate-calcium ration 60 and pH value 8.5 (Figure 4.27).
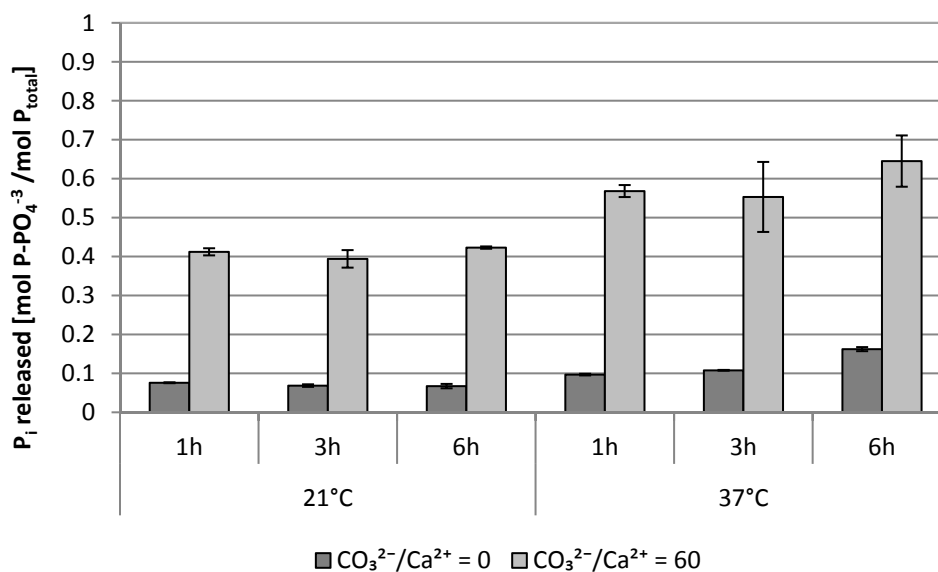
Figure 4.27 Effect of time and temperature on P_i release

For both temperatures, there was no significant difference between 1 h, 3 h and 6 h mixing time. Regarding the temperature effect, a significant higher P release, up to 64.5% was measured at a temperature of 37°C.

The effect of time and temperature on the calcium and magnesium solubility is shown in Figure 4.28, for a mixing time of 1 hour. In the case of magnesium, there is no significant difference in the release for 20°C and 37°C, with a maximum release of about 40%. The calcium release at 21°C was statistically significant higher than at 37°C, however the difference was only 1.6%.
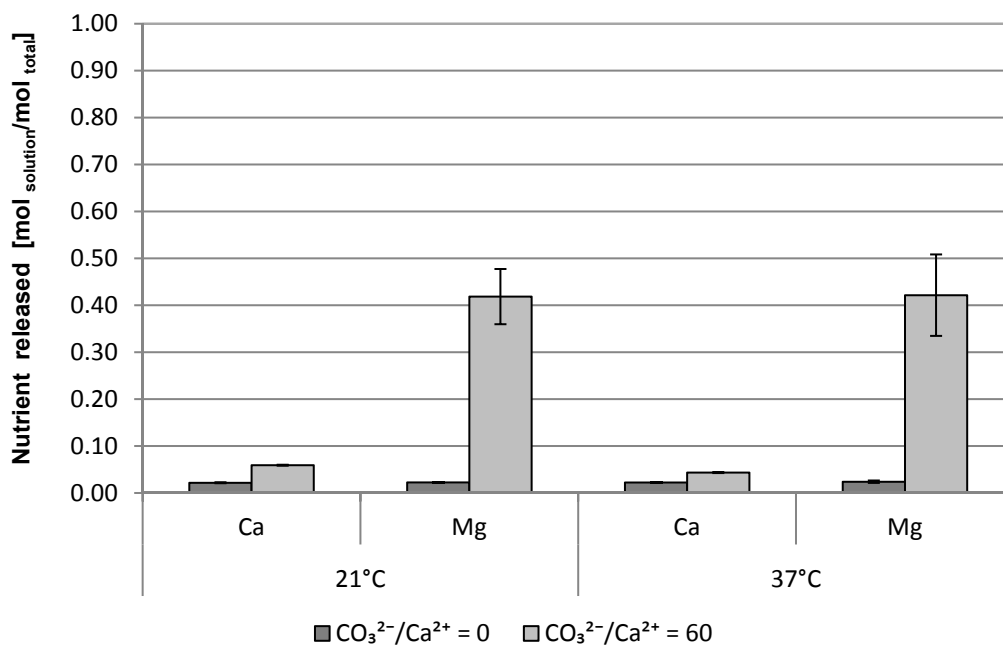
Figure 4.28 Effect of time and temperature on Ca and Mg release

4.3.3 Thermodynamic modeling

i. Precipitation from acidified liquid fraction

The output of the precipitation of the acidified liquid fraction at pH 9 (Table 4.11) showed that hydroxylapatite, struvite and magnesium phosphate were mineral phases likely to form (SI>0). However, from the equilibrium simulation it was determined that only hydroxylapatite and struvite precipitated.

Table 4.11 Output of the simulation of the acidification process

Precipitated product	Non-equilibrium	Equilibrium
	Saturation index (SI)	Concentration [mmol·l^{-1}]
Hydroxylapatite (Ca$_5$(PO$_4$)$_3$(OH))	20.56	1.81
Struvite (MgNH$_4$PO$_4$·6H$_2$O)	2.784	9.82
Calcium sulfate (CaSO$_4$)	-0.508	0
Magnesium phosphate (Mg$_3$(PO$_4$)$_2$)	3.352	0

The quantity of each element that precipitated from the acidified liquid by increasing the pH value to 9 was calculated from the simulation results (Table 4.12). The results of the simulation showed that more than 99% of P and Ca could be recovered as insoluble salts. Regarding N and Mg, the quantity was lower, namely 34.4% and 88.4% respectively, which corresponded to the struvite formation. Potassium and sulfur remained in solution as soluble ions.

Table 4.12 Quantity of nutrient precipitated in the product

Element	Fraction of total content [%$_{mol}$]
P	99.4
N-NH$_4$	34.4
K	0.0
Ca	99.9
Mg	88.4
S	0.0

From the simulation results the nutrient composition of the precipitated product could be calculated (Table 4.13). As mentioned, the precipitated salt consisted of a mixture of hydroxylapatite (27.4% w/w) and of struvite (72.6% w/w).

Table 4.13 Nutrient composition of the precipitated salt

Element	Mass fraction [%$_{weight}$]
P	14.2
N-NH$_4$	4.1
K	0.0
Ca	10.9
Mg	7.2

ii. Bicarbonate addition process

The output of the simulation of the carbonate addition process at pH 9.2 and a carbonate-calcium ratio of 60 (Table 4.14) showed that the different minerals that could form are hydroxylapatite, struvite, calcium carbonate, calcium sulfate and magnesium phosphate (SI>0). However, from the non-equilibrium simulation it was determined that struvite and calcium carbonate precipitated.

Table 4.14 Output of the simulation of the carbonate addition process

Precipitated product	Non-equilibrium condition	Equilibrium condition
	SI	Concentration [mmol·l^{-1}]
Hydroxylapatite ($Ca_5(PO_4)_3(OH)$)	21.68	0
Struvite ($MgNH_4PO_4·6H_2O$)	2.966	15.17
Calcium carbonate ($CaCO_3$)	5.343	13.47
Calcium sulfate ($CaSO_4$)	0.2	0
Magnesium phosphate ($Mg_3(PO_4)_2$)	4.014	0

The results of the model showed that after the bicarbonate addition process only 19% P and 0.3% Mg^{2+} are released into solution. These results differed with the measured values in Section 4.3.2. , where about 60% of these elements were released into solution.

Table 4.15 Element content in the liquid fraction after bicarbonate addition

Element	Concentration [mmol·l^{-1}]	Fraction of total content [%$_{mol}$]
P-PO$_4$$^{3-}$	3.56	19
N-NH$_4$	13.39	46.9
K$^+$	0.43	100
Ca^{2+}	0.0	0
Mg^{2+}	0.05	0.3
CO$_3$$^{2-}$	776.4	98.29

5 Discussion

In this thesis, different processes to increase the inorganic phosphate (PO_4^{3-}) content in the liquid fraction after solid-liquid separation of agricultural residues were studied. In this way, the P recovery from the liquid fraction as valuable phosphate salts could be significantly increased. The first step consisted of the characterization of different agricultural residues and the determination of the P forms in the residue matrix, since this influences the availability of free phosphate in the liquid fraction.

Subsequently, different process for the release and solubilisation of refractory phosphorus were investigated, namely: i) Mineralization of the organic phosphorus compounds in model solutions and agricultural residues, ii) Dissolution of insoluble phosphate minerals and iii) Solubilization and release of the adsorbed phosphates. Finally, a concept for the integrated nutrient recovery from agricultural residues is proposed. The discussion of the findings is presented in the following sections.

5.1 Characterization of agricultural residues and determination of P fractions

5.1.1 Total nutrient determination

The nutrient composition of the manure and digestate samples depends on many factors, such as type and state of the animal (e.g. age, health condition) and manure management system (storage system, storage time, pretreatments). Moreover, environmental conditions such as temperature, rainfall, microorganisms, etc. play a main role in the nutrient distribution in the solid and liquid fractions. Therefore, a general characterization of the samples was necessary to determine the nutrient content and recovery potential of the residues.

Since the samples were collected in different periods, certain variability in the nutrient concentration was expected. In spite of this, the nutrient concentration in most of the samples was relatively constant, with standard deviations under 1%. The exception was the calcium and magnesium concentration of laying hen manure Ch-1, which was above 3% and 4%, respectively. This variability can be explained by the fact that only two collections of this type of manure were carried out.

The nutrient composition of the residues can be compared with literature values. In this case, to have a more precise impression of the actual nutrient content in the samples, the concentrations are presented in fresh matter content. The comparison for the different types of residues analyzed is presented as follows.

i. Pig manure

For the pig slurry sample Pig-1a, the total solid content and nutrient concentrations measured are middle values within the range found in literature (Table 5.1). The exception was the magnesium content, which was relatively high in the upper limit. The high ammonia concentration, which is characteristic of this type of manure, could be explained by the presence of urine.

Table 5.1 Comparison of nutrient content and total solids of pig slurry (Pig-1) with literature values

Nutrient	Nutrient content [%FM]	
	Measured	**Literature [12, 111, 112]**
$P-PO_4^{3-}$	0.11 ± 0.01	0.01-0.22
$N-NH_4^+$	0.37 ± 0.04	0.19-0.61
K^+	0.23 ± 0.04	0.14-0.33
Ca^{2+}	0.13 ± 0.02	0.12-0.16
Mg^{2+}	0.06 ± 0.01	0.03-0.06
%TS	3.6 ± 0.50	1.5-9.2

In the case of solid pig manure Pig-2, the range of $P-PO_4^{3-}$ and $N-NH_4^+$ found in literature was 0.07% to 0.65% and 0.05 to 0.6 %, respectively [12]. The values obtained in the characterization were between this reported range, namely 0.23 ± 0.05 % for phosphorus and 0.22 ± 0.05% for ammonium nitrogen.

In this thesis, an extensive characterization, which included micronutrients, heavy metals and other pollutants, was carried out. This kind of information was not found in literature.

The differences in the total solids and nutrient concentration of the pig manure samples Pig-1a and Pig-2 were related to the different management practices and animal diets in the farms. The composition of the feedstuff provided to the pigs in both farm is shown in Table 5.2. In order to determine the relation between food intake and nutrient excretion in feces and manure composition, information about feedstuff consumption and nutrient assimilation by the pigs is necessary. This detailed nutrient uptake/excretion balance was beyond the scope of this study, but it should be consider in further investigations. This would provide important information to minimize overfeeding to animals and avoid nutrient losses.

Table 5.2 Composition of the feedstuff for pigs

Component	Content [%]	
	Commercial farm A	**Research farm**
Ingredients		
Soy shred	61.9	15.0
Rape shred	19.4	-
Residue cereals from fermentation	3.2	-
Vegetable oil	-	0.6
Peas	-	7.0
Barley	-	34.75
Triticale	-	20
Wheat	-	20
Calcium carbonate	7.3	-
Sodium chloride	1.7	-
Mono calcium phosphate	1.2	-
Mineral supplement	-	2.75
Nutrients and proteins		
Phosphorus	0.4	1.0
Calcium	0.6	3.5
Magnesium	-	0.18
Sodium	0.7	0.16
Raw protein	16.0	39.5
Lysine	1.0	3.9

ii.   Anaerobic digestate

The nutrient content of the digestate Dig-1 and Dig-2 were comparable. This was expected because both samples came from agricultural based plants; this means they use a mixture of animal manure and different kinds of crops as main substrate. The results of the macronutrient content were also compared with values reported in literature (Table 5.3).

Table 5.3 Comparison of nutrient content of anaerobic digestate

Nutrient	Nutrient content [%$_{FM}$]		
	Measured		**Literature [11, 27]**
	(Dig-1)	**(Dig-2)**	
P-PO$_4$$^{3-}$	0.13 ± 0.01	0.11 ± 0.00	0.07 - 0.15
N-NH$_4$$^{+}$	0.37 ± 0.00	0.31 ± 0.01	0.18 - 0.31
K^{+}	0.37 ± 0.02	0.39 ± 0.07	0.34 - 0.44
Ca^{2+}	0.29 ± 0.02	0.25 ± 0.03	0.11 - 0.16
Mg^{2+}	0.11 ± 0.02	0.07 ± 0.02	0.04 - 0.07
%TS	9.2 ± 0.3	8.7 ± 0.7	6.1 - 9.1

In general, the nutrient content of the measured digestate samples agreed with literature. The exceptions were the $N-NH_4^+$ and Mg^{2+} content for Dig-1, which was around 0.07% and 0.04% higher than the literature maximum value, respectively. Moreover, both digestate samples had more Ca^{2+} content in comparison with the literature values. These oscillations could be explained by differences in all the different factors that can influence the nutrient composition in anaerobic digestate, such as type and composition of substrate, type of reactor, digestion technologies, etc.

iii.   Poultry manure

The P content of the laying hen manure Ch-1 was about 60% higher than the maximum value reported in literature (Table 5.4). Opposite to this, the potassium content in the sample is relatively low respect to the literature values. The magnesium content was higher but due to the considerable standard deviation of the sample the difference was not significant.

Table 5.4 Comparison of nutrient content of laying hen manure

Nutrient	Nutrient content [%FM]	
	Measured	Literature [12, 113]
$P-PO_4^{3-}$	0.49 ± 0.05	0.3-1.2
$N-NH_4^+$	0.56 ± 0.04	1.1-6.0
K^+	0.62 ± 0.01	1.1-1.6
Ca^{2+}	1.02 ± 0.87	-
Mg^{2+}	1.41 ± 1.19	0.1-0.4
%TS	27.3 ± 1.0	22-55

The total solids and nutrient content of the samples varied according its type and farm management system (Table 5.5). Pig-1a showed the higher P concentration in the dry matter basis. However, this type of manure had the higher water content. An important finding was the high calcium and magnesium content in all samples. This can be explained by the feeding of the animals with mineral supplements. Calcium and magnesium have a considerable influence in the solubility of phosphate and must therefore be considered for the development of the recovery process from the separated liquid. P could be precipitated as insoluble calcium and magnesium salts. This means, that contrary to the case of P recovery from municipal wastewater, where these ions have to be added in the process (for example as $Mg(OH)_2$ or $MgCl_2$), no further chemicals addition is necessary.

Table 5.5 Comparison of the P, Ca and Mg content of the samples

Sample	TS [%]	Content in fresh matter [%FM]			Content in dry matter [%DM]		
		P	Ca	Mg	P	Ca	Mg
Pig-1a	3.6±0.5	0.11±0.01	0.13±0.02	0.06±0.01	2.97±0.36	3.62±0.66	1.57±0.31
Pig-2	16.8±5.5	0.23±0.05	0.24±0.08	0.18±0.02	1.37±0.29	1.43±0.48	1.06±0.13
Dig-1	9.2±0.3	0.13±0.01	0.23±0.02	0.11±0.02	1.39±0.07	2.53±0.20	1.17±0.25
Dig-2	8.7±0.7	0.11±0.00	0.25±0.03	0.07±0.02	1.22±0.04	2.88±0.34	0.75±0.20
Ch-1	27.3±1.0	0.49±0.05	1.02±0.87	1.41±1.19	1.80±0.19	3.75±3.20	5.16±4.35
Ch-2	80±n.a	1.16±n.a	0.32±n.a	7.21±n.a	1.45±n.a	0.40±n.a	9.01±n.a

For the P recovery approach investigated in this thesis, which is the recovery by precipitation from the liquid phase, the most appropriate samples were the pig slurry Pig-1 and both digestate samples. This is because they fulfilled two important conditions: First, the P content was high and second the high water content make more suitable the solubilisation and recovery of the refractory phosphorus. The solid pig manure sample, together with the chicken samples had a higher P content, but since they are solids external water should be added for the P release process.

