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EXECUTIVE SUMMARY

The primary goal of the Circular Agronomics project was to evaluate upcoming technologies and procedures to improve carbon and nutrient management in the EU. The main drives to do so are to assist the transition to circular economy, reduce EU's dependency on imported nutrients and mitigate related environmental issues to surplus of no recycled nutrients including the associated GHG emissions. As a technology provider NuReSys focused on applying its developed struvite process for phosphorus recovery to attain the project goals. Soybean agro-industrial processing was selected for its high potential in terms of nutrient recovery (waste water rich in NPK) and given the high quantities of soybean imported in the EU to support as feed ingredient. A major challenge was the occurrence of phosphorus as phytic acid in soybean wastewater, this particular form of phosphorus cannot be recycled and needs to be converted first into free PO4-P.

Conversion of phytic acid to recoverable PO4-P was achieved by addition of commercially available phytase enzyme or could also be achieved by in vivo cultivation of selected strains of yeast/fungi producing the phytase enzyme. Although in vivo cultivation was successful the addition of readily available phytase enzyme (with a broad operational range of temperature and pH) did give the best results in terms of conversion efficiency. Up to 70-80% of the phosphorus present could be converted into recoverable PO4-P. This enzyme was dosed upfront the anaerobic processing under low pH (3.50 – 4.00) and elevated temperature (45-55°C) at low dosage of 5-10 µg/g COD.

Detailed analysis of the raw wastewater revealed the interesting feature that magnesium content is higher compared to the phosphorus content (Molar ratio of 1.2) and that the potassium to phosphorus ratio was even more pronounced (Molar ratio of 5.3). This opened the opportunity to produce NH4-struvite after the anaerobic stage without any addition of reagents. This could be achieved by increasing the pH due to CO2 stripping. Even of more importance was the finding that if not considering this feature of excess magnesium versus phosphorus a high risk of scaling does exists. An adapted anaerobic reactor design configuration was developed to prevent this and actually combine NH4-struvite recovery and scaling control (Fig. 26). This will be of major importance in further upscaling to industrial application level.

A second major achievement was the development of a process focused on potassium recovery. Potassium is an essential metabolic nutrient with a high water-solubility. Recovering potassium as a solid pure crystalline product would be a real market changer. This was achieved during the project by producing K-struvite on aerobically processed wastewater. The process did require an additional pH increase but could be intensified by applying on reverse osmosis concentrate containing even higher concentrations. A point of attention remains the crystal growth into sized pellet of 1-2 mm ready for reuse.

The results have also shown that compared to conventional treatment using Fe of Al coagulants the newly developed methods do produce far less amorphous P-sludge (50 to 100 % reduction). In addition, this amorphous P-sludge has little to no recovery use. And finally avoiding the use of technical grade coagulant such as FeCl3 40% prevent cross contamination with heavy metals such as cobalt and cadmium.

To summarize in brief:

1. Release of phosphorus from phytic acid to maximize P-recovery was shown to be possible by commercial phytase enzyme addition.
2. Non-reagent NH4-struvite formation can be of major importance but does require caution in reactor design and operation. A novel reactor approach combining P-recovery and scaling control is proposed
3. The possibility to recover potassium as a solid was demonstrated and is a unique technique to include potassium within the spectrum of recoverable nutrients.
4. Combining increase PO4-P conversion with K-struvite formation after the aerobic stage has additional benefits in terms of overall reduced sludge production.
5. The technologies proposed are already in some case at TRL level 9 and require only some further research to be combined into one consistent technology sequence.
6. Once brought to TRL 9 these technologies can contribute to the EU Green Deal goals related to fertilizer use.

1. INTRODUCTION

Our work does consisted out of two major parts to achieve the final goal of constructing and running a pilot unit on site of a major soy-bean processing plant to evaluate the possibilities of recovering phosphorus as a reusable resource using struvite technology. To do this, at first a number of knowledge based theoretical assumptions were made to select the best hotspots for the recovery of phosphorus in this typical agro-food industry. In particular, the fact that phosphorus is mainly present as phytic acid is an extra challenge since phosphorus recovery as struvite is only possible when the phosphorus is present a soluble PO4-P. This means that all will start and depend on the conversion of the phytic acid bound phosphorus into free recoverable phosphate (ic PO_4^{3-}-P) . The options retained for successful phosphorus recovery as struvite were the following ones:

a. Conversion of phytic acid bound P into PO_4^{3-}-P by means of adding phytase enzyme(s) in the raw incoming wastewater prior to the anaerobic stage.
b. Conversion of phytic acid bound P into PO_4^{3-}-P by means of adding phytase enzyme(s) after the first anaerobic treatment stage.
c. Leaching PO_4^{3-}-P by phytase enzymatic treatment out of solid fodder solid residue
d. Production of K-struvite as an alternative for NH4-struvite after the aerobic treatment stage.