5.1.2 Sequential and parallel extraction for determination of P fractions

Sequential extraction procedures have been used by researchers with the aim to assess the P runoff potential from soils and soils amended with animal manures. The procedure consists of multi-step sequential extractions using different solutions (e.g. water, bicarbonate, HCl, NaOH) from dried samples to determine labile and refractory P pools. Water and bicarbonate extractable P are used as an indicator of the labile P fraction available for plant intake [114]. Bicarbonate extractable P includes additionally some labile solid phase inorganic P, such as loosely sorbed P_i [115].

In this thesis, the extraction methodology was used with the aim to quantify the fraction of P_i available in the manure and digestate residues for the P precipitation as salt. This fraction could be compared directly with the plant available P because plants are able to adsorb only inorganic phosphate ions. As a consequence, the water and bicarbonate extraction procedures were selected for the characterization.

As a further step, a one-step extraction procedure using 0.5 M $NaHCO_3$ was proposed for the determination of the available P_i fraction in the samples. Furthermore, it was determined that the drying and milling pretreatment of the samples had an effect in increasing the extractable P_i content of the samples.

i.   Sequential and parallel extraction using pretreated samples

The first approach in the extraction method was to use dry and milled samples. This is the procedure recommended in soil science literature to assure homogenous samples. Two extraction methods were successfully tested: First, a sequential extraction with water and bicarbonate solution and second, a simplified method with only one extraction step using bicarbonate solution.

The results confirmed that only a fraction of total P was available in the liquid fraction for precipitation, the rest P was refractory. A comparison of the sequential and parallel extraction is showed in Table 5.6. Moreover, for the sequential extraction, the mean value and standard deviation of literature data from Table 2.5 is presented.

Table 5.6 Comparison of the P_i extracted from the sequential and one-step method using pretreated samples

Sample	P_i extracted [% mol $PO_4^{3-} \cdot$ mol P_{total}^{-1}]			
	Sequential*			Parallel
	Water	0.5 M $NaHCO_3$	Sum of fractions	0.5 M $NaHCO_3$
Pig-2	49 ±5 (59± 5)	18 ±1 (14 ± 5)	67	77 ±1
Dig-1	35 ± 0	17±0	52	69±1
Dig-2	23±4	42±3	65	84±1
Ch-1	35±11 (27 ± 3)	17±1 (11±7)	52	69±1

* The numbers in parenthesis are the average values found in literature

Although there are differences in the type of sample and the exact methodology used by different authors, the results of the sequential extraction with water and bicarbonate of Pig-2 and Ch-1 agreed with the literature findings (Table 2.5).

When comparing the sum of the two-step sequential extractions with the parallel bicarbonate extraction it was noticeable that the parallel extracted more P from the sample (6% to 33% difference) than the sequential. This could be explained in part by sample loses in the sequential extraction. After centrifugation and filtration of the water mixture, a part of the

solids remained in the flasks and filters. Therefore, the material available for the bicarbonate extraction was lower.

As a conclusion, the sequential method was selected for the characterization of available P_i using fresh residues samples in the following section. Compared with the sequential extraction methods, this simplified procedure had the advantage that the time for the experiment was reduced to the half and the material losses during centrifugation and filtration of the samples minimized. In this way, sources of error during the procedure were reduced.

ii.   **One step extraction with fresh samples**

The available P using fresh samples was considerable lower in comparison with the pretreated ones. For instance, for the sample Pig-2 the extracted P from fresh sample was about 55% lower than from the pretreated. This confirmed the hypothesis that the drying and milling increased the P solubility of the samples. This finding agreed with the investigation of Worsfold *et al.* [104] , who reported that water extractable P was influenced by mild drying of soil. This phenomenon could be caused, in one hand, by the osmotic shock and cell rupture of the microbial cells caused by rewatering of the samples [116, 117]. Additionally, physical stresses induced by drying and milling disrupt organic matter coatings mineral surfaces, which may contribute to solubilisation of both organic and inorganic P [118].

The results suggested that mechanical and thermal treatment could be an effective method to increase the available P in residues, especially in those with low water content. However, in the approach of this investigation, which is to recover the P from the liquid fraction, the drying and rewetting of the samples would not be cost and time effective. Moreover, ammonium nitrogen would be lost by drying.

5.1.3 Mass balance and element distribution after solid-liquid separation of pig manure and anaerobic digestate

The determination of the mass balances and element distribution provided information about the recovery potential of P and other nutrients in the solid and liquid fraction of the residues. Moreover, this information provided an overview of the interactions of the different elements in the samples in the solid and liquid fractions, which was essential for the development of the integrated nutrient recovery concept presented in section 5.4.

In the case of pig manure, the detail mass balance, including macronutrient, micronutrients and pollutants distributions after mechanical separation of the liquid and solid fraction study

was a novel finding that was not found in literature. For anaerobic digestate, only information about phosphorus, nitrogen and potassium distributions were found in literature [11].

From the mass balances of the solid-liquid separation step of both, Pig-1 and digestate Dig-1, it can be concluded that phosphorus, magnesium and calcium were mainly present in the solid fraction. This confirmed the hypothesis that P is combined with these two cations and form insoluble compounds. The slightly higher soluble P_i content in sample Dig-1, in comparison with Pig-1, can be explained by the mineralization of organic P compounds during the anaerobic digestion process. The findings agreed with values reported in literature for anaerobic digestion, in which 55% to 65% of the P remains in the solid fraction after separation [11]. Contrary to this behavior, potassium and nitrogen were mainly present in the liquid fraction, which demonstrate that they form soluble compounds.

Concerning the secondary macronutrients in the Pig-1 sample, iron was equally distributed in the solid and liquid fractions. This suggested that this element could form insoluble (e.g. iron phosphates) and soluble (e.g. iron soluble complexes) compounds. On the other hand, manganese was present in the solid fraction, which suggested that it formed insoluble salts. The high standard deviation of the micronutrients measurements could be explained by the heterogeneity of the manure samples. Since these elements were present in very small concentrations, the differences between samples were more evident.

The heavy metals and other possible pollutants present in the Pig-1 fractions were compared with the threshold values of the German Fertilizer Ordinance (Table 5.7) [119].

Table 5.7 Pollutant concentration in Pig-1 sample compared with threshold values

Element	Content [mg/kg DM]			Threshold value
	Sample Pig-1			
	Raw	Liquid	Solid	
Hg	< 0.05	< 0.05	< 0.05	1
Tl	< 0.2	< 0.2	< 0.2	1
As	< 4.00	< 4.00	< 4.00	40
Pb	< 5.00	< 5.00	< 5.00	150
Cd	< 0.5	< 0.5	< 0.5	1.5
Ni	9.5 ± 3.4	14.5 ± 3.5	8.3 ± 2.0	80
Cu	223.0 ±100	230.3 ± 90.0	225.7 ± 69.8	*
Zn	1325.0 ± 115.0	1280.0 ± 128.3	1230.0 ±37.4	*

* Threshold values not reported

For all the elements, the concentrations in the raw manure and the solid and liquid fractions were below the threshold value according to the ordinance. In the case of zinc and copper,

no threshold values are reported. The measurement of the pollutant concentration was essential to determine if the residues could be used as raw material for the P recovery process, without any concern of contamination with these metals. Especially for the solid fraction, which could be dried and used as soil conditioner (Section 5.4), it was important to guarantee that the pollutant concentrations were below the threshold values of the regulations.

The information of the characterization section was used for the selection of the most suitable substrate for the further steps of the investigation. Pig-1 was selected for this purpose due to different reasons. First, the production of pig slurry in Europe is considerable. It accounts for more than 10% of the total manure produced (Table 2.2). This quantity is only surpassed by cattle slurry (32%). However, cattle slurry was not selected for the characterization because ruminants metabolize most of the organic phosphorus converting it into phosphate; therefore it was expected a much lower organic P content than in pig manure. Since the idea of the process to be developed is to precipitate and recover phosphorus on a liquid solution, the high water content of pig slurry represents an advantage. As discussed in Section 5.5, the liquid fraction can be treated and used as recycling water in the process. In contrast, for residues with lower water content, such as, solid pig manure Pig-2, and the poultry manure Ch-1 and Ch-2 another approach has to be developed for the treatment.

5.2 Enzymatic process for the mineralization of organic P compounds

Phosphorus can be present in agricultural residues, such as animal manure and anaerobic digestate not only as inorganic phosphate but also as organic bounded compounds. According to literature, the quantity of organic P in these residues can varied from about 25% to 80% (Table 2.6 and Table 2.7). Based on this information, it was expected that the P_o fraction in the sample could be released by mineralization using of phosphatases, this means by conversion of the organic P molecules into inorganic phosphate. As a first step, the performance of the enzymes was tested under optimal conditions using model P compounds. Subsequently, the enzymatic process was tested using pig manure under batch and continuous operation.

5.2.1 Enzyme selection and determination of the enzyme activity

The different enzymes used in the experimental part were selected from literature research. They represent the phosphatases which can mineralize the organic P compounds that can

be present in organic residues. The enzyme activity assay was performed successfully as a methodology to control the quality of the enzymes. In general, the activities measured agree with the values reported by the suppliers. This means, that the catalytic activity of the enzymes was not reduced during storage.

5.2.2 Enzyme specificity assay and determination of process parameters using model phosphorus compounds

The objective of the enzyme specificity study was to determine in which organic P compounds the phosphatases have a mineralization effect and the extension of this hydrolytic activity. The experiments were successfully performed using different P model compounds under optimal enzyme pH and temperature conditions.

i.   Assay with individual model compound and enzyme solutions

The results confirmed that all phosphatases had no activity toward phosphonates and therefore no phosphate release was measured. Phosphonates have a similar structure than phosphates except that they have a C–P bond instead of the C–O–P linkage. This C-P bond is very resistant to enzymatic and thermal hydrolysis [34]. Moreover, due to their structural similarity to phosphate esters, phosphonates often act as inhibitors of enzymes [120].

As expected, the refractory C-O-P bond in inositol phosphate could be hydrolyzed only by the plant and fungal phytases. Wheat phytase (WPhy) released a maximum of 58% P_i after 16 h incubation, which was 26% more P_i than *Aspergillus Niger* phytase (AsPhy). This result indicated that a complete mineralization of the organic P could not be reached. Theoretically, phytases dephosphorylate phytic acid (also called *myo*-inositol hexakisphosphate) to a series of lower inositol phosphate esters (from *myo*-inositol pentaphosphate to *myo*-inositol monophosphate) and eventually to inositol and phosphate [73, 83]. However, the results suggested that the sequential dephosphorylation was not completed. This behavior is also reported by Barrientos *et al.* [35], which tested the hydrolysis of inositol phosphate by pollen phytase. They reported that the final product of the reaction was *myo*-inositol triphosphate and no further hydrolysis was detected even after extensive periods of incubation (48 h). Moreover, Wyss *et al*. [33] investigated the catalytic properties of different fungal and bacterial phytases. They concluded that all phytases where able to hydrolyse only five phosphates groups from phytic acid, which left as final product *myo*-inositol 2-monophosphate. This behavior can be explained by the fact that this phosphate group is not located in an energetically favorable position for the enzymatic hydrolysis.

The difference between the performances of the two phytases WPhy and AsPhy could rely, first on the different production and purification processes of the enzymes and second on the hydrolysis mechanism of them. Wheat phytase starts the hydrolysis in the carbon-6 position whereas *Aspergillus Niger* phytase in the carbon-3 position [83]. Probably the sequential dephosphorilation starting in C-6 favors the subsequently hydrolysis of the low inositol phosphates.

The phosphate monoesters α-D-Glucose-1-phosphate and D-Glucose-6-phosphate have labile C-O-P bonds. As expected, the alkaline phosphatases (AlkPhos-1 and AlkPhos-2) released more than 72% P_i since phosphate monoesters are the substrates where they have their higher activity (Section 2.3). Wheat phytase (Wphy) was also able to release up to 85% P_i. This confirmed that Wphy have a calatytic activity over a wide range of substrates. In general, phytases from plants have a low specificity, this means they act over different organic P compounds. This can be explained by the presence of other enzymes as impurities, especially alkaline phosphatases during the production processes [82].

In nature, the hydrolysis of polyphosphates is extremely low. The half-life time of their P-O-P bonds at neutral pH and room temperature linkages is of the order of magnitude of years. However, enzymes can affect the degradation rate as much as 10^5-10^6 times faster [6]. This affirmation was proved by the results where tripolyphosphate could be degraded mainly by WPhy (up to 87%) and AlkPhos-2 (up to 53%).

This finding suggested that these enzymes could be used for the mineralization of substrates with high content of polyphosphates, for example sewage sludge from bio-P treatment plants. This application should to be investigated in further studies.

The nucleotides AMP and ATP are phosphate monoesters with C-O-P bonds, this explained that they could be mineralized mainly by alkaline phosphatases. In the case of ATP, Wphy had also a considerable effect in the phosphate release from this molecule (> 80% P_i release). The reason of this can be explained by the hydrolysis of the condensed polyphosphate in the ATP molecule. Phosphodiesterase exhibited activity towards both nucleotides, especially AMP (~80% P_i release). The enzymes with higher activitiy toward RNA was, as expected, the phosphodiesterase and wheat phytase (>80%P_i released).

Under the experimental conditions, acid phosphatase showed very low or no activity toward most of the substrates. A maximum P_i released of about 20% was detected only for AMP and RNA. The reason of the low performance of acid phosphatase is not clear. If further investigations with this enzyme are required, it is suggested to increase the substrate-enzyme ratio and to test enzymes from different providers.

Regarding the incubation time, a significant difference in the phosphate release between 1h and 6 h incubation time for all the substrates was determined. After 6 h incubation, the enzymatic hydrolysis reached its maximum only for inositol phosphate, α-D-glucose-1-phosphate, AMP and ATP. This means that the minimum retention time for the enzymatic process has to be 6 h and for a maximum hydrolysis of all the organic P compounds preferably 16 h.

ii.   Assays with individual and mixture of model compounds using analytic and industrial enzymes

In this part the results of Sections 4.2.2-ii and 4.2.2-iii are compared and discussed. The experiments confirmed that the analytical grade enzymes with higher activity toward a wider range of substrates were Wphy and AlkPhos-2; therefore, a mixture of both enzymes were tested (Figure 4.14). In this case, the influence of the incubation time on the P_i extracted is significant for all substrates during the first 6 h incubation. After this time, only the mineralization of inositol phosphate and tripolyphosphate reached its maximum, which means that for a more extensive mineralization of all model compounds 16 hours incubation are necessary. This is also proved by the results of the substrate mixture, where there is a significant increase of about 65% in the P_i released between 6h and 16h incubation.

A comparison in the P_i released between the individual WPhy and AlkPhos-2 and the mixture of both is shown in Table 5.8 . The results suggested that the use of an enzyme mixture of wheat phytase and alkaline phosphatase would be meaningful only in the case of organic residues with high concentrations of phosphate monoesters (e.g. α-D-Glucose-1-phosphate, D-Glucose-6-phosphate, AMP) except inositol phosphate. In the opposite case, the use of only wheat phytase would be enough for the P mineralization of the P compounds.

The substrate specificity assay was also performed using industrial phytase Natuphos (Table 5.8).This phytase type, which is produced industrially, derives from *Aspergillus Ficcum*. Since the results in the experiments with analytical grade enzymes showed that the fungal phytase activity toward most of the substrate was low, it was decided to triplicate the enzyme-substrate ratio to 60 U·mmol P^{-1}. Similar to the analytical grade fungal phytase, this industrial phytase had a higher activity toward inositol phosphate followed by tripolyphosphate. For the rest of model compounds, the activity of Natuphos was considerable lower if compared with the analytical wheat phytase. This verified that under the experimental conditions the performance of the analytic grade enzymes was higher than the industrial grade since they could act over a wider range of substrates.

Table 5.8 Overview of P$_i$ released by analytical and industrial phosphatases

Substrate mixture	P$_i$ released [% mol P-PO$_4^{3-}$·mol P$_{total}^{-1}$]			
	WPhy	AlkPhos-2	Wphy+ Alkphos-2	Natuphos*
Inositol phosphate	58 ±1.0	2.2 ±1.5	64 ±9.8	56 ±1.5
Glucose-1-phosphate	35 ±6.0	87 ±6.9	64 ±4.1	7 ± 1.0
Glucose-6-phosphate	85 ±6.0	100 ±6.1	90 ±4.9	10 ±1.3
AMP	43 ±3.0	87 ±9.6	76 ±0.4	13 ±0.3
ATP	82 ±9.0	77 ±6.6	100 ±12.3	9 ±0.3
RNA	100 ±2.0	16 ±3.2	100 ±0.6	22 ±1.6
Tripolyphosphate	87 ±1.0	52 ±2.9	92 ±7.2	38 ±2.6
Substrate mixture	n.a	n.a	87 ±5.6	27 ±0.6

* The enzyme-substrate ratio of Natuphos is 3 X higher than the analytical enzymes (60U·mmol P$_{total}^{-1}$)

If the selection criterion for the optimal phytase were merely based on the enzyme performance, wheat phytase would be clearly the more appropriate selection. Nevertheless, for the development of the process was also necessary to consider economic factors. The price of wheat phytase (with an activity of only 0.05 U·mg$_{enz}^{-1}$) was about 52,000 €·kg$_{enz}^{-1}$. On the other hand, the industrial phytase (with an activity of 10 U·mg$_{enz}^{-1}$) costed 12.5 €·kg$_{enz}^{-1}$. The difference in cost was considerable. For this reason for the experiments in a larger scale, industrial phytase was preferred.