As obvious, out of the listed options above, the use of phytase enzymes will be a of major importance. There are two ways to implement the enzymatic phytase treatment; either by adding commercial available phytase enzymes or inducing in vivo phytase enzymatic activity as part of the pilot unit. Both options were investigated.

The initial work was the establish, taking into account all considerations above, what would be the most appropriate way to continue in terms of selecting the best location for struvite formation and what means of using of phytase enzyme(s) would be suited.

An important extra notification is that the struvite process is mostly an add-on to an existing treatment plant. This makes an eventual full-scale implementation much easier since basically there will be no major changes needed in the existing treatment. However, this also means that specific situation on a possible application site will depend on what is already present. So, a site-specific approach will always be needed and might result in the fact that there is not a “single cover it all” solution. This needed flexibility will be retained to its maximum extend (what is technically possible) within the pilot design.

To gather, and/or confirm, all needed information a number of initial lab-scale trials were performed. These trials did consist out of a number of basic analysis but also included a number of incubation and batch wise treatment simulations. As results became available, a number of additional or clarifying trials were initiated.

The lab-trials studies were performed by Avecom NV (which is has been a long-term partner of NuReSys for all analytical and specific developed trials procedures in regard with struvite phosphate recovery) under supervision of Nuresys. The lab-scale trials were mainly focused on:

a. To liberate the P bound in phytic acid by addition of phytase, on the one hand commercially available phytase enzyme and on the other hand phytase obtained by fungal activity
b. To define optimum process parameters to convert the liberated PO_4^{3-}-P towards struvite in view of P-recovery.

2. CHARACTERIZATION OF WASTEWATER AND SOLID WASTE SAMPLES FROM SOYBEAN PROCESSING

2.1. Basic chemical composition

Since soybean, one of the major food and feed substrates, contains phosphorus as phytic acid, wastewater and solid waste samples from soybean processing were used for the tests.

Different samples were collected by NuReSys (October and November 2018 and Januari 2019) and were analyzed by Avecom. Not all of these samples showed an important difference between the total P and the free orthophosphate. An overview of the main characteristics of the 'suited' wastewater samples is presented in Table 1. A solid paste sample (sample delivery 1) was also obtained and analyzed (see Table 2). In Figure 1, a pH titration curve of both influent samples is graphically presented. The pH titration curve of the anaerobic effluent (sample delivery 2) is shown in Figure 2.

Table 1: Main characteristics of different wastewater samples from soybean processing

Parameter	Unit	Samples delivery 1		Samples delivery 2	
		Raw wastewater	Effluent aerobic treatment	Raw wastewater	Effluent anaerobic treatment
pH		3.7	8.2	3.8	7.0
COD total	mg/L	16830	83	15094	-
COD soluble	mg/L	13185	79	13070	1166
TSS*	mg/L	2320	20	1150	1630
VSS*	mg/L	2140	ND**	1150	1550
Kj-N total	mg/L	2282	14	-	-
NH_4^+-N	mg/L	32	1	-	360
NO_2^--N	mg/L	0	0	-	-
NO_3^--N	mg/L	18	0.3	-	-
P total	mg/L	162	-	176	130
P soluble	mg/L	141	3.7	154	85
PO_4^{3-}-P	mg/L	59	3.4	62	80
K^+	mg/L	1200	1100	-	-
Mg^{2+}	mg/L	160	110	-	-
Na^+	mg/L	500	400	-	-
Ca^{2+}	mg/L	160	120	-	-

*TSS/VSS: Total suspended solids and volatile suspended solids
**ND: Not detectable
-: Not determined

Table 2: Main characteristics of a paste sample from soybean processing

Parameter	Unit	Samples delivery 1
Dry Matter	%	14.64
Ash	%	0.61
Ash/Dry Matter	%	4.2
P	mg/g wet weight	77

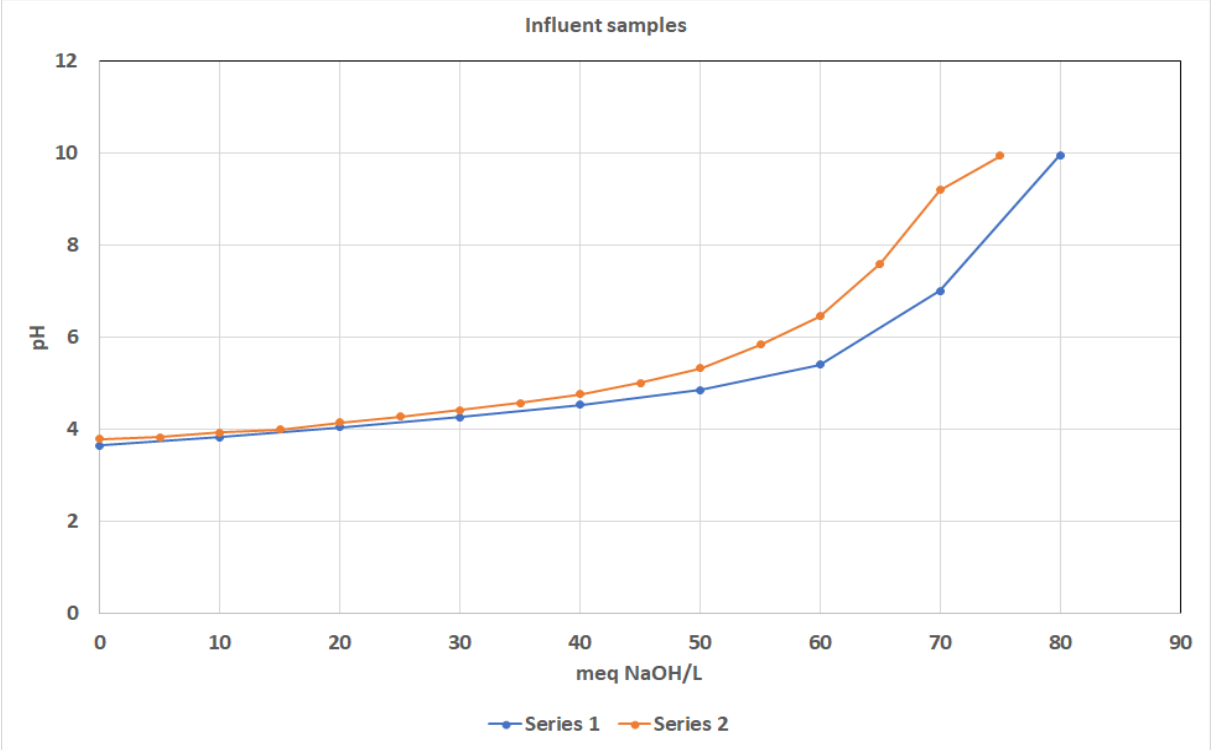


Figure 1: pH titration curves of the influent samples

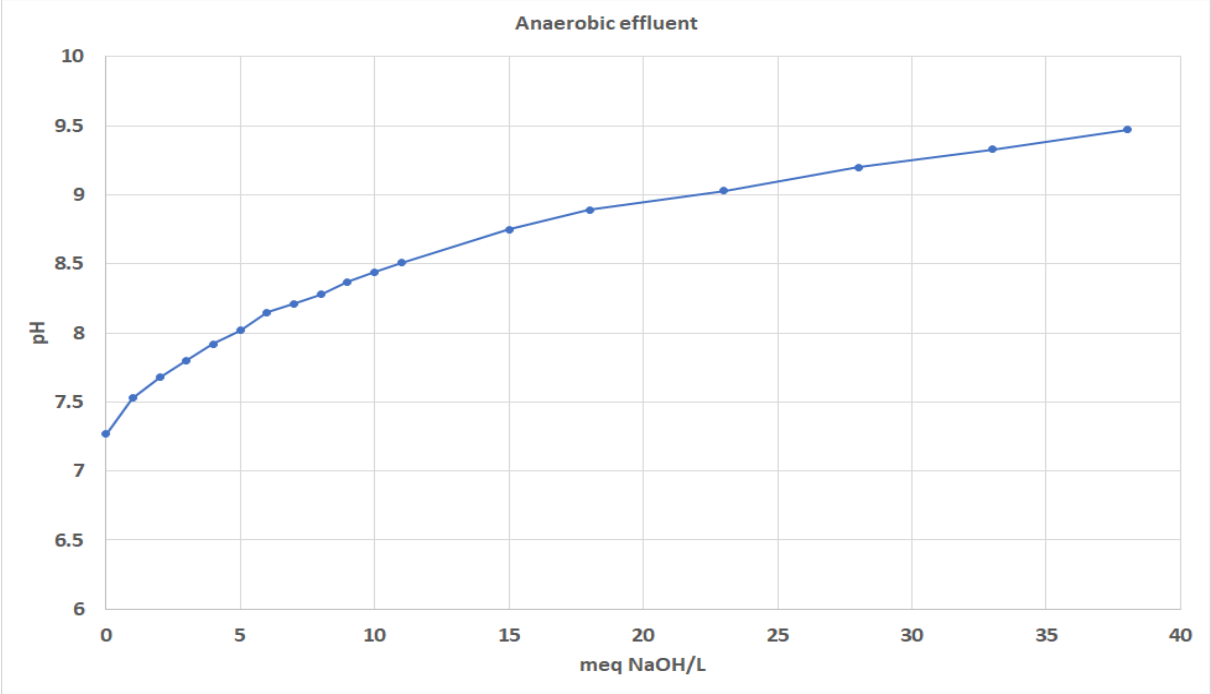


Figure 2: pH titration curve of the anaerobic effluent sample (delivery 2)

2.2. Chemical properties

The data show that the raw wastewater is at a relatively low pH (< 4), which is way to low to induce any kind of struvite formation. However, the levels of Mg 160 mg Mg/l or 6,5 mM and K (average 1200 mg K/l or 30 mM) are plenty versus even the total P of 162 mg P/l (or 5,1 mM). The level of NH4-N in the raw wastewater of 32 mg NH4-N/l (2,3 mM) is to low to produce struvite. The needed alkalinity to raise the pH form <' 4 up to 8 (a suitable value for struvite formation) was in the order of 70 meq NaOH what would correspond to nearly 8 liters of NaOH (29% w/w) per m3 of raw wastewater.

The low pH and relatively high incoming wastewater temperature (45 – 50 °C) are, although not that biologically friendly, well within the optimum conditions of some commercially available phytase enzymes. This could be a major advantage to have the phytic acid bound phosphorus liberated by the enzymatic limiting bacterial interference. Also cultivating the in vivo phytase active producing fungal cultures are more likely to run stable under these conditions.

2.3. Distribution different phosphorus species

Phosphorus levels present were split-up into three different fractions:

- Total P = measured as total phosphorus content measured of a well-mixed sample
- P soluble = measured as total phosphorus after centrifugation in supernatant
- PO4-P = measured as ortho-phosphate after centrifugation/filtration

The latter PO4-P is the one of interest for struvite formation. Both raw influent wastewater samples showed an important difference between the total P and free PO4-P, indicating the presence of phytic acid. The difference between Total P and P soluble is considered as the phosphorus related or present in the suspended solids. The raw influent samples were used for testing with the phytase enzyme.

The anaerobic effluent contained ortho-P (80 mg/L or about 2.6 mM) and ammonium-N (360 mg/l or about 26 mM). This sample was taken the second time, at which moment the anaerobic stage was working correctly. Although it should be noted that the effluent pH was only 7,0 (which for a normal stable anaerobic stage is normally somewhat higher between 7,50 to 8,50). The anaerobic effluent stage pH will turn out to be an important parameter, opening an alternative option for struvite recovery not considered initially. As such the lab-scale trials have already contributed substantially towards the goal to define the pilot plant to be included features.