According to literature, the organic P content in agricultural residues includes different types of phosphate monoesters, phosphate diesters and polyphosphates. With the enzyme specificity assays it was probed that it was feasible to mineralize the organic P content from these compounds using different kinds of phosphatases. For most of the compounds more than 80% of the organic P could be released under the different experimental conditions. The exception was for the compound inositol phosphate where a maximum of 65% was released. This information has to be considered for the treatment of residues in which most of the organic P is present as inositol phosphate.

5.2.3 Batch enzymatic process using real residues

The enzymatic process was demonstrated to be successful in mineralizing organic P from model compounds under optimal pH and temperature conditions and without the presence of any inhibitory compound. However, real residues contain other organic and inorganic compounds which could interference with the performance of the enzymes. As a result,

experiments with agricultural residues were carried out to determine the efficiency of the enzymatic process under real conditions.

i.   Enzymatic treatment of pretreated residues using analytical-grade enzymes

The objective of this experiment was to determine the effect of analytical grade phytase on the phosphorus mineralization of a solution 1% TS of the pretreated (dry and milled) residues samples. Following a similar procedure than the study with model compounds, this experiment was carried out in a micro scale volume (2,000 µl). Due to the small reaction volume, pretreated residues samples were used to assure homogeneity.

Under the experimental conditions, no significant effect of the phytase on the P_i released was demonstrated for most of the samples. Except for the Pig-2 and Ch-1 samples without buffer, where a difference between the blanks and the samples with enzyme addition could be measured. In the case of Pig-2, the differences in the P_i release could be caused by the oscillations in the pH value during the 16 h incubation time, since no buffer solution was used. It was determined that the pH value increased in more than 1.1 units during the 16 hours incubation. This higher pH value could have caused that less P is available in the liquid fraction. For Ch-1 the higher P release could only been explained by differences in the sample composition or an analytical error. Since the sample volume was only 2,000 µl, small changes in the manure composition could results in a considerable change in the P_{total} content of the sample.

In the samples Dig-1 and Pig-2 there was a significant increase in the P_i release only by decreasing the pH value to 5, achieving a release of up to 80%. This means that independently from the enzyme hydrolysis, the pH value have an effect in the P solubility. This was an important finding that is further discussed in Section 5.3.

The reason of the apparent low performance of the phytase could be the absence of organic P in the samples. Due to the pretreatment steps, drying and milling, it was possible that the organic P hydrolyzed during those treatments. Another possible explanation is that the organic phosphorus compounds were chemically hydrolyzed due to the acidic conditions in the experiments. However, this is not likely because in samples Pig-2 and Ch-1 no significant difference in the P_i release between pH 7 (which are milder acidic conditions) and pH 5 was measured.

ii.   Enzymatic treatment of fresh residues using analytical grade enzymes. Parameter variation experiment

The total solids content of the fresh manure sample had a considerable effect on the P_i release from the samples. This finding can be explained by the solubility of phosphate salts (e.g. calcium phosphates), which is determined by the ion saturation in the solution. For instance, when the total solids increased from 1.0% to 9.3% the P in solution decreased in around 25%, at a fixed pH value of 5. These results also suggested, that due to the ion saturation in solutions with higher %TS, the phosphate released by enzymatic mineralization precipitated and therefore no changes in the P_i concentration could be measured. This phenomenon is explained in Section 5.2.3.4.

The model generated with the program Design Expert® 8 suggested that the addition of phytase to the samples had a measurable effect for high enzyme-substrate ratio (16 U·mmol P_{total}^{-1}). However, experiments with this condition were carried out only once (Table 4.3), due to the high amounts of enzyme needed. For this reason it cannot be complete neglected that the differences in the P_i release were caused by the variability in the sampling and analytic measurements. Especially if considering that for the following higher enzyme-substrate ratio of 12 U·mmol P_{total}^{-1} no significant difference in the P_i release was measured when compared to the blanks.

iii.   Enzymatic treatment of fresh residues using industrial enzymes

The utilization of different industrial enzymes (i.e. phytase, xylanase, glucanase and cellulase) showed no significant effect in the P_i release under the experimental conditions. The exact reason of this finding is not clear. As presented in Section 2.3, the possible explanations are:

1) The organic P content in the samples was too low and it could have been degraded by the pH and temperature conditions

2) The organic P was not available to the enzymes because it was attached to the biomass or it formed part of the microbial cells

3) The enzymes were inhibited by the components of the biomass matrix reducing partly or totally their performance.

In these cases, the possible small changes in the P_i concentration in the liquid phase could not be detected.

In order to discard the performance of the enzymes in real residues with high organic P content, experiments were performed adding a spike of inositol phosphate to the pig manure mixture. It was demonstrated that the phytase was able to mineralize 24.2% of the organic P spiked; however, the enzyme activity was reduced in almost 50% compared with the activity measured in the experiment with model compounds in buffer solutions (Section 4.2.3).

As discussed in Section 2.3, there are different explanations for reduction in the enzyme activity [39, 82, 84]:

1) Presence of divalent ions, such as calcium and magnesium, which could act as enzyme inhibitors

2) The high P concentration in the samples inhibited the progress of the enzymatic reaction due to common ion effect (since the product, phosphate is already in solution the equilibrium is forced to the reactants side)

3) The enzyme was adsorbed to the minerals and organic matter in the sample affecting its activity

4) The inositol phosphate interacted with the component of the residues matrix. Due to its large negative charge, it attracted divalent ions forming complex bonds (Figure 5.1). These ions interfered with the active site of the enzyme diminishing their activity toward the inositol phosphate ring.
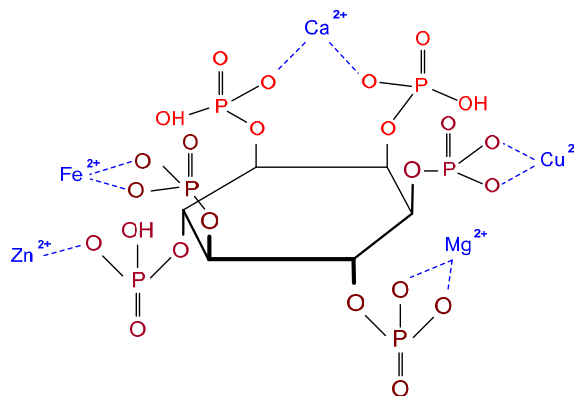
Figure 5.1 Formation of complexes of inositol phosphate with divalent ions

   iv.   **Interferences in the enzymatic treatment by the residue matrix components**

The purpose of this experiment was to determine the fate of inorganic P_i added to the manure samples. It was demonstrated that the added phosphate ions reacted with the ions in the solution or were adsorbed until a maximum value of 34 mg $P_{bound} \cdot g_{manureDM}^{-1}$. Afterwards,

the solution was saturated and the added phosphate was not adsorbed anymore. This finding proved the hypothesis that the phosphate ions released from organic compounds during the enzymatic treatment reacted with the manure components and therefore could not be measure in solution. This means, that measuring the increase in the P_i concentration in solution is not an completely accurate methodology to evaluate the performance of the enzymes in this type of samples.

 The equilibrium relation between the amount of refractory P adsorbed and the concentration of the solution (Figure 4.18) are known as adsorption isotherm. This method is used in soil science to model the P adsorption and desorption process. The procedure consists in fitting the adsorption isotherm using a mathematical equation. The two terms Langmuir isotherm is one of the most common used equations [76, 121]. The Langmuir equation is shown below:

$$\frac{C}{S} = \frac{1}{kS_{max}} + \frac{C}{S_{max}}$$

Where,

C: Total P concentration in the solution (mg P·l^{-1})

S: P adsorbed (mg P· g manure $_{DM}$$^{-1}$)

k: Bonding energy constant of the Langmuir model (l·mg P^{-1})

S_{max}:   Maximum amount of P adsorbed

If it is assumed that all the refractory P is adsorbed P, the different parameters of the Langmuir equation can be calculated from the curve C/S vs. C (Figure 5.2).

Figure 5.2 Plot for the determination of the Langmuir parameters

From the graphic it was calculated that the theoretical maximum phosphorus adsorption was S_{max}: 37.45 mg $P \cdot g_{manureDM}^{-1}$, this value is slightly lower than the measured experimentally. The bonding energy constant or Langmuir coefficient (k) was calculated at 0.013 l·mg P^{-1}. This value described the affinity of the P to the surface of the manure [76].

The Langmuir sorption isotherms are widely used in soil science for characterization of the P adsorption capacity of soils [122]. However, the information provided by this method has to be interpreted thoughtfully. Sorption isotherms describe the sorption equilibrium but provide no information about the kinetics of the adsorption- or desorption process [76]. Moreover, the Langmuir is an empiric equation that was developed under the following assumptions: The surface is homogeneous, the adsorbed molecule has no interaction between them and only one sorption mechanism is present. In the case of soil and organic residues this conditions are not completely fulfilled [76].

The results from the Langmuir isotherms are highly dependent of the experimental conditions. In the case of this investigation, they were 37°C, 16 h incubation time and using 1% TS of fresh manure. In order to use this method to compare the sorption capacity of different organic residues the same conditions must be maintained.

As a conclusion of this section, the results of the enzymatic batch process using real residues provided novel information about the performance of the enzymes in the residues biomass matrix. In previous investigations the enzymatic mineralization was carried out in liquid extraction solutions of manure or soil with solely analytical purposes [40, 41, 46, 48, 50]. These extraction solutions contained no solids and low interference ions because the samples were centrifuged and filtered before the enzymes were added. This means, that in this case the enzymes had access to freely soluble organic P compounds. Opposite to this, as demonstrated in this study, if the enzymes are added directly to the biomass matrix different interactions can occurred between the enzymes and released P with the other residues components, which reduces the enzymes performance.

5.2.4 Continuous enzymatic process using real residues

The enzymatic process using spiked manure samples was successfully verified in a continuous process. Compared with the batch test, the continuous operation revealed a higher performance in the total P release (68% versus 51.5 %). There are different explanations for this behavior. First, the 1% TS manure sample in the supply container was mixed continuously before being pumped into the reactor; this mechanical procedure could

have induced the release from P_i form the manure. Moreover the pH value had an oscillation of ± 0.5 in the reactor. As explained in Section 5.3, the pH value has a considerable effect on the solubilisation of P_i. As a result, at a lower pH value of 4.5, more P_i could have been solubilized. Considering only the P mineralization from the inositol phosphate spike, around 5% more P_i was released in comparison to the batch experiments. This could be explained by a possible chemical hydrolysis of the inositol phosphate spike under these conditions. Moreover, the contact area of the enzyme with the substrate could have been favor by the mixing in the reactor.

A continuous enzymatic process for the P release from agricultural residues was firstly verified in this study and was not found in previous studies. It was determined that phytase could mineralize, at least partially, the organic P content of the sample, although the performance in the residue matrix was reduced. For the process development this is an important finding because it proved that the enzymatic process can be suitable for residues with high organic P concentration (e.g. from inositol phosphate) and low concentration of other interfering ions, such as Ca^{2+}. To determine this, further characterization and investigation using other organic residues from agriculture or, for example, from the food industry is necessary.

5.3   Chemical process for the release of adsorbed P and dissolution of phosphate minerals

In agricultural residues the inorganic P ions can interact with the residue components. As a result, this P_i fraction is not available in solution for the P recovery from the liquid fraction of the residues. For instance, P_i can be adsorbed to the biomass matrix or precipitate as an insoluble phosphate salt. For this reason, the aim of this section was to investigate chemical methods for the release of adsorbed and insoluble P from a pig manure sample. The chemical processes investigated were acidification and addition of carbonate ions.

5.3.1 Acidification process using real residues

i.   Effect of the pH value in the release of phosphate from fresh residues

The dependency of the phosphate solubility with the pH value in the pig manure sample was demonstrated. The P_i in solution increased significantly between a pH value of 6 and 7. This demonstrated that the chemical acidification treatment was successful to release more than 90% of P from the samples. The increase in the P_i release by acidification can be explained

by the solubilisation of phosphate salts, such as calcium phosphates (i.e brushite, monetite, tricalcium phosphate and hydroxyapatite) and struvite. The solubility of these salts increases as the pH value decreases [77].

On the other hand, it is still unknown, whether the addition of sulfuric acid had an effect in the hydrolysis of the organic P in the samples. It is possible that the low pH values, together with the strong oxidizing effect of sulfuric acid disrupted the microbial cells and released organic P. This affirmation seems contradictory with the findings in the enzymatic experiments using model compounds (Section 4.2.2) where no chemical hydrolysis of the compounds was measured at pH 5. However, in this case acetate buffer were used instead of sulfuric acid, which could result in milder conditions. Moreover, changes in the pH value could have also released the P fraction that is adsorbed to the biomass matrix due to a variation in the absorbent surfaces charges [123].

ii. Quantification of the acid consumption for the process

The acid requirement for acidification of the agricultural residues was successfully determined by the standard method (DIN 38409-7) and by the procedure at pH 5. The quantity of acid needed to modify the pH value depends on the concentration of the buffer substances present in the sample. Important buffer systems are for instance hydrogencarbonate/carbonate, ammonium/ammonia, phosphate and sulfur [77, 124, 125]:

- Hydrogencarbonate / Carbonate

$$CO_3{}^{2-} + H^+ \leftrightarrow HCO_3{}^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

- Ammonium/ ammonia

$$NH_4{}^+ + OH^- \leftrightarrow NH_3 + H_2O$$

- Phosphate

$$H_3PO_4 \leftrightarrow H_2PO_4{}^- + H^+ \leftrightarrow HPO_4{}^{2-} + 2H^+$$

- H_2S/HS^-

$$H_2S \leftrightarrow HS^- + H^+$$

Moreover organic acids and humic acids present in the agricultural residues have also an influence in the buffer capacity of the samples. The method described in the norm DIN 38409-7 is developed for wastewater characterization and it is based in the equilibrium of the hydrogencarbonate/carbonate buffer system. However, if other buffer systems are present they are also included in the acid consumption. Therefore, this parameter expresses the buffer capacity of all buffer systems present in the samples.

The acid consumption (expressed as mmol $H^+ \cdot l_{sample}^{-1}$) determined by the standard method at pH 4.3 and the method at pH 5 is comparable (Table 4.8). This was expected because both acids used, HCl and H_2SO_4, are strong acids and dissociate completely. Moreover, the results suggest that after reaching a pH value of 5 the buffer effect of the different systems diminished. For this reason, the additional acid necessary to decrease the pH value from 5 to 4.3 was minimal.

As a conclusion the methodology of adjustment at pH value 5 predicted the acid consumption of the process presented in this study. Though, if a standard method has to be used for this measurement, the methodology based on the norm DIN 38409-7 reproduces similar results.

iii.   Continuous operation process using fresh residues and phosphate precipitation from the liquid fraction after solid-liquid separation

The acidification of pig manure in continuous operation was successfully performed. The results confirmed that more than 80% of the P was released after only one hour retention time. The effect of the temperature was not significant. This is an advantage for the process development because the process can be carried out at room temperature no heating is necessary.

The composition of the liquid fraction after solid-liquid separation showed that in pig manure the concentration of Ca^{2+} and Mg^{2+} is sufficient in comparison to $P-PO_4^{3-}$ to performed the precipitation process without addition of external chemicals (e.g. $MgCl_2$) as source of divalent ions. It is only necessary to increase the pH value by alkali addition. Moreover, the quantity of base (i.e. sodium hydroxide) needed for this purpose was very low (>0.1% weight). This finding represents a difference with wastewater treatment technologies were normally additional ions, such as Mg^{2+}, have to be added to carry out the precipitation process (e.g. as struvite).The phosphate salts obtained were identified as a mixture of different calcium and magnesium phosphates (Table 4.10). Moreover, the presence of $N-NH_4^+$ indicated that struvite was also present.

The concentrations of heavy metals and other potential pollutants were below the threshold values of the values of the German Fertilizer Ordinance (Table 5.9) [119]. This successfully proved that the product obtained could be used as a fertilizer, which is of central importance for further commercialization of the technology.