3. LAB-SCALE TESTS WITH PHYTASE ENZYME

3.1. Tests with the wastewater samples of soybean processing

It was agreed on to express the phytase dosage as µg per g COD. For each of the influents tested, the samples were used as such and after separation of the suspended solids by centrifugation (centrates). An overview of the tests with the influent sample of delivery 1 is presented in Table 3 and in Figure 3. In Table 4, the corresponding results of the phytase tests with the influent sample of delivery 2 are given.

Table 3: Overview of the phytase tests (series A, B and C) with the influent sample of delivery 1 - At 37°C and reaction time of 3 - 4 hours

Series A	**Sample**	**Influent COD**	**Phytase addition**		**pH**	**PO_4^{3-}-P**	**P sol**
	*	g/L	µg/g COD	µg/L		mg/L	mg/L
Control 1	T	16.8	0	0	3.7	69	143
Test 1	T	16.8	10	168	3.7	104	160
Test 2	T	16.8	25	420	3.7	108	155
Test 3	T	16.8	50	840	3.7	107	156
Control 2	S	13.2	0	0	3.6	72	136
Test 4	S	13.2	10	132	3.6	95	144
Test 5	S	13.2	25	330	3.6	98	143
Test 6	S	13.2	50	660	3.6	98	144
Series B	**Sample**	**Influent COD**	**Phytase addition**		**pH**	**PO_4^{3-}-P**	**Psol**
	*	g/L	µg/g COD	µg/L		mg/L	mg/L
Control 1	T	16.8	0	0	3.7	78	
Test 1	T	16.8	2.5	42		105	149
Test 2	T	16.8	5	84		110	142
Test 3	T	16.8	7.5	126		113	164
Control 2	S	13.2	0	0	3.6	75	
Test 4	S	13.2	2.5	33		97	168

Test 5	S	13.2	5	66		99	169
Test 6	S	13.2	7.5	99		101	155
Series C	**Sample**	**Influent COD**	**Phytase addition**		**pH**	**PO_4^{3-}-P**	**P sol**
	*	**g/L**	**µg/g COD**	**µg/L**		**mg/L**	**mg/L**
Control 1	T	16.8	0	0	3.7	72	142
Test 1	T	16.8	0.5	8.4		94	166
Test 2	T	16.8	1	16.8		99	163
Test 3	T	16.8	1.5	25.2		97	163
Control 2	S	13.2	0	0	3.6	76	149
Test 4	S	13.2	0.5	6.6		89	153
Test 5	S	13.2	1	13.2		89	149
Test 6	S	13.2	1.5	19.8		90	149

*T= total influent: S = centrate (soluble fraction)

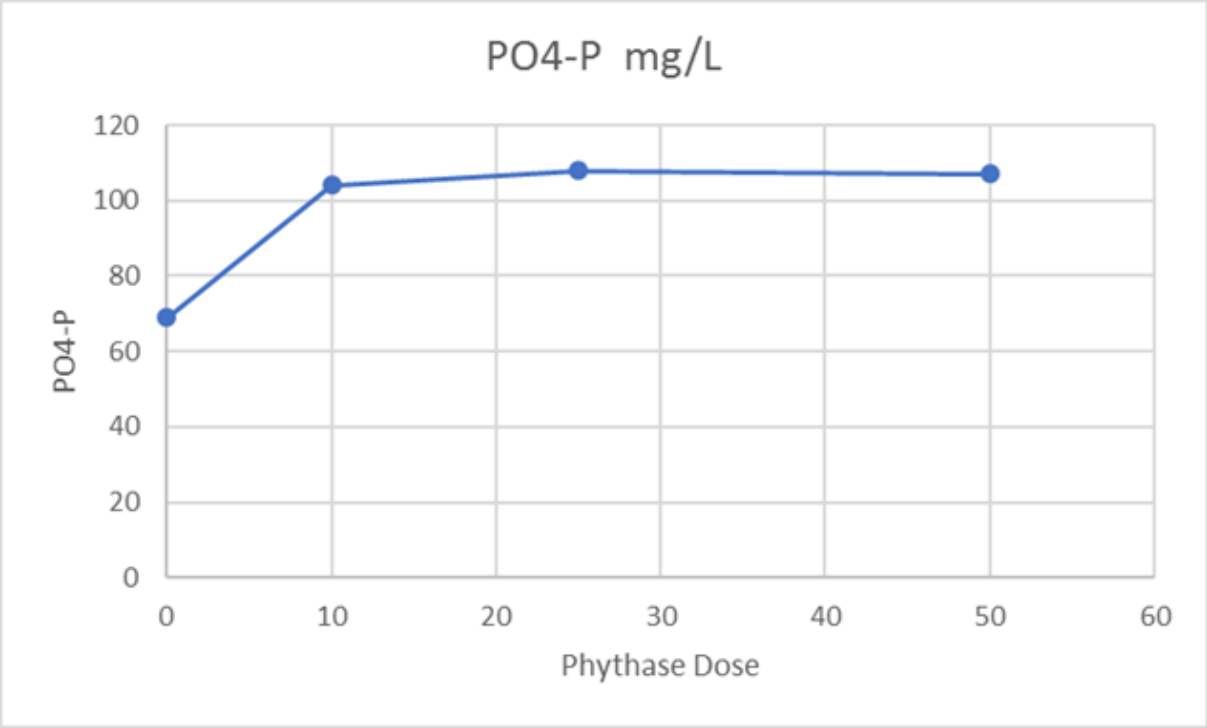


Figure 3: Correlation between the phytase dosage (µg/g COD) and the ortho-P concentration - Test with influent sample (delivery 1)

Table 4: Overview of the phytase tests with the influent sample of delivery 2 - At 33°C and reaction time of 3 hours

	Sample	**Influent COD**	**Phytase addition**		**pH**	**PO_4^{3-}-P**	**Psol**
	*	**g/L**	**µg/g COD**	**µg/L**		**mg/L**	**mg/L**
Control 1	T	15.09	0	0	3.8	66	152
Test 1	T	15.09	1	15	3.8	82	154
Test 2	T	15.09	2.5	38	3.8	82,5	171
Test 3	T	15.09	5	75	3.8	111	177
Test 4	T	15.09	10	151	3.8	114	173
Control 2	S	13.07	0	0	3.8		138
Test 5	S	13.07	1	13	3.8	77	138
Test 6	S	13.07	2.5	33	3.8	86.5	148
Test 7	S	13.07	5	65	3.8	90	141
Test 8	S	13.07	10	131	3.8	89	140

* T= total influent: S = centrate (soluble fraction)

Figure 4 gives a more graphic presentation of the results obtained using the commercial enzyme to liberate PO_4^{3-}-P out of the phytic acid present in the raw wastewater. The data shown in Figure 4 relate to the first sample of raw influent (second sample not shown but do confirm the results of first sample). The results in Figure 4 are those obtained on untreated raw influent (this means no removal of suspended solids). The latter considers the operational mode at the full-scale treatment plant on site.

Figure 4 show that PO_4^{3-}-P levels go up after phytase treatment, but not significant further increase is obtained at higher phytase dosage levels. Also, apparently the enzymatic treatment does somehow increase the P soluble level compared to the control. The reason why is not immediately clear but it seems the enzymatic additive affects the total phosphorus included in the suspended solids.

Figure 3 clearly show that maximum conversion is already achieved with the lowest dosage of enzyme initially tested. Additional trials (series B & C - Table 3) showed that the dosage level could even further be reduced without lower conversion rates.