Table 5.9 Pollutant concentration the phosphate salts compared with threshold values

Element	Content [$mg \cdot kg_{DM}^{-1}$]		
	Phosphate salt	Threshold value	Declaration form
Hg	< 0.05	1	0.5
Tl	< 0.2 5	1	0.5
As	0.15	40	20
Pb	0.23	150	100
Cd	0.13	1.5	1
Ni	2.9	80	40
Cu	28	-	500
Zn	280	-	1,000

Calcium phosphate as a product of the process can have important applications besides of those for the fertilizer industry. The use of calcium phosphates has become very important in material science and technology, for example in the fabrication of ceramics, artificial bones and teeth. Moreover, there is a recent interest in using calcium phosphates as raw material on the field of electronics and surface (e.g. gas sensors, catalyst and chromatographic adsorbers) [126]. In these industries, calcium phosphate may be preferred over struvite since it is the same substance as the current raw material used, namely phosphate rock [127]. One of the problems regarding the use of phosphate rock, besides its scarcity, is its low quality [9]. The use of recycled calcium phosphate, with a high phosphate grade could, therefore mitigate this problem.

5.3.2 Carbonate addition process using real residues

The P concentration in solution was successfully increased by the addition of carbonate ions. The results of the experiments varying the carbonate concentration and pH value demonstrated that at pH 9.2 the optimal carbonate-calcium ratio was 60. A further addition had not a significant increase in the P_i release. This is an important finding because it verified that the quantity of carbonate salts added in the process can be minimized by adjusting the pH value. The increased in the P_i release at pH 9.2 can be explained by an enhanced precipitation of the calcium ions with carbonate under this pH condition.

Regarding the effect of time, no significant effect was determined under the experimental conditions. However, further studies should be carried out with longer times. It is possible that the reaction time of the carbonate ions with other components of the residues (which leads to an increase in the soluble phosphate) was too slow to be determined.

As expected, an increase in temperature led to an increase in the P_i release from the samples. This was anticipated because the solubility of calcium carbonates decreases with higher temperatures and, in this way, the phosphate ions are released to the solution. The difference between 21°C and 37°C reaction temperature was about 15% for the different reaction times. It is anticipated that at temperatures higher than 37°C the P_i released would increase. However, this affirmation has to be investigated in further studies because higher temperatures could also have an effect in other components of the samples, which could affect the P_i solubility.

The effect of the carbonate ions on the phosphate solubility is also reported in literature [65, 128]. This phenomenon is explained by the fact that carbonate ions can inhibit the formation and precipitation of insoluble phosphate salts by several mechanisms:

1) As mentioned before, carbonate precipitates with calcium as calcium phosphate. If the carbonate concentration is high enough, the Ca^{2+} ions in the hydroxylapatite (HAP) molecule, for example, react with carbonate, due to common ion effect, releasing the phosphates ions:

$$Ca_{10}(PO_4)_6(OH)_2\downarrow \rightarrow 10Ca^{3+} + 6PO_4^{3-} + 2OH^-$$

$$Ca^{3+} + 2\ HCO_3^- \rightarrow Ca(CO_3)_2 \downarrow + 2\ H^+$$

2) The second mechanism, as explained in Section 2.1.1. is the interference of carbonate ions in the crystal structure of calcium phosphates, for instance hydroxylapatite (HAP). According to literature, carbonate interfere by competing with the PO_4^{3-} and OH^- sites in the HAP structure. This reduces the crystallinity of HAP, thus increasing the phosphate solubility [128].

3) Another posible mechanim is the formation of complex compounds between carbonate and calcium [76]. Since calcium is unavailable in the solution, P is then released. This complex molecule is represented in Figure 5.3.

Figure 5.3 Ca-Carbonate complex

A combination of the different mechanism could have ocurred during the experiments. However, the low calcium concentration in comparison with phosphate after carbonate addition suggest that the precipitation of calcium as carbonate was the predominant effect.

The results demonstrated that the carbonate addition also increased the magnesium release. This suggest that magnesium did not precipitate as $MgCO_3$ under the experimental conditions. This finding was contradictory to other authors [64, 129] that suggest tha under certain conditions Mg^{2+} coprecipitate with calcium carbonate and reduce the effect of the carbonate ions in the P release.

As a conclusion, the addition of carbonate ions to the pig manure samples caused an increase in the P and Mg^{2+} ions in the liquid phase and the decrease of Ca^{2+} ions. These novel approach of adding carbonate ions for the P release from organic residues was not found in previous studies. The finding suggested that this method could be applied in the case that the phosphate ions need to be precipitated in salts other than calcium phosphates, for example as magnesium ammonium phosphate and potassium ammonium phosphate.

5.3.3 Thermodynamic simulation

i. Precipitation from acidified liquid fraction

The results of the simulation and experimental values of the precipitation from the acidfied liquid by increasing the pH value to 9 are comparable (Table 5.10). This finding proved that the precipitated product obtained consisted of a mixture of aproximatelly 27% hydroxylapatite and 73 % struvite (weight percentages repect to the dry precipitated product).

Moreover, from the output of the simulation it was calculated a phosphate salts production of 3.32 grams per liter of acidified liquid pig manure.

Table 5.10 Comparison of the precipitated salt composition between measured values and simulation

Element	Mass fraction [% weight]	
	Measurement	Simulation
P	13.8	14.6
N-NH$_4^+$	2.6	3.8
K$^+$	0.1	0.0
Ca^{2+}	11.2	13.6
Mg^{2+}	5.8	6.5

The small differences in the nutrient composition of the simulation respect to the experimetal value can explained by the fact that not all ions in the sample, such as micronutrients and heavy metas were considerer for the simulation. Moreover, for the simulation average concentration values from the different samples collected were used as an input. This average value could slightly differ from the specific nutrient content from the samples used in the experimets.

ii. Bicarbonate addition process

The fraction of P, Ca^{2+} and Mg^{2+} released during the carbonate addition process was compared for the simulation and measured values (Table 5.11).

Table 5.11 Comparison of the macronutrients release after carbonate addition between measured values and simulation

Element	Fraction of total content [%$_{mol}$]	
	Measurement	Simulation
P	64.5	19.0
Ca^{2+}	5.3	0.0
Mg^{2+}	58.5	0.3

For P and Mg^{2+} the simulation resulted in values considerable lower than those measured experimentally, namely 45.5% and 58.2% lower release, respectively. This lower P and Mg^{2+} content in the liquid fraction is explained by the fact that the simulation predicted that at a pH value of 9 this ions precipitate as struvite [68]. The results from the real samples suggest that the struvite precipitation did not occur. Different explanations for this finding are possible: First, the experiments were carried out at 37°C, however for the simulation the effect of temperature was not considered. Secondly, it is possible that carbonate ions reduces or

inhibit the formation of struvite crystals in a similar way that with hydroxylapatite, this would have as an effect that P is released into solution.

On the other hand, the model predicted that no hydroxylapatite could precipitate under these conditions, although this is a stable phase. Moreover, calcium precipitated completely as calcium carbonate. This suggests that the main effect of carbonate on the P release is the reaction with the calcium ions as calcium carbonate.

5.4 Applicability of the enzymatic and chemical processes

The P release from a pig manure sample with 1% TS was investigated by means of an enzymatic and two chemical processes, namely acidification and bicarbonate addition. In this investigation, it was first expected that the enzymatic and chemical processes would be combined for the effective P release since the refractory P in these samples could be present as both organic and unavailable inorganic P.

In the enzymatic process with industrial phytase, the pH value of the sample had to be adjusted to an optimal value of 5 for a better performance of the enzyme. This represented already a chemical treatment of the pig manure samples that caused a considerable increase in the P released, from around 20% to more than 65%. A further measureable P release after the incubation with phytase was not determined. This means that the enzymatic treatment is not necessary for those residues samples in which the solely acidification release most of the refractory P. Conversely, as demonstrated in the experiments with the addition of inositol phosphate spike, for residues with higher amounts of organic P compounds, the enzyme addition could result in an increase of the P release.

One limitation of the enzymatic process respect to acidification is that longer retention times are needed for the P release (16 h compared to 1 h). Consequently, for the process design higher reactor volumes are necessary for the residues treatment by the same flow rate by the enzymatic process. Moreover, the enzymatic reactor has to be heated to assure the optimal temperature for the enzymes.

In the carbonate addition process more than 64% of P and 58% Mg was released and, simultaneosly, the Ca content removed from the liquid fraction. This represent the advantage that P could be recovered as salts other than calcium phosphates. Moreover, the mixing time for the reactor was only 1 h and the pH value could be kept in the basic range, which was the same that the original pH value of the samples. In spite of this, a main disadvantage of this process was the high quantity of carbonate (e.g. as sodium bicarbonate)

that had to be added to have an effect in the P released, namely 66.4 mg $NaHCO_3$ per gramm sample.

5.5 Integrated nutrient recovery concept

A general design of a pilot plant to treat 100 kg·h^{-1} of pig slurry is presented in this section. The information and assumptions for the mass balances and process design were based on the results and interpretation of the experimental part and on literature research. An overview of the process including a general mass balance and a nutrient mass balance is shown in Figure 5.4 and Figure 5.5. The explanation and discussions of the different process steps are presented as follows:

1) Preliminary solid-liquid separation

Pig manure is separated into a solid (s_1) and a liquid fraction (l_1) by mechanical separation, for instance by a decanter. For the mass balances it is determined that 90.7% of the mass remains in the liquid fraction and 9.3% in the solid fraction. This step could also be omitted and the residues could be acidified directly. This would have the advantage that the investment and operating costs of the separation equipment would be avoided. However, a preliminary separation could also be favorable. First, most of the carbonate in the residue is removed from the solid fraction by mechanical separation, since most of the carbonate is dissolved in the liquid fraction. In this way, the problem of foam formation in the acidification step is minimized. Moreover, the quantity of acid needed for the pH adjustment is reduced because the carbonate buffering ions are removed. Secondly, most of the ammonium nitrogen and potassium are separated in the liquid fraction. If the nutrient recovery approach is focused on nitrogen and potassium recovery, or if these nutrients are to be recovered independently of phosphorus, the preliminary separation is a suitable option.

2) Nutrient recovery from liquid fraction

The nutrient concentration in the liquid fraction l_1 (Table 5.12) was calculated from the mass balances. The total P content in this fraction (~300 mg·l^{-1}) was relatively low if compared with the content in solid fraction. However, the P recovery from this fraction is still attractive for this concentration. A possible process is to precipitate the phosphorus as calcium, magnesium and ammonium salts (e.g. calcium phosphate, magnesium phosphate, struvite) by increasing the pH value to 9. In this case, additional Ca^{2+} or Mg^{2+} ions have to be added since the content already present is not enough for the precipitation. Considering an initial pH value of the manure liquid fraction of 8, it is estimated that less than 0.1% of base (e.g. sodium hydroxide) has to be added.

Table 5.12 Nutrient concentration in the liquid fraction l_1

Nutrient	Concentration [mg·l^{-1}]
N-NH$_4$$^+$	4,029
K$^+$	3,235
P$_{total}$	333
Ca^{2+}	173
Mg^{2+}	44

Due to the high N-NH$_4$$^+$ and K$^+$ concentrations on this liquid fraction, the recovery of these ions could be attractive. For this purpose, several processes can be used. For instance, ammonium nitrogen can be recovered by stripping (by means of pH or temperature increase) and precipitation as ammonium sulfate [130-132]. In the case of potassium, since the K$^+$ salts are very soluble, they can be recovered by an evaporation step, in which the solutions are concentrated and the salts crystallized [133].

For the mass balances, it is assumed that all ions in this liquid fraction (which are consider in the total solid content of 2.4%) and the soluble ammonia are recovered as salts and that water losses are only 1%. Consequently, 87.25% of the liquid can be reused in the process as recycled water.

3) Acidification (and enzymatic treatment) of the manure solution

The solid fraction s_1 is mixed in a tank with recycled water to obtain a solution with maximum 1% of total solids. This value of 1% TS was used for the calculations to keep the same conditions investigated in the experimental work. Nevertheless, for a real process a higher solid concentration could be considered. To minimize the use of fresh water in the process, the water output of step 2) Nutrient recovery from liquid fraction and 5) Enhanced phosphorus recovery, can be used as recycled water. For the calculations, it was assumed that the pH value of the recycled water did not change significantly the pH value of the manure solution. In a real process, this possibility has to be considered, because the recycled water coming from step 5) is alkaline, with a pH around 9. In this case, more acid will be necessary to modify the pH value.

After the manure sample is mixed, acid is added to decrease the pH value to 5. If sulfuric acid 96% is used for this purpose, the quantity needed is less than 0.25% w/w respect to the manure mixture mass. However, due to corrosion and security reasons the use of more diluted acid is recommended. For instance, for an acid concentration of 31% (6 M) the quantity added to the manure would be around 0.8%w/w.

In Section 4.3.1 it was determined, that modifying the pH value to 6 or lower was enough to release up to 95% of the P into the liquid fraction. Nevertheless, in the case that a lower

release occurs due to a high organic P concentration of the sample, the addition of enzymes for the mineralization of P_o could be considered. The quantity of enzyme necessary would be less than 0.01% of the sample (assuming an enzyme-substrate ratio of 60 $U \cdot mmol\ P_{organic}^{-1}$ and enzyme activity of 10 $U \cdot g_{enzyme}^{-1}$).

4) Solid-liquid separation

A second separation is carried out to obtain a liquid fraction (l_2) with higher P concentration. It was assumed that 95% of the mass remained in the liquid fraction and 5% in the solid fraction and that 95 % of the nutrients were released in the liquid fraction l_2 (Table 5.13).

Table 5.13 Nutrient concentration in the liquid fraction (l_2)

Nutrient	Concentration	
	$[mg \cdot l^{-1}]$	$[mmol \cdot l^{-1}]$
$P-PO_4^{3-}$	519	16.5
Ca^{2+}	593	14.8
Mg^{2+}	298	12.3
$N-NH_4^{+}$	392	27.8
K^{+}	183	4.7

5) Enhanced phosphorus recovery

The liquid fraction (l_2) is the input for the precipitation process. The precipitation can be carried out by increasing the pH value by addition of a base (e.g. sodium hydroxide) to a value of 9. The quantity of base needed is lower than 0.15% respect to the total liquid mass. The product of the precipitation step is a mixture of phosphate salts. The output water can be used as recycling water (r_2) to minimized the used of fresh water in the process. For the calculations, it was assumed that all the solids in the liquid fraction l_2, which is 0.59%, precipitate as salts. Moreover, this phosphate salt product is still wet with a solid content of 13%. The further drying of the salts (not shown in Figure 5.4) can be carried out by aeration at room temperature.