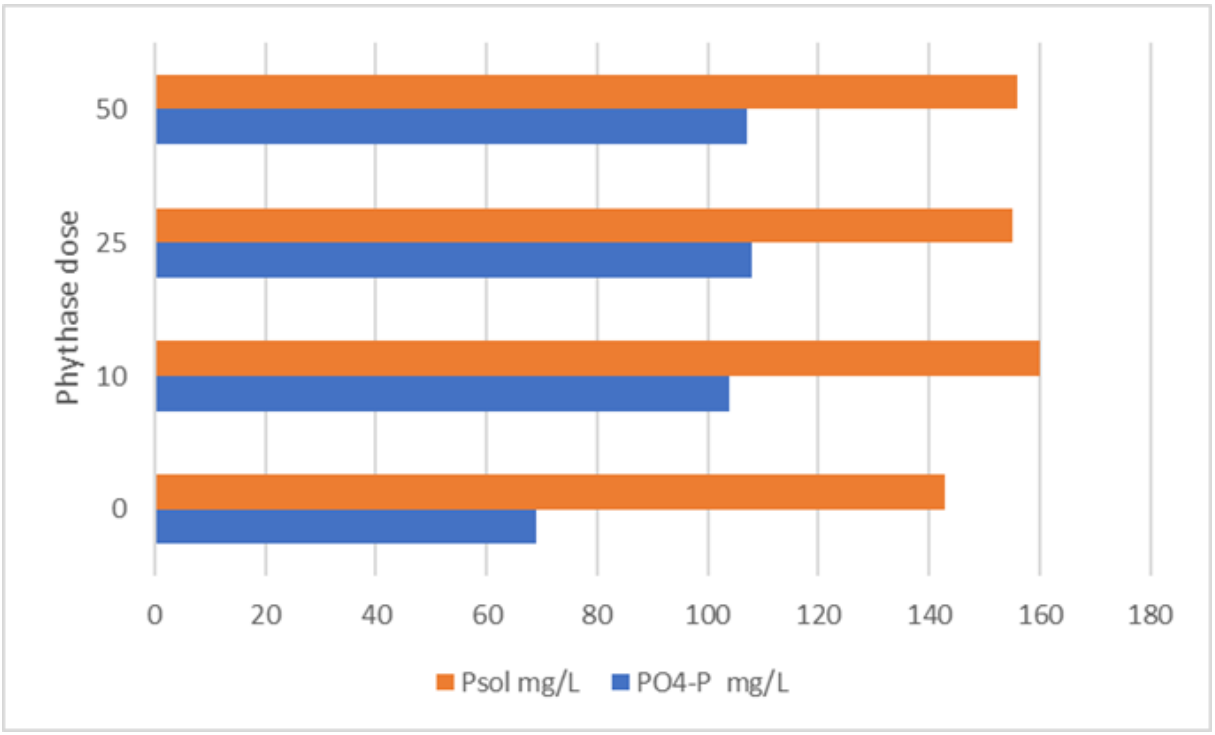


Figure 4: P soluble and PO4-P levels with different dosage levels of phytase enzyme (µg/g COD)

3.2. Evaluation of the tests with phytase enzyme

As shown in the tables above, all of the phytase dosages tested resulted in an increase of ortho-P. Hence, dosage of relatively small amounts of the phytase enzyme to the soybean processing wastewater resulted in liberation of PO_4^{3-}-P.

However, in none of the treatments a complete conversion of bounded P in solution (P soluble) into free PO_4^{3-}-P was obtained at this stage treating raw influent. In the tests with the first influent sample, the highest PO_4^{3-}-P concentrations were obtained at phytase dosages, ranging between 5 and 7.5 µg/g COD. In the tests with the second influent sample, the highest phytase dosages tested (5 to 10 µg/g COD) gave the highest liberation of PO_4^{3-}-P.

In general, good results for the release of free ortho-P from phytic acid were obtained with relatively low dosages of the commercially available phytase enzyme product. An additional benefit is that a phytase enzyme could be selected with optimum operational conditions in perfect line with the characteristics of the raw wastewater in terms of pH and temperature.

3.3. Tests with the solid paste sample of soybean processing

The following test procedure was followed:

- The paste sample was previously diluted with tap-water to obtain about 5 % dry matter (340 g wet weight/kg);
- The dilution was shaken during 1 h;
- Analyses were carried out on the dilution;
- Phytase tests were performed with the paste dilution. The phytase dosage was expressed as µg/g dry matter.

Diluting the sample to 5% dry weight was needed to obtain a mixture which could be used to dose the enzyme. Enzymatic treatment of the dry fodder product as such is not possible. Also, on has to take into account that the dry fodder product is flow generated in the soybean processing line. So, there is no possibility to apply the enzymatic process somewhere upstream in then soybean processing line. The needed dilution step, almost certainly needing a second dewatering treatment phase, is therefore a serious drawback to be considered. This means that this type of process approach will only be feasible if a major quantity of phosphorus could be recovered.

The pH of the diluted paste sample was 4.2 and the soluble P and ortho-P were respectively 27 mg/L and 19 mg/L. The low phosphate levels obtained in the diluted solution are in accordance with the low phosphorus content of the solid waste fodder as such (77 mg P/gram of wet material – Table 2).

The pH titration curve of the paste dilution is shown in Figure 5. Result did indicate a need of about 26 meq NaOH to attain a struvite favorable pH of 8, or in practice nearly 3 liters of 29% (w/w) NaOH per m3.