6) Drying and pelletizing of the solid fraction

The solid fraction (s_2) can be dried and then pelletized. During the pelletization the dried organic residues can be mixed with the phosphate salts obtained. In this way, a fertilizer product can be obtained, which have a nutrient ratio that can be modify on demand, depending on the specific crop needs.
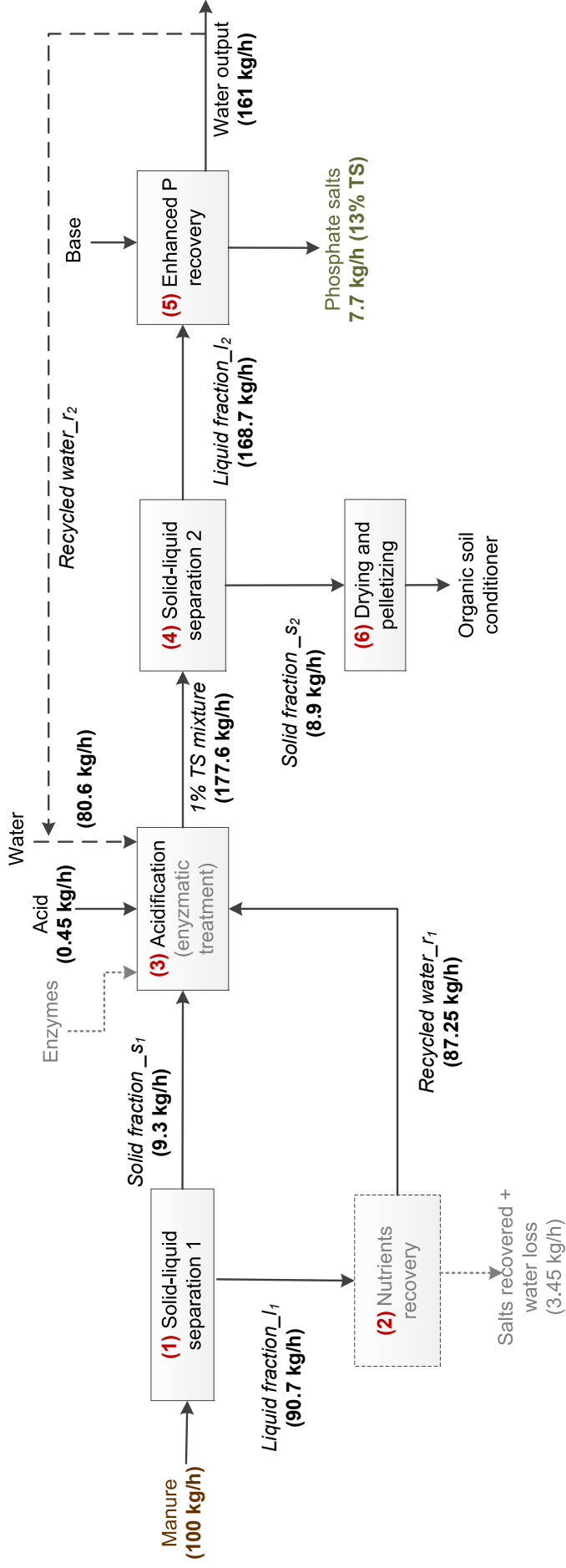
Figure 5.4 General mass balance of the process

Nutrient [g/h]

Ammonium-Nitrogen (NH4-N)
Phosphorus (P)
Potassium (K)
Calcium (Ca)
Magnesium (Mg)

Raw digestate (100%)

435
121
326
121
57

Solid-liquid separation 1

Liquid fraction 1

365.4 (84%)
30.2 (25%)
293.4 (90%)
15.7 (13%)
4.0 (4%)

Nutrient recovery (N, K)

Acidification (enzymatic treatment)

Solid fraction 1

Solid -liquid separation 2

Drying/pelletizing (organic soil conditioner)

Solid fraction 2

3.5 (0.8%)
4.6 (3.8%)
1.6 (0.5%)
5.3 (4.4%)
2.7 (4.7%)

Liquid fraction 2

66.1 (15.2%)
86.2 (71.2%)
31.0 (9.5%)
100.0 (82.6%)
50.3 (88.2%)

Enhanced P recovery (>99% P release from solid fraction 1)
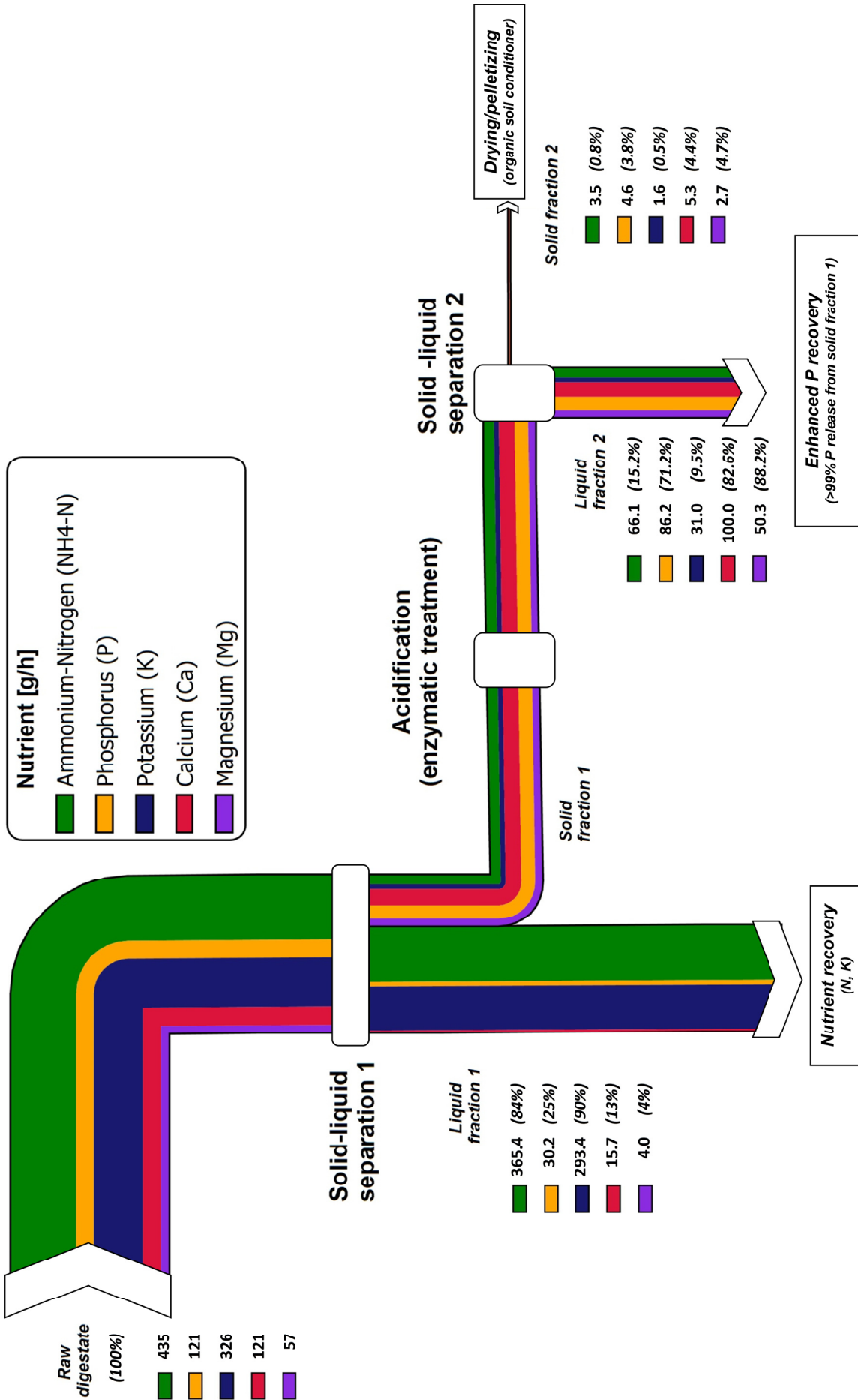
Figure 5.5 Nutrient mass balance of the process

6 Conclusions

The potential for P recycling from agricultural residues, such as animal manure and anaerobic digestate, is significant. However, state-of-the-art technologies have failed to effectively recover this nutrient because the recovery is limited by the low inorganic phosphate content (P_i) in the liquid fraction of the residues. In this thesis, one enzymatic process and two chemical processes were investigated to enhance the phosphorus recovery from agricultural residues. It was successfully confirmed that it is possible to increase the quantity of soluble phosphate in the liquid fraction after solid-liquid separation by chemical processes. Moreover, phosphate salts were successfully precipitated from the liquid fraction as a valuable fertilizer product. In this way, the efficiency of P recovery from agricultural residues can be increased. Regarding the enzymatic process, a measurable increase in the P released was only determined by the addition of an inositol phosphate spike to the sample.

The bicarbonate extractable P analysis using fresh samples confirmed that 80% of the P in pig manure and 60% in anaerobic digestate were refractory. Moreover, the mass balances of the solid-liquid separation proved that P in these samples remained mainly in the solid fraction. Additionally, the results suggested that P interacts with divalent ions, specifically calcium and magnesium, as insoluble phosphate compounds. In contrast, the other macronutrients, ammoniacal nitrogen and potassium, were more soluble and remain in the liquid fraction. These findings proved the limitation of the current technologies respect to P recovery, because for an efficient recovery the refractory P compounds have first to be released into solution.

The enzymatic process was successfully performed using P model compounds. The results confirmed that the enzymes with higher mineralization performance toward a wider range of substrates were principally the analytical grade wheat phytase followed by alkaline phosphatase. In the case of a mixture of these two enzymes, the P_i release varied from 64% up to 100% depending on the specific substrate. This suggested that an enzyme mixture is the most appropriate selection for the effective mineralization of a mixture of the substrates investigated. In spite of this, for the batch and continuous experiments, the industrial fungal phytase was selected due to economic reasons.

The enzymatic treatment experiments using pig manure samples and industrial fungal phytase showed no significant effect on the P_i release from the samples. On the other hand, when the samples were spiked with inositol phosphate, the phytase was able to mineralize 24.2% of the added organic P. Though this performance was about 50% lower compared

with the experiments with model compounds. These findings strongly suggest that the content of organic P in the sample was not high enough to detect significant differences in the P_i release after incubation with the enzymes. Probably the organic P in the samples was already chemically hydrolyzed due to the sulfuric acid addition to modify the pH value to 5. Additionally, the apparent reduction in the enzyme activity when using a spiked sample compared to model compounds, suggested that the enzyme was inhibited or that the P_i enzymatically released was bound with the sample components, for instance calcium ions, and could not be detected in the liquid phase. This second option was confirmed experimentally. It was determined that the sample could adsorb a maximum of 34 mg of P per gram of dry manure. This means that the P_i released was adsorbed into the biomass matrix and could not be detected in the solution.

It was demonstrated in the investigation that the acidification of the manure samples had a significant effect in the P solubility. The P_i release was successfully increased by 80% to 100% by acidifying the samples to a pH value of 5. The quantity of acid needed for this purpose could be effectively predicted by a developed methodology or by standard methods (DIN 38409-7) giving similar results. Furthermore, the acidified liquid fraction after solid-liquid separation contained a high concentration of calcium and magnesium ions. As a result, the precipitation of phosphate could be carried out by only increasing the pH value to 9, without the addition of other external chemicals to the process. The product obtained was rich in P (13.8%) and other important macronutrients. As determined in the thermodynamic assessment, P was present in the product as a mixture of hydroxylapatite and struvite. Additionally, the concentrations of heavy metals and other possible pollutants remained below the threshold values established by the German Fertilizer Ordinance. This means, that this product can be used as a valuable fertilizer.

The addition of carbonate ions to the manure samples successfully increased the P_i released more than 65% for the optimal parameters: pH value of 9.2 and carbonate-calcium ratio 60. On the other hand, no differences in the calcium release were detected, which confirmed that it remained in the solid fraction as insoluble calcium carbonate. Besides the precipitation of calcium carbonate, the mechanisms that explain the increase of phosphate by bicarbonate addition are interferences in the crystal structure of calcium phosphate and formation of complex calcium carbonate compounds. Regarding magnesium, similar to P its release was increased by the carbonate addition. As a consequence, phosphorus and magnesium could be precipitated together with other ions such as ammonium and potassium the insoluble phosphate salts struvite (magnesium ammonium phosphate) or K-struvite (potassium magnesium phosphate).

A comparison of the enzymatic and chemical processes showed that for the residues studied, the quantity of P released by the enzymes is significantly lower than the P release by chemical methods. In the case of acidification, the process could be carried out at room temperature and 1 h retention time, this means that the acidification process was more efficient in terms of P released and also energy consumption, because no heating was needed.

An integrated nutrient recovery concept was proposed for the sustainable use of pig manure. The process includes an acidification step for increasing the P_i release into the liquid fraction after separation of the solids. This acidification process was selected because it released more refractory P than the enzymatic and the carbonate addition process. The main product of the process is the precipitated phosphate salts. It was calculated that about 7.7 $kg \cdot h^{-1}$ wet phosphate salts product (1 $kg \cdot h^{-1}$ dry product) can be produced per 100 $kg \cdot h^{-1}$ manure. Moreover, organic soil conditioner and other nitrogen and potassium salts are additional valuable products that can be produced.

7 Outlook

The results of this thesis verified that enzymes can effectively mineralize a wide range of organic P model compounds.   However, the enzymatic process failed to achieve the expected results with real substrates such as animal manure. Thus, this enzymatic mineralization process should be investigated using other organic substrates with higher organic P content and lower inhibiting ions. For example, sewage sludge from bio-P treatment plants, residues for the food industry, etc. Additionally, the samples could be treated using processes for cell disintegration such as pulsed electric field [134, 135]. In this way, the organic P that is attached to the biomass or is part of the microorganism cells could be solubilized so that the enzymes can act more effectively.

Wheat phytase and alkaline phosphatase could effectively mineralize a wide range of organic P compounds. However, a considerable limitation for the experimental work was high price of these analytical grade enzymes. It is necessary to develop processes for the production of these enzymes on industrial scale. In addition, for analytical application the enzyme production is focused on obtaining a product with high specificity. In contrast, for the application proposed in this study it is required that the enzymes can hydrolyzed a wide range of compounds.

In the carbonate addition process, the quantity of sodium bicarbonate needed to the process has to be minimized by optimization of the operational parameters. For instance, the reaction kinetics of calcium carbonate formation has to be studied at different conditions of time and temperature. Moreover, the precipitation of the phosphate salts from the liquid fraction rich in carbonate ions after solid liquid separation should be investigated. Since the calcium is removed from the liquid fraction, a possibility would be the precipitation as struvite. For this, the molar ratio of P, Mg^{2+} and NH_4^+ has to be measured under the optimal conditions to determine if it is necesary the addition of one of the cations. Subquently, the equilibrium and kinetics of struvite under conditions of high carbonate concentratios has to be determined.

 An interesting approach for the further investigation of the carbonate addition process is the development of a integrated technology for P recovery and pathogen reduction in animal manure. Different investigations [136-139] suggest that adding carbonate reduces and even eliminate the concentration of pathogens in manure because carbonate has antimicrobial properties [136]. In this way, the use of relatively high quantities of carbonate salts in the process could be justified.

For the development of the nutrient recovery concept proposed in Section 5.4, further studies have to be carried out regarding the recovery of nitrogen and potassium, followed by long term experiments and scale up of the process. Additionally, the drying and pelletization process of the solid fraction should be investigated and optimized to obtain an organic product that can be commercialized.

8 References

1.   Rosemarin, A., G. de Brujine, and I. Caldwell *The next inconvenient truth: Peak Phosphorus*. 2009. 6-9.

2.   Cordell, D., J.-O. Drangert, and S. White, *The story of phosphorus: Global food security and food for thought.* Global Environmental Change, 2009. **19**(2): p. 292-305.

3.   Smit, A.L., et al. *Phosphorus in agriculture: global resources, trends and developments*. 2009.

4.   *Phosphorus availability in the 21st century. Management of a non-renewable resource.* 1998 [cited 2013 May 2013]; Available from: http://www.nhm.ac.uk/research-curation/research/projects/phosphate-recovery/p&k217/steen.htm.

5.   Young, R.S., *Chemical Analysis of Phosphate Rock*. Topics in Phosphorus Chemistry, ed. E.J. Griffith and M. Grayson. Vol. 8. 1976, New York: John Wiley & Sons, Inc.

6.   van Wazer, J.R., *Phosphorus and its Compounds*. Volume 1 Chemistry. 1958: Interscience Publishers

7.   Morse, G.K., et al., *Review: Phosphorus removal and recovery technologies.* Science of The Total Environment, 1998. **212**(1): p. 69-81.

8.   Schmundt, H. *Essential Element Becoming Scarce: Experts Warn of Impending Phosphorus Crisis*. 2010; Available from: http://www.spiegel.de/international/world/essential-element-becoming-scarce-experts-warn-of-impending-phosphorus-crisis-a-690450.html.

9.   de Ridder, M., et al. *Risks and Opportunities in the Global Phosphate Rock Market*. 2012.

10.   Sims, J.T., R.R. Simard, and B.C. Joern, *Phosphorus Loss in Agricultural Drainage: Historical Perspective and Current Research.* Journal of Environmental Quality, 1998. **27**(2).

11.   Fuchs, W. and B. Drosg, *Technologiebewertung von Gärrestbehandlungs- und Verwertungskonzepten*. 2010, Tulln: Universität für Bodenkultur Wien.

12.   Burton, C.H., C. Turner, and I. Silsoe Research, *Manure management : treatment strategies for sustainable agriculture*. 2003, Silsoe: Silsoe Research Institute.

13.   Menzi, H., et al., *Impacts of Intensive Livestock Production and Manure Management on the Environment*. Livestock in a Changing Landscape, ed. H. Steinfeld, et al. Vol. 1. 2010, Washington: Island Press.

14.   Foged, H.L., et al., *Inventory of Manure Processing Activities in Europe*, in *Technical Report to the European Commission*. 2011: Tjele.

15.   Stratful, I., M.D. Scrimshaw, and J.N. Lester, *Conditions influencing the precipitation of magnesium ammonium phosphate.* Water Res, 2001. **35**(17): p. 4191-9.

16.   Bouropoulos, N.C. and P.G. Koutsoukos, *Spontaneous precipitation of struvite from aqueous solutions.* Journal of Crystal Growth, 2000. **213**(3–4): p. 381-388.

17.   Dao, T.H. and R.C. Schwartz, *Mineralizable phosphorus, nitrogen, and carbon relationships in dairy manure at various carbon-to-phosphorus ratios.* Bioresour Technol, 2010. **101**(10): p. 3567-74.

18.   Fordham, A.W. and U. Schwertmann, *Composition and Reactions of Liquid Manure (Gülle), with Particular Reference to Phosphate: II. Solid Phase Components1.* J. Environ. Qual., 1977. **6**(2): p. 136-140.

19.   Oenema, O., D. Oudendag, and G.L. Velthof, *Nutrient losses from manure management in the European Union.* Livestock Science, 2007. **112**(3): p. 261-272.

20.   Sims, J.T., et al., *Nutrient management for intensive animal agriculture: policies and practices for sustainability.* Soil Use and Management, 2005. **21**(1): p. 141-151.

21.   *Common Manure Handling Systems.* [cited 2013; Available from: http://www.epa.gov/agriculture/ag101/porkmanure.html.

22.   Harrison, J.D. and D.R. Smith. *Nutrient Concentrations in Manure Storage Facilities.* 2004; Available from: http://digitalcommons.usu.edu/extension_histall/1168/.

23.   Fulhage , C. and D. Pfost. *Swine Manure Management Systems in Missouri.* 2001; Available from: http://extension.missouri.edu/p/EQ350.

24.   Honeyman, M.S., *Extensive bedded indoor and outdoor pig production systems in USA: current trends and effects on animal care and product quality.* Livestock Production Science, 2005. **94**(1): p. 15-24.

25.   Sorensen, J.T., et al., *Animal production systems in the industrialised world.* Rev Sci Tech, 2006. **25**(2): p. 493-503.

26.   Lukehurst, C.T., P. Frost, and T. Al Seadi *Utilisation of digestate from biogas plants as biofertiliser.* 2010.

27.   *Leitfaden Biogas. Von der Gewinnung zur Nutzung.* 5 ed. 2010, Gülzow: Fachagentur Nachwachsende Rohstoffe e.V.

28.   Hobbs, P., *Phosphorus recovery from unprocessed manure.* Phosphorus in Environmetal Technology, ed. E. Valsami-Jones. 2001, Padstow: IWA Publishing.

29.   FAOSTAT. [cited 2013; Available from: http://faostat.fao.org/site/729/default.aspx#ancor.

30.   Rigby, H. and S. Smith *New Markets for Digestate from Anaerobic Digestion.* 2011.

31.   Holm-Nielsen, J.B., T. Al Seadi, and P. Oleskowicz-Popiel, *The future of anaerobic digestion and biogas utilization.* Bioresource Technology, 2009. **100**(22): p. 5478-5484.

32. Burke, S., L. Heathwaite, and N. Preedy, *Transfer of phosphorus to surface waters; eutrophication*. Phosphorus in Environmetal Technology, ed. E. Valsami-Jones. 2001, Padstow: IWA Publishing.

33. Hansen, W., M. Christopher, and M. Verbuecheln. *EU Waste Policy and Challenges for Regional and Local Authorities*. in *Seminar on Household Waste Management "Capacity Building on European Community's Environmental Policy"*. 2002.

34. *EC Press release: Competitiveness (Internal Market, Industry and Research) (21 and 22 May 2007)*, in *2801st Council Meeting*. 21-22 May 2007.

35. *Fact Sheets on the European Union*, E. Parliament, Editor. 2009.

36. Oenema, O., *Governmental policies and measures regulating nitrogen and phosphorus from animal manure in European agriculture.* Journal of Animal Science, 2004. **82**(13 suppl): p. E196-E206.

37. Bomans, E., et al., *Addressing phosphorus related problems in farm practice* Final report to the European Commission, 2005.

38. Valsami-Jones, E., *The geochemistry and minerology of phosphorus*. Phosphorus in Environmetal Technology, ed. E. Valsami-Jones. 2001, Padstow: IWA Publishing.

39. Mattingly, G.E.G. and O. Talibudeen, *Progress in the Chemistry of Fertilizer and Soil Phosphorus*. Topics in Phosphorus Chemistry, ed. E.J. Griffith and M. Grayson. Vol. 8. 1976, New York: John Wiley & Sons, Inc.

40. Turner, B.L., et al., *Extraction of soil organic phosphorus.* Talanta, 2005. **66**(2): p. 294-306.

41. Turner, B.L. and A.B. Leytem, *Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure.* Environ Sci Technol, 2004. **38**(22): p. 6101-8.

42. Leinweber, P., L. Haumaier, and W. Zech, *Sequential extractions and 31P-NMR spectroscopy of phosphorus forms in animal manures, whole soils and particle-size separates from a densely populated livestock area in northwest Germany.* Biology and Fertility of Soils, 1997. **25**(1): p. 89-94.

43. Wienhold, B.J. and P.S. Miller, *Phosphorus Fractionation in Manure from Swine Fed Traditional and Low-Phytate Corn Diets* J. Environ. Qual., 2004. **33**(1): p. 389-393.

44. Zhang, Y.S., et al., *Effect of organic manure on organic phosphorus fractions in two paddy soils.* Biology and Fertility of Soils, 1994. **17**(1): p. 64-68.

45. Turner, B.L., *Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy.* J Environ Qual, 2004. **33**(2): p. 757-66.

46. He, Z., et al., *Phosphorus in Poultry Litter and Soil: Enzymatic and Nuclear Magnetic Resonance Characterization* Soil Sci. Soc. Am. J., 2008. **72**(5): p. 1425-1433.

47.   He, Z., et al., *Phosphorus forms in conventional and organic dairy manure identified by solution and solid state p-31 NMR spectroscopy.* J Environ Qual, 2009. **38**(5): p. 1909-18.

48.   He, Z., et al., *Comparison of phosphorus forms in wet and dried animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy and enzymatic hydrolysis.* J Environ Qual, 2007. **36**(4): p. 1086-95.

49.   Turner, B.L., N. Mahieu, and L.M. Condron, *Phosphorus-31 Nuclear Magnetic Resonance Spectral Assignments of Phosphorus Compounds in Soil NaOH–EDTA Extracts.* Soil Sci. Soc. Am. J., 2003. **67**(2): p. 497-510.

50.   He, Z., et al., *An enzymatic hydrolysis approach for characterizing labile phosphorus forms in dairy manure under mild assay conditions.* Bioresour Technol, 2006. **97**(14): p. 1660-8.

51.   Toor, G.S., B.J. Cade-Menun, and J.T. Sims, *Establishing a Linkage between Phosphorus Forms in Dairy Diets, Feces, and Manures.* J. Environ. Qual., 2005. **34**(4): p. 1380-1391.

52.   Bannink, A., et al., *Efficiency of Phosphorus and Calcium Utilization in Dairy Cattle and Implications for the Environment*. Phosphorus and Calcium Utilization and Requirements in Farm Animals, ed. D.M.S.S. Vitti and E. Kebreab. 2010, Oxfordshire: CAB International.

53.   Vitti, D.M.S.S., et al., *Phosphorus and Calcium Utilization in Ruminants Using Isotope Dilution Technique*. Phosphorus and Calcium Utilization and Requirements in Farm Animals, ed. D.M.S.S. Vitti and E. Kebreab. 2010, Oxfordshire: CAB International.

54.   Kiarie, E. and C.M. Nyachoti, *Bioavailability of Calcium and Phosphorus in Feedstuffs for Farm Animals*. Phosphorus and Calcium Utilization and Requirements in Farm Animals, ed. D.M.S.S. Vitti and E. Kebreab. 2010, Oxfordshire: CAB International.

55.   Lopes, J.B., et al., *Phosphorus and Calcium Utilization in Non-ruminants Using Isotope Dilution Technique*. Phosphorus and Calcium Utilization and Requirements in Farm Animals, ed. D.M.S.S. Vitti and E. Kebreab. 2010, Oxfordshire: CAB International.

56.   Lei, X.G. and J.M. Porres, *Phytase and Inositol Phosphates in Animal Nutrition: Dietary Manipulation and Phosphorus Excretion by Animals*. Inositol Phosphates. Linking Agriculture and the Environment, ed. B.L. Turner, A.E. Richardson, and E.J. Mullaney. 2007 Oxfordshire: CAB International.

57.   Knowlton, K., et al., *Phosphorus and Calcium Requirements of Ruminants*. Phosphorus and Calcium Utilization and Requirements in Farm Animals, ed. D.M.S.S. Vitti and E. Kebreab. 2010, Oxfordshire: CAB International.

58.   Holleman, A. and N. Wiberg, *Lehrbuch der anorganischen Chemie*. 101 ed. 1995, Berlin: Walter de Gruyter & Co.

59.   Butler, J.N., *Ionic Equilibrium: Solubility and pH calculations*. 1998: John Wiley & Sons.

60.   Cordbridge, D.E.C., *The Structural Chemistry of Phosphorus*. 1974, Amsterdam: Elsevier Scientific Publishing Company.

61.   Dorozhkin, S., *Calcium orthophosphates.* Journal of Materials Science, 2007. **42**(4): p. 1061-1095.

62.   Koutsoukos, P.G. and E. Valsami-Jones, *Principle of phosphate dissolution and precipitation*. Phosphorus in Environmetal Technology, ed. E. Valsami-Jones. 2001, Padstow: IWA Publishing.

63.   Xinde Cao, W.G.H., Manohardeep S. , *Inhibition of calcium phosphate precipitation under environmentally-relevant conditions.* Science of the total environment, 2007: p. 205-215.

64.   Cao, X. and W. Harris, *Carbonate and Magnesium Interactive Effect on Calcium Phosphate Precipitation.* Environmental Science & Technology, 2007. **42**(2): p. 436-442.

65.   Ferguson, J.F. and P.L. McCarty, *Effects of carbonate and magnesium on calcium phosphate precipitation.* Environmental Science & Technology, 1971. **5**(6): p. 534-540.

66.   Barralet, J., S. Best, and W. Bonfield, *Carbonate substitution in precipitated hydroxyapatite: an investigation into the effects of reaction temperature and bicarbonate ion concentration.* J Biomed Mater Res, 1998. **41**(1): p. 79-86.

67.   Vignoles, M., et al., *Influence of preparation conditions on the composition of type B carbonated hydroxyapatite and on the localization of the carbonate ions.* Calcif Tissue Int, 1988. **43**(1): p. 33-40.

68.   Doyle, J.D. and S.A. Parsons, *Struvite formation, control and recovery.* Water Research, 2002. **36**(16): p. 3925-3940.

69.   Huchzermeier, M.P. and W. Tao, *Overcoming Challenges to Struvite Recovery from Anaerobically Digested Dairy Manure.* Water Environment Research, 2012. **84**(1): p. 34-41.

70.   James D. Doyle, S.A.P. *Struvite formation, control and recovery*. 2002 25.01.2013]; Available from: http://144.206.159.178/FT/1092/72646/1241688.pdf.

71.   Turner, B.L., et al., *Inositol phosphates in the environment.* Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 2002. **357**(1420): p. 449-469.

72.   Shears, S.B. and B.L. Turner, *Nomenclature and Terminology of Inositol Phosphates: Clarification and a Glossary of Terms*. Inositol Phosphates. Linking Agriculture and the Environment, ed. B.L. Turner, A.E. Richardson, and E.J. Mullaney. 2007, Oxfordshire: CAB International.

73.   Selle, P.H., et al., *Phytate and phytase: consequences for protein utilisation.* Nutr Res Rev, 2000. **13**(2): p. 255-78.

74.   Alberts, B., et al., *Molecular Biology of the Cell*. 3 ed. 1994, New York: Garland Science.

75.   Cordbridge, D.E.C., *Phosphorus. An Outline of its Chemistry, Biochemistry and Uses*. Fifth ed. 1995, Amsterdam: Elsevier Scientific Publishing Company.

76.   Scheffer, F. and P. Schachtschabel, *Lehrbuch der Bodenkunde*. 16 ed. 2010: Spektrum Akademischer Verlag

77.   Stumm, W. and J.J. Morgan, *Aquatic Chemistry. Chemical Equilibria and Rates in Natural Waters*. 3rd ed. 1996, New York: John Wiley & Sons.

78.   Palmer, T., *Principles of Enzymology for Technological Applications*. 1993, Oxford: Butterworth-Heinemann.

79.   Dixon, M., et al., *Enzymes*. Third ed. 1979, London: Longman Group Limited.

80.   Coleman, J.E., *Structure and mechanism of alkaline phosphatase.* Annu Rev Biophys Biomol Struct, 1992. **21**: p. 441-83.

81.   Heinrikson, R.L., *Purification and Characterization of a Low Molecular Weight Acid Phosphatase from Bovine Liver.* Journal of Biological Chemistry, 1969. **244**(2): p. 299-307.

82.   Quiquampoix, H. and D. Mousain, *Enzymatic Hydrolysis of Organic Phosphorus*. Organic Phosphorus in the Environment, ed. B.L. Turner, E. Frossard, and D.S. Baldwin. 2005, Oxfordshire: CAB International.

83.   Brinch-Pedersen, H., L.D. Sørensen, and P.B. Holm, *Engineering crop plants: getting a handle on phosphate.* Trends in Plant Science, 2002. **7**(3): p. 118-125.

84.   George, T.S., et al., *Interactions Between Phytases and Soil Constituents: Implications for the Hydrolysis of Inositol Phosphate*. Inosito Phosphates. Linking Agriculture and the Environment, ed. B.L. Turner, A.E. Richardson, and E.J. Mullaney. 2001, Oxfordshire: CAB International.

85.   Choi, E., *Piggery Waste Management. Towards a Sustainable Future*. 2007, London: IWA Publishing.

86.   Burton, C.H., *The potential contribution of separation technologies to the management of livestock manure.* Livestock Science, 2007. **112**(3): p. 208-216.

87. Stevens, R.J., R.J. Laughlin, and J.P. Frost, *Effect of acidification with sulphuric acid on the volatilization of ammonia from cow and pig slurries.* The Journal of Agricultural Science, 1989. **113**(03): p. 389-395.

88. Schoumans, O.F., et al. *Phosphorus recovery from animal manure. Technical opportunities and agro-economical perspectives*. 2010.

89. Battistoni, P., et al., *Phosphorus removal from a real anaerobic supernatant by struvite crystallization.* Water Res, 2001. **35**(9): p. 2167-78.

90. Uludag-Demirer, S., G.N. Demirer, and S. Chen, *Ammonia removal from anaerobically digested dairy manure by struvite precipitation.* Process Biochemistry, 2005. **40**(12): p. 3667-3674.

91. Le Corre, K.S., et al., *Impact of calcium on struvite crystal size, shape and purity.* Journal of Crystal Growth, 2005. **283**(3–4): p. 514-522.

92. Bauer, P.J., A.A. Szogi, and M.B. Vanotti, *Agronomic Effectiveness of Calcium Phosphate Recovered from Liquid Swine Manure* Agron. J., 2007. **99**(5): p. 1352-1356.

93. Rückert, D., et al., *Rückgewinnung von Phosphaten (MAP) aus Gülle durch Membranfiltration und Fällung*, in *Rückgewinnung von Phosphor in der Landwirtschaft und aus Abwasser und Abfall*. 2003: Berlin.

94. Giesen, A., *Cristalactor: Crystallization Process Enables Environmental Friendly Phosphate Removal at Low Costs.*

95. Liberti, L., D. Petruzzelli, and L. De Florio, *REM NUT Ion Exchange Plus Struvite Precipitation Process.* Environmental Technology, 2001. **22**(11): p. 1313-1324.

96. *Jahresbericht des Ausschusses der Versuchstation "Unterer Lindenhof" 2011*. 2011, Universität Hohenheim: Eningen.

97. *Jahresbericht des Ausschusses der Versuchstation "Unterer Lindenhof" 2008*. 2008, Universität Hohenheim: Eningen.

98. Lemmer, A., et al., *Research biogas plant „Unterer Lindenhof".* Landtechnik, 2008(63): p. 80-81.

99. Naegele, H.-J., et al., *Electric Energy Consumption of the Full Scale Research Biogas Plant "Unterer Lindenhof": Results of Longterm and Full Detail Measurements.* Energies, 2012. **5**(12): p. 5198-5214.

100. Hedley, M.J. and J.W.B. Stewart, *Method to measure microbial phosphate in soils.* Soil Biology and Biochemistry, 1982. **14**(4): p. 377-385.

101. Cross, A.F. and W.H. Schlesinger, *A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems.* Geoderma, 1995. **64**(3–4): p. 197-214.

102. *Biogas*. 5 ed. 2012, Gülzow: Fachagentur Nachwachsende Rohstoffe e.V.

103.   Heinonen, J.K. and R.J. Lahti, *A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase.* Analytical Biochemistry, 1981. **113**(2): p. 313-317.

104.   Worsfold, P.J., et al., *Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils.* Talanta, 2005. **66**(2): p. 273-293.

105.   Murphy, J. and J.P. Riley, *A modified single solution method for the determination of phosphate in natural waters.* Analytica Chimica Acta, 1962. **27**(0): p. 31-36.

106.   Bezerra, M.A., et al., *Response surface methodology (RSM) as a tool for optimization in analytical chemistry.* Talanta, 2008. **76**(5): p. 965-977.

107.   Merkel, B.J. and B. Planer-Friedrich, *Groundwater Geochemistry: A Practical Guide to Modeling of Natural and Contaminated Aquatic Systems*. 2005: Springer-Verlag Berlin Heidelberg.

108.   Appelo, C.A.J. and D. Postma, *Geochemistry, groundwater and pollution*. 2nd ed. 2005: A.A. Balkema Pubishers

109.   Frank, D., *Experimentelle Untersuchung und Modellierung der Fällung von Kalium-Magnesium-Phosphat.* 2013, University of Stuttgart: Stuttgart.

110.   Söhnel, O. and J. Garside, *Precipitation : Basic principles and industrial applications*. 1992: Butterworth-Heinemann

111.   Sánchez, M. and J.L. González, *The fertilizer value of pig slurry. I. Values depending on the type of operation.* Bioresource Technology, 2005. **96**(10): p. 1117-1123.

112.   Bernal, M.P., A. Roig, and D. García, *Nutrient balances in calcareous soils after application of different rates of pig slurry.* Soil Use and Management, 1993. **9**(1): p. 9-14.

113.   Nicholson, F.A., B.J. Chambers, and K.A. Smith, *Nutrient composition of poultry manures in England and Wales.* Bioresource Technology, 1996. **58**(3): p. 279-284.

114.   Thiessen, H. and J.O. Moir, *Characterization of Available P by Sequential Extraction*, in *Soil Sampling and Methods of Analysis*, M.R. Carter and E.G. Gregorich, Editors. 2008, Taylor & Francis Group: Boca Raton.

115.   Schoenau, J.j. and I.P. O'Halloran, *Sodium Bicarbonate-Extractable Phosphorus*, in *Soil Sampling and Methods of Analysis*, M.R. Carter and E.G. Gregorich, Editors. 2008, Taylor & Francis Group: Boca Raton.

116.   Kieft, T.L., E. soroker, and M.K. firestone, *Microbial biomass response to a rapid increase in water potential when dry soil is wetted.* Soil Biology and Biochemistry, 1987. **19**(2): p. 119-126.

117. Turner, B.L., et al., *Potential contribution of lysed bacterial cells to phosphorus solubilisation in two rewetted Australian pasture soils.* Soil Biology and Biochemistry, 2003. **35**(1): p. 187-189.

118. Bartlett, R. and B. James, *Studying Dried, Stored Soil Samples — Some Pitfalls1.* Soil Sci. Soc. Am. J., 1980. **44**(4): p. 721-724.

119. in *Verordnung über das Inverkehrbringen von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln (Düngemittelverordnung - DüMV)*, L.u.V. Bundesministerium für Ernährung, Editor. 2012.

120. Nowack, B., *Environmental chemistry of phosphonates.* Water Research, 2003. **37**(11): p. 2533-2546.

121. Sharpley, A., P. Kleinman, and J. Weld, *Environmental Soil Phosphorus Indices*. 2 ed. Soil Sampling and Methods of Analysis, ed. M.R. Carter and E.G. Gregorich. 2006, Boca Raton: Taylor & Francis Group.

122. Zhou, A., H. Tang, and D. Wang, *Phosphorus adsorption on natural sediments: Modeling and effects of pH and sediment composition.* Water Research, 2005. **39**(7): p. 1245-1254.

123. Bleam, W.F., *Soil and Environmental Chemistry*. 2012, Boston: Academic Press.

124. Sommer, S.G. and S. Husted, *The chemical buffer system in raw and digested animal slurry.* The Journal of Agricultural Science, 1995. **124**(01): p. 45-53.

125. Husted, S., L.S. Jensen, and S.S. Jørgensen, *Reducing ammonia loss from cattle slurry by the use of acidifying additives: The role of the buffer system.* Journal of the Science of Food and Agriculture, 1991. **57**(3): p. 335-349.

126. Kanazawa, T., *Inorganic Phosphate Materials*, ed. K. Elsevier. 1989, Tokyo.

127. Valsami-Jones, E., *Mineralogical controls on phosphorus recovery from wastewaters.* Mineralogical Magazine, 2001. **65**(5): p. 611-620.

128. Cao, X., et al., *Inhibition of calcium phosphate precipitation under environmentally-relevant conditions.* Science of The Total Environment, 2007. **383**(1–3): p. 205-215.

129. Reddy, M.M. and K.K. Wang, *Crystallization of calcium carbonate in the presence of metal ions: I. Inhibition by magnesium ion at pH 8.8 and 25°C.* Journal of Crystal Growth, 1980. **50**(2): p. 470-480.

130. Saracco, G. and G. Genon, *High temperature ammonia stripping and recovery from process liquid wastes.* Journal of Hazardous Materials, 1994. **37**(1): p. 191-206.

131. Zeng, L., C. Mangan, and X. Li, *Ammonia recovery from anaerobically digested cattle manure by steam stripping.* Water Sci Technol, 2006. **54**(8): p. 137-45.

132. Laureni, M., et al., *Influence of pig slurry characteristics on ammonia stripping efficiencies and quality of the recovered ammonium-sulfate solution.* Journal of Chemical Technology & Biotechnology, 2013. **88**(9): p. 1654-1662.

133.   Garrett, D.E., *Potash - Deposits, Processing, Properties and Uses*. 1996, London: Chapman & Hall.

134.   Salerno, M.B., et al., *Using a Pulsed Electric Field as a Pretreatment for Improved Biosolids Digestion and Methanogenesis.* Water Environment Research, 2009. **81**(8): p. 831-839.

135.   Donsì, F., G. Ferrari, and G. Pataro, *Applications of Pulsed Electric Field Treatments for the Enhancement of Mass Transfer from Vegetable Tissue.* Food Engineering Reviews, 2010. **2**(2): p. 109-130.

136.   Arthurs, C.E., G.N. Jarvis, and J.B. Russell, *The Effect of Various Carbonate Sources on the Survival of Escherichia coli in Dairy Cattle Manure.* Current Microbiology, 2001. **43**(3): p. 220-224.

137.   Diez-Gonzalez, F., et al., *Use of Carbonate and Alkali To Eliminate Escherichia coli from Dairy Cattle Manure.* Environmental Science & Technology, 2000. **34**(7): p. 1275-1279.

138.   Jarvis, G.N., et al., *The mechanism of carbonate killing of Escherichia coli.* Letters in Applied Microbiology, 2001. **33**(3): p. 196-200.

139.   Park, G.W. and F. Diez-Gonzalez, *Utilization of carbonate and ammonia-based treatments to eliminate Escherichia coli O157:H7 and Salmonella Typhimurium DT104 from cattle manure.* Journal of Applied Microbiology, 2003. **94**(4): p. 675-685.

9 Appendix

Appendix I: Element concentrations of the raw pig manure and fractions after solid-liquid separation (sample Pig-1)

Parameters	Units	Raw Manure	Liquid fraction	Solid fraction
Total Solids	%	3.6 ± 0.9	2.4 ± 0.4	19.2 ± 0.4
Macro nutrients				
Nitrogen total (N)	$\%_{DM}$	13.6 ± 1.5	19.6 ± 4.0	5.8 ± 0.4
Ammonium Nitrogen (NH_4-N)		11.3 ± 0.8	18.6 ± 3.5	4.0 ± 0.5
Phosphorus (P)		3.1 ± 0.3	1.5 ± 0.0	5.8 ± 1.7
Potassium (K)		8.6 ± 1.9	15.0 ± 0.7	1.7 ± 0.2
Calcium (Ca)		3.1 ± 0.1	1.5 ± 0.5	5.4 ± 1.0
Mg (Mg)		1.4 ± 0.2	0.2 ± 0.1	3.7 ± 1.2
Sulfur (S)		0.9 ± 0.0	1.0 ± 0.1	0.6 ± 0.0
Micronutrients				
Boron total (B)	mg/kg_{DM}	77.9 ± 10.5	101.1 ±17.3	50.5 ± 9.9
Copper (Cu)		223.0 ± 100.0	230.3 ± 90.0	225.7 ± 69.8
Manganese (Mn)		789.3 ± 224.3	311.0 ± 137.8	1,544.3 ± 582.0
Molybdenum (Mo)		9.4 ± 0.7	11.1 ± 1.1	7.9 ±0.7
Zinc (Zn)		1,325.0 ± 115.0	1,280.0 ± 128.3	1,230.0 ± 37.4
Iron (Fe)		2,433.3 ± 531.2	2,100.0 ± 588.8	2,833.3 ± 590.7
Heavy metals and other pollutants				
Lead (Pb)	mg/kg_{DM}	<5.00	<5.00	<5.00
Cadmium (Cd)		<0.50	<0.50	<0.50
Chromium (Cr)		6.3 ± 0.7	5.8 ± 0.9	8.6 ±0.3
Nickel (Ni)		9.5 ±3.4	14.50 ± 3.54	8.3 ± 2.0
Mercury (Hg)		<0.50	<0.50	<0.50
Cobalt (Co)		<5.00	6.0 ± 0.2	<5.00
Arsenic (As)		<4.00	<4.00	<4.00
Thalium (Tl)		<0.2	<0.20	<0.2
Aluminium (Al)		265.0 ± 55.0	380.0 ± 21.6	426.7 ± 20.6

Appendix II: Total mass and element balances after solid liquid separation of sample 1,000 kg Pig-1

i) Total mass distribution after solid liquid separation of samples Pig-1

Fraction	Mass distribution [%]			
	Pig1-a	Pig1-b (n=1)	Pig1-b (n=2)	Average
Raw manure	100	100	100	100
Liquid fraction	16.6	3.1	9.3	9.7 ± 0.5
Solid fraction	83.4	96.9	90.7	90.3 ± 0.5

ii) Element mass balances

Parameters	Unit	Raw manure					Liquid fraction					Solid fraction				
		Pig1-a	Pig1-b(1)	Pig1-b(2)	Average	Std dev	Pig1-a	Pig1-b(1)	Pig1-b(2)	Average	Std dev	Pig1-a	Pig1-b(1)	Pig1-b(2)	Average	Std dev
Total Solids	Kg	40.0	32.0	45.0	39.0	5.4	15.8	23.3	27.2	22.1	4.7	32.7	5.8	17.9	18.8	11.0
Macronutrients	Kg															
Total Nitrogen (N)		5.4	5.0	5.3	5.2	0.2	4.0	3.9	4.6	4.2	0.3	1.7	0.4	1.1	1.0	0.6
Ammonium Nitrogen (NH_4–N)		4.4	4.0	4.7	4.3	0.3	3.7	3.8	4.3	3.9	0.2	1.1	0.3	0.7	0.7	0.3
Other Nitrogen (N_{other})		1.0	1.0	0.6	0.9	0.2	0.3	0.0	0.3	0.2	0.1	0.6	0.1	0.4	0.4	0.2
Phosphorus (P)		1.1	0.9	1.6	1.2	0.3	0.2	0.4	0.4	0.3	0.1	1.2	0.5	1.0	0.9	0.3
Potassium (K)		2.6	3.6	3.5	3.3	0.4	2.4	3.6	3.8	3.3	0.6	0.5	0.1	0.3	0.3	0.2
Calcium (Ca)		1.3	1.0	1.4	1.2	0.2	0.3	0.5	0.4	0.4	0.1	1.9	0.4	0.8	1.0	0.6
Magnesium (Mg)		0.6	0.4	0.8	0.6	0.1	0.0	0.1	0.0	0.0	0.0	0.8	0.3	0.6	0.6	0.2
Sulfur (S)		0.4	0.3	0.4	0.3	0.1	0.2	0.2	0.3	0.2	0.0	0.2	0.0	0.1	0.1	0.1
Micronutrients	g															
Boron (B)		2.6	2.9	3.5	3.0	0.4	1.2	2.7	2.9	2.3	0.8	2.1	0.2	0.8	1.1	0.8
Manganese (Mn)		21.4	24.1	48.6	31.4	12.2	1.9	10.1	10.3	7.4	3.9	24.0	12.0	32.8	22.9	8.5
Molybdenum (Mo)		0.4	0.3	0.5	0.4	0.1	0.2	0.2	0.3	0.3	0.1	0.3	0.0	0.2	0.2	0.1
Copper (Cu)		4.9	8.0	14.5	9.2	4.0	1.7	6.1	8.8	5.5	2.9	4.2	1.6	5.0	3.6	1.4
Zinc (Zn)		48.4	39.0	64.8	50.7	10.6	18.1	29.1	39.5	28.9	8.7	38.9	7.4	21.9	22.7	12.9
Iron (Fe)		72.0	76.8	139.5	96.1	30.8	20.6	53.5	73.5	49.2	21.8	65.4	19.1	57.4	47.3	20.2
Total		149.7	151.1	271.4	190.7	57.0										
Heavy metals and other pollutants	g															
Chromium (Cr)		0.3	0.2	0.3	0.2	0.0	0.1	0.1	0.1	0.1	0.0	0.3	0.1	0.1	0.2	0.1
Nickel (Ni)		0.2	0.4	0.6	0.4	0.1	0.2	0.4	0.5	0.3	0.1	0.2	0.1	0.2	0.1	0.1
Aluminium (Al)		8.4	10.2	10.8	9.8	1.0	6.2	9.3	9.5	8.3	1.5	14.7	2.5	7.2	8.1	5.0
Total		8.9	10.8	11.7	10.5	1.2										

Appendix III: Thermodynamic model

Carried out with the software Engineering Equation solver (EES)

- **Conditions in the reactor at t=0 (input)**

n_K2SO4_init =   "Moles in the reactor"

n_MgCl2_init =

n_NaH2PO4_init =

n_CaCl2_init =

n_NH4Cl_init =

n_NaOH_init =

n_Al_init =

n_Fe_init =

n_Zn_init =

n_Cu_init =

V_init = 1,0   "Volumen of the reactor "

- **Total ion concentration in the liquid fraction**

C_T_K = (2*n_K2SO4_init/V_init)

C_T_Mg = (n_MgCl2_init/V_init)

C_T_S = (n_K2SO4_init/V_init)

C_T_Cl = ((2*n_MgCl2_init + n_NH4Cl_init + 2*n_CaCl2_init)/V_init)

C_T_P = (n_NaH2PO4_init/V_init)

C_T_Na = ((n_NaH2PO4_init + n_NaOH_init)/V_init)

C_T_N = (n_NH4Cl_init/V_init)

C_T_Ca = (n_CaCl2_init/V_init)

C_T_Al = (n_Al_init/V_init)

C_T_Fe = (n_Fe_init/V_init)

C_T_Zn = (n_Zn_init/V_init)

C_T_Cu = (n_Cu_init/V_init)

- **Overview of the state forms considered**

C_T_K = C_K +C_KSO4 + C_KHPO4 + (n_KMP/V_init)

C_T_Mg = C_Mg + C_MgOH + C_MgSO4 + C_MgPO4 + C_MgHPO4 + C_MgH2PO4 + (n_KMP/V_init) + (n_MOH/V_init) + (n_MAP/V_init) + (3*n_MP/V_init) + (n_MHP/V_init)

C_T_S = C_SO4 + C_MgSO4 + C_KSO4 + C_HSO4 + C_NaSO4 + C_NH4SO4 + C_CaSO4

C_T_Cl = C_Cl

C_T_P = C_MgPO4 + C_HPO4 + C_MgHPO4 + C_PO4 + C_NaHPO4 + C_KHPO4 + C_H2PO4 +

      C_MgH2PO4 + C_H3PO4 + C_CaPO4 + C_CaHPO4 + C_CaH2PO4+ (n_KMP/V_init) +

      (n_MAP/V_init) + (2*n_MP/V_init) + (n_MHP/V_init) + (n_AlP/V_init) + (3*n_HAP/V_init) +

      ((2*n_FeP + n_FeP2)/V_init) + (2*n_ZnP/V_init) + (2*n_CuP/V_init)

C_T_Na = C_Na + C_NaSO4 + C_NaHPO4

C_T_N = C_NH3 + C_NH4 + C_NH4SO4 + C_CaNH3 + C_CaNH3_2 + (n_MAP/V_init)

C_T_Ca = C_CaPO4 + C_Ca + C_CaSO4 + C_CaHPO4 + C_CaNH3 + C_CaOH + C_CaNH3_2 +

      C_CaH2PO4 + (5*n_HAP/V_init)

C_T_Al = C_Al + (n_AlP/V_init)

C_T_Fe = C_Fe+ ((n_FeP + n_FeP2)/V_init)

C_T_Zn = C_Zn+ (n_ZnP/V_init)

C_T_Cu = C_Cu+ (n_CuP/V_init)

- **Definition of the state forms concentration**

C_H = 10^(log_C_H)

C_OH = 10^(log_C_OH)

C_Mg = 10^(log_C_Mg)

C_MgOH = 10^(log_C_MgOH)

C_MgSO4 = 10^(log_C_MgSO4)

C_SO4 = 10^(log_C_SO4)

C_K = 10^(log_C_K)

C_KSO4 = 10^(log_C_KSO4)

C_HSO4 = 10^(log_C_HSO4)

C_MgPO4 = 10^(log_C_MgPO4)

C_HPO4 = 10^(log_C_HPO4)

C_MgHPO4 = 10^(log_C_MgHPO4)

C_PO4 = 10^(log_C_PO4)

C_NaHPO4 = 10^(log_C_NaHPO4)

C_KHPO4 = 10^(log_C_KHPO4)

C_H2PO4 = 10^(log_C_H2PO4)

C_MgH2PO4 = 10^(log_MgH2PO4)

C_Na = 10^(log_C_Na)

C_NaSO4 = 10^(log_C_NaSO4)

C_H3PO4 = 10^(log_C_H3PO4)

C_NH3 = 10^(log_C_NH3)

C_NH4 = 10^(log_C_NH4)

C_NH4SO4 = 10^(log_C_NH4SO4)

C_CaPO4 = 10^(log_C_CaPO4)

C_Ca = 10^(log_C_Ca)

C_CaSO4 = 10^(log_C_CaSO4)

C_CaHPO4 = 10^(log_C_CaHPO4)

C_CaNH3 = 10^(log_C_CaNH3)

C_CaOH = 10^(log_C_CaOH)

C_CaNH3_2 = 10^(log_C_CaNH3_2)

C_CaH2PO4 = 10^(log_C_CaH2PO4)

C_Al = 10^(log_C_Al)

C_Fe = 10^(log_C_Fe)

C_Zn = 10^(log_C_Zn)

C_Cu = 10^(log_C_Cu)

- **Charge balances**

Z_0 = 0

Z_1 = 1

Z_2 = 2

Z_3 = 3

CB = 0

CB = (C_H*Z_1 + C_K*Z_1+ C_Mg*Z_2 + C_MgOH*Z_1 + C_Na*Z_1 + C_MgH2PO4*Z_1 + C_NH4*Z_1 + C_Ca*Z_2 + C_CaNH3*Z_2 + C_CaNH3_2*Z_2 + C_CaOH*Z_1 + C_CaH2PO4*Z_1 + C_Al*Z_3 + C_Fe*Z_3 + C_Fe*Z_2 + C_Zn*Z_2 + C_Cu*Z_2) - (C_OH*Z_1 + C_KSO4*Z_1 + C_SO4*Z_2+ C_Cl*Z_1 + C_HSO4*Z_1 + C_NaSO4*Z_1 + C_NaHPO4*Z_1+ C_MgPO4*Z_1 + C_HPO4*Z_2 + C_PO4*Z_3 + C_KHPO4*Z_1 + C_H2PO4*Z_1 + C_NH4SO4*Z_1 + C_CaPO4*Z_1)

- **Ionic strength and activity coefficients (Davies equation)**

$$I = 0{,}5*(C\_H*Z\_1^2 + C\_OH*Z\_1^2 + C\_SO4*Z\_2^2 + C\_K*Z\_1^2 + C\_KSO4*Z\_1^2+$$
$$C\_Mg*Z\_2^2+ C\_MgOH*Z\_1^2 + C\_Cl*Z\_1^2 + C\_HSO4*Z\_1^2 + C\_Na*Z\_1^2 +$$
$$C\_MgH2PO4*Z\_1^2+ C\_NaSO4*Z\_1^2 + C\_NaHPO4*Z\_1^2 + C\_MgPO4*Z\_1^2 +$$
$$C\_HPO4*Z\_2^2 + C\_PO4*Z\_3^2 + C\_KHPO4*Z\_1^2 + C\_H2PO4*Z\_1^2 +$$
$$C\_NH4*Z\_1^2 + C\_NH4SO4^Z\_1^2 + C\_Ca*Z\_2^2 + C\_CaNH3*Z\_2^2 +$$
$$C\_CaNH3\_2*Z\_2^2 + C\_CaOH*Z\_1^2 + C\_CaH2PO4*Z\_1^2 + C\_CaPO4*Z\_1^2 +$$
$$C\_Al*Z\_3^2 + C\_Fe*Z\_3^2 + C\_Fe*Z\_2^2 + C\_Zn*Z\_2^2 + C\_Cu*Z\_2^2)$$

A = 0,509

gamma_0 = 1

-LOG10(gamma_1) = (A*Z_1^2)*(I^0,5/(1+(I^0,5)) - 0,3*I)

-LOG10(gamma_2) = (A*Z_2^2)*(I^0,5/(1+(I^0,5)) - 0,3*I)

-LOG10(gamma_3) = (A*Z_3^2)*(I^0,5/(1+(I^0,5)) - 0,3*I)

- **Calculation of the activites from the measured concentrations**

log_act_H = log10(gamma_1) + log_C_H

log_act_OH = log10(gamma_1) + log_C_OH

log_act_Mg = log10(gamma_2) + log_C_Mg

log_act_MgOH = log10(gamma_1) + log_C_MgOH

log_act_MgSO4 = log10(gamma_0) + log_C_MgSO4

log_act_SO4 = log10(gamma_2) + log_C_SO4

log_act_K = log10(gamma_1) + log_C_K

log_act_KSO4 = log10(gamma_1) + log_C_KSO4

log_act_HSO4 = log10(gamma_1) + log_C_HSO4

log_act_MgPO4 = log10(gamma_1) + log_C_MgPO4

log_act_HPO4 = log10(gamma_2) + log_C_HPO4

log_act_MgHPO4 = log10(gamma_0) +log_C_MgHPO4

log_act_PO4 = log10(gamma_3) + log_C_PO4

log_act_NaHPO4 = log10(gamma_1) + log_C_NaHPO4

log_act_KHPO4 = log10(gamma_1) + log_C_KHPO4

log_act_H2PO4 = log10(gamma_1) + log_C_H2PO4

log_act_MgH2PO4 = log10(gamma_1) + log_MgH2PO4

log_act_Na = log10(gamma_1) + log_C_Na

log_act_NaSO4 = log10(gamma_1) + log_C_NaSO4

log_act_H3PO4 = log10(gamma_0) + log_C_H3PO4

log_act_NH3 = log10(gamma_0) + log_C_NH3

log_act_NH4 = log10(gamma_1) + log_C_NH4

log_act_NH4SO4 = log10(gamma_1) + log_C_NH4SO4

log_act_CaPO4 = log10(gamma_1) + log_C_CaPO4

log_act_Ca = log10(gamma_2) + log_C_Ca

log_act_CaSO4 = log10(gamma_0) + log_C_CaSO4

log_act_CaHPO4 = log10(gamma_0) + log_C_CaHPO4

log_act_CaNH3 = log10(gamma_2) + log_C_CaNH3

log_act_CaOH = log10(gamma_1) + log_C_CaOH

log_act_CaNH3_2 = log10(gamma_2) + log_C_CaNH3_2

log_act_CaH2PO4 = log10(gamma_1) + log_C_CaH2PO4

log_act_Al = log10(gamma_3) + log_C_Al

log_act_Fe = log10(gamma_3) + log10(gamma_2) +log_C_Fe

log_act_Zn = log10(gamma_2) + log_C_Zn

log_act_Cu = log10(gamma_2) + log_C_Cu

 -pH = log_act_H

log_K_w = log_act_H + log_act_OH

log_K_w = -13,997

pH = 11

- **Equilibrium reaction of aqueous complexes**

"Mg(2+) + OH(1-) = MgOH(1+)"

log_K_MgOH = 2,603

log_K_MgOH = log_act_MgOH - (log_act_Mg + log_act_OH)

"Mg(+2) + SO4(-2) = MgSO4 "

log_K_MgSO4 = 2,26

log_K_MgSO4 = log_act_MgSO4 - (log_act_Mg + log_act_SO4)

"K(+) + SO4(-2) = KSO4(-)"

log_K_KSO4 = 0,85

log_K_KSO4 = log_act_KSO4 - (log_act_K + log_act_SO4)

"H(+) + SO4(-2) = HSO4(-)"

log_K_HSO4 = 1,99

log_K_HSO4 = log_act_HSO4 - (log_act_H + log_act_SO4)

"Na(+) + SO4(-2) = NaSO4(-)"

log_K_NaSO4 = 0,73

log_K_NaSO4 = log_act_NaSO4 - (log_act_Na + log_act_SO4)

"Na(+) + H(+) + PO4(-3) = NaHPO4(-)"

log_K_NaHPO4 = 13,445

log_K_NaHPO4 = log_act_NaHPO4 - (log_act_Na + log_act_H + log_act_PO4)

"Mg(+2) + PO4(-3) = MgPO4(-)"

log_K_MgPO4 = 4,654

log_K_MgPO4 = log_act_MgPO4 - (log_act_Mg + log_act_PO4)

"PO4(-3) + H(+) = HPO4(-2)"

log_K_HPO4 = 12,375

log_K_HPO4 = log_act_HPO4 - (log_act_PO4 + log_act_H)

"Mg(+2) + H(+) + PO4(-3) = MgHPO4"

  log_K_MgHPO4 = 15,175

log_K_MgHPO4 = log_act_MgHPO4 - (log_act_Mg + log_act_H + log_act_PO4)

"K(+) + H(+) + PO4(-3) = KHPO4(-)"

log_K_KHPO4 = 13,255

log_K_KHPO4 = log_act_KHPO4 - (log_act_K + log_act_H + log_act_PO4)

"PO4(-3) + 2H(+) = H2PO4(-)"

log_K_H2PO4 = 19,573

log_K_H2PO4 = log_act_H2PO4 - (log_act_PO4 + 2*log_act_H)

"Mg(+2) + 2H(+) + PO4(-3) = MgH2PO4(+)"

log_K_MgH2PO4 = 21,2561

log_K_MgH2PO4 = log_act_MgH2PO4 - (log_act_Mg + 2*log_act_H + log_act_PO4)

"3H(+) + PO4(-3) = H3PO4"

log_K_H3PO4 = 21,721

log_K_H3PO4 = log_act_H3PO4 - (3*log_act_H + log_act_PO4)

"NH4(+) = NH3 + H(+)"

log_K_NH4 = -9,244

log_K_NH4 = log_act_NH3 + log_act_H - log_act_NH4

"NH4(+) + SO4(2-) = NH4SO4(-)"

log_K_NH4SO4 = 1,03

log_K_NH4SO4 = log_act_NH4SO4 - (log_act_NH4 + log_act_SO4)

"Ca(2+) + PO4(3-) = CaPO4(-)"

log_K_CaPO4 = 6,46

log_K_CaPO4 = log_act_CaPO4 - (log_act_Ca + log_act_PO4)

"Ca(2+) + SO4(2-) = CaSO4"

log_K_CaSO4 = 2,36

log_K_CaSO4 = log_act_CaSO4 - (log_act_Ca + log_act_SO4)

"Ca(2+) + H(+) + PO4(3-) = CaHPO4"

log_K_CaHPO4 = 15,035

log_K_CaHPO4 = log_act_CaHPO4 - (log_act_Ca + log_act_H + log_act_PO4)

"Ca(2+) + NH4(+) = CaNH3(2+) + H(+)"

log_K_CaNH3 = -9,144

log_K_CaNH3 = (log_act_CaNH3 + log_act_H) - (log_act_Ca + log_act_NH4)

"Ca(2+) + 2NH4(+) = CaNH4_2(2+) + 2H(+)"

log_K_CaNH3_2 = -18,788

log_K_CaNH3_2 = (log_act_CaNH3_2 + 2*log_act_H) - (log_act_Ca + 2*log_act_NH4)

"Ca(2+) + 2H(+) + PO4(3-) = CaH2PO4(+)"

log_K_CaH2PO4 = 20,923

log_K_CaH2PO4 = log_act_CaH2PO4 - (log_act_Ca + 2*log_act_H + log_act_PO4)

"Ca(2+) + OH(-) = CaOH(+)"

log_K_CaOH = 1,303

log_K_CaOH = log_act_CaOH - (log_act_Ca + log_act_OH)

- **Equilibrium reactions of possible solids**

"Mg(OH)2(s) --------------------------------------> Mg(2+) + 2OH(1-); Magnesiumhydroxid MOH"

log_Ksp_MOH = -11,15

log_IAP_MOH = log_act_Mg + 2*log_act_OH

SI_MOH = log_IAP_MOH - log_Ksp_MOH

"SI_MOH = 0"

"n_MOH = 0"

"MgNH4PO4(s) ----------------------> Mg(2+) + NH4(1+) + PO4(3-) ; Struvit MAP"

log_Ksp_MAP = -13,26

log_IAP_MAP = log_act_Mg + log_act_NH4 + log_act_PO4

SI_MAP = log_IAP_MAP - log_Ksp_MAP

"SI_MAP = 0"

"n_MAP = 0"

"MgKPO4(s) -------------------------------> Mg(2+) + K(1+) + PO4(3-); K-Struvit KMP"

log_Ksp_KMP = -10,62

log_IAP_KMP = log_act_Mg + log_act_K + log_act_PO4

SI_KMP = log_IAP_KMP - log_Ksp_KMP

"SI_KMP = 0"

"n_KMP = 0"

"Mg3(PO4)2(s) ---------------------------> 3Mg(2+) + 2PO4(3-); Boberrit MP"

log_Ksp_MP = -23,28

log_IAP_MP = 3*log_act_Mg + 2*log_act_PO4

SI_MP = log_IAP_MP - log_Ksp_MP

"SI_MP = 0"

"n_MP = 0"

"MgHPO4(s) ------------------------------------> Mg(2+) + HPO4(2-); Newberyit MHP"

log_Ksp_MHP = -5,8

log_IAP_MHP = log_act_Mg + log_act_HPO4

SI_MHP = log_IAP_MHP - log_Ksp_MHP

"SI_MHP = 0"

n_MHP = 0

"Ca5(PO4)3OH(s) -----------------------> 5Ca(2+) + 3PO4(3-) + OH(1-); Hydroxyalpatit HAP"

log_Ksp_HAP = -58,33

log_IAP_HAP = 5*log_act_Ca + 3*log_act_PO4 + log_act_OH

SI_HAP = log_IAP_HAP - log_Ksp_HAP

SI_HAP = 0

n_HAP = 0

"Al3PO4(s) ----------------------------------> Al(3+) + PO4(3-); Aluminium phosphate AlP"
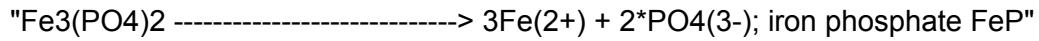
log_Ksp_AlP = -18,24

log_IAP_AlP = log_act_Al + log_act_PO4

SI_AlP = log_IAP_AlP - log_Ksp_AlP

"SI_AlP = 0"

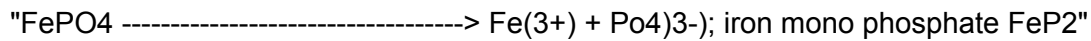n_AlP = 0

"Fe3(PO4)2 ----------------------------> 3Fe(2+) + 2*PO4(3-); iron phosphate FeP"

log_KsP_FeP = - 36

log_IAP_FeP = 3*log_act_Fe + 2*log_act_PO4

SI_FeP = log_IAP_FeP - log_KsP_FeP

"Si_FeP = 0"

n_FeP = 0

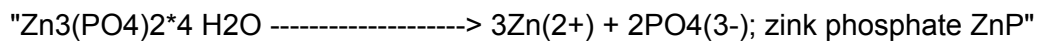"FePO4 -----------------------------------> Fe(3+) + Po4)3-); iron mono phosphate FeP2"

log_KsP_FeP2 = -26,4

log_IAP_FeP2 = log_act_Fe + log_act_PO4

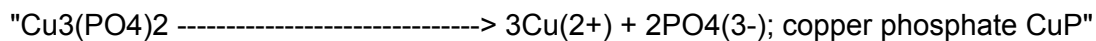SI_FeP2 = log_IAP_FeP2 - log_KsP_FeP2

"Si_FeP2 = 0"

n_FeP2 = 0

"Zn3(PO4)2*4 H2O --------------------> 3Zn(2+) + 2PO4(3-); zink phosphate ZnP"

log_KsP_ZnP = - 35,301

log_IAP_ZnP = 3*log_act_Zn + 2*log_act_PO4

SI_ZnP = log_IAP_ZnP -log_KsP_ZnP

"Si_ZnP = 0"

n_ZnP = 0

"Cu3(PO4)2 -------------------------------> 3Cu(2+) + 2PO4(3-); copper phosphate CuP"

log_KsP_CuP = - 36,854

log_IAP_CuP = 3*log_act_Cu + 2*log_act_PO4

SI_CuP = log_IAP_CuP - log_KsP_CuP

"SI_CuP = 0"

n_CuP = 0

Phosphorus (P) can be recovered in a sustainable way from agricultural residues (e.g. animal manure, digestate). However, current state of the art technologies can only recover the soluble inorganic P in the liquid fraction after mechanical separation, which can be as low as only 20% of the total P content. In this thesis, enzymatic and chemical processes for the increase of this soluble P content in the liquid fraction were investigated. In addition, a concept for the integrated nutrient recovery from pig manure was proposed.