In Table 5, an overview of the phytase tests release test. Only a limited increase in both PO4-P and soluble P were noted. Orthophosphate increase form 19 to a maximum of 30 mg PO_4^{3-}-P/l as for soluble a maximal increase of 26.5 to 39 mg P/l was obtained.

In order to assess whether more P could be liberated from the pasta in case of a heat pretreatment, the diluted pasta was submitted to autoclaving (10 min at 120 °C). The soluble P and ortho-P concentrations were respectively 46 mg/L and 29 mg/L. So, no important increase of soluble P was obtained by means of a heat treatment of the diluted paste.

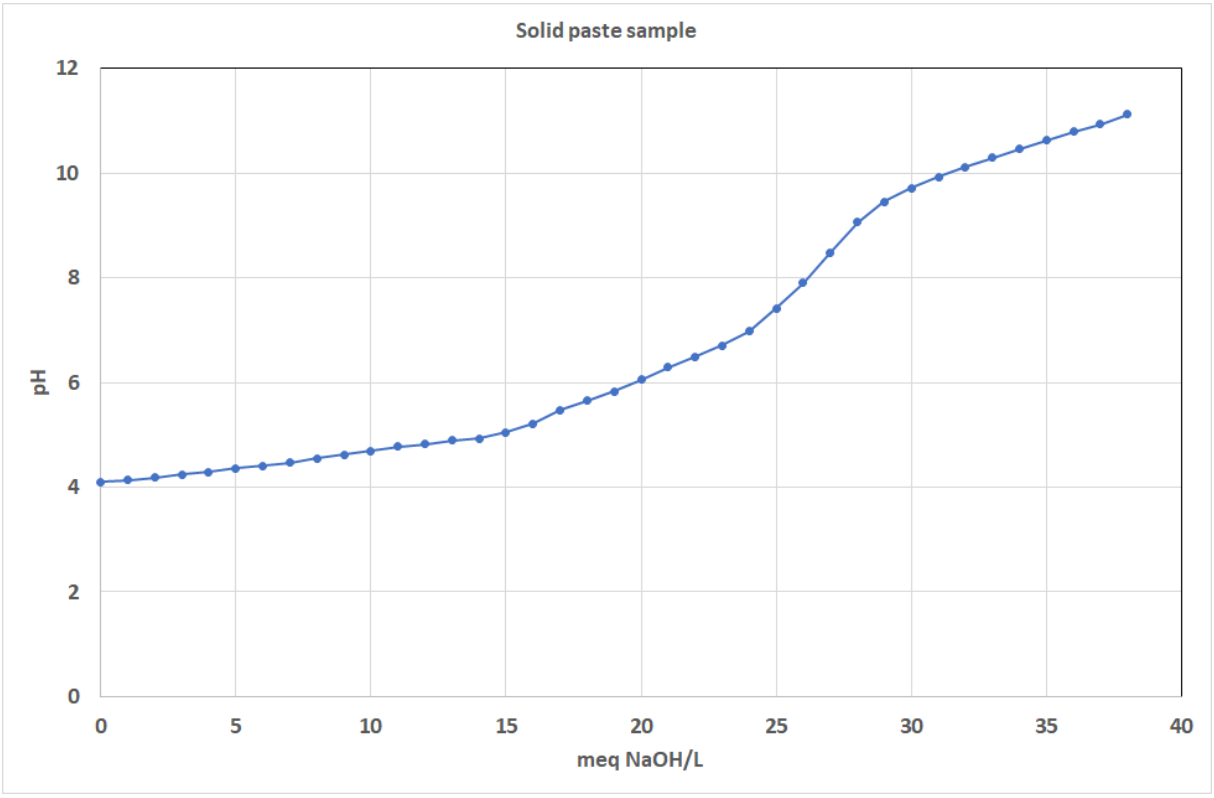


Figure 5: pH titration curve of diluted solid paste of soybean processing

Table 5: Overview of the phytase tests with the diluted paste sample - pH 4.2, at 37°C and reaction time of 3 hours

	Influent DM	Phytase addition		PO_4^{3-}-P	Psol
	g/L	µg/g DS	µg/L	mg/L	mg/L
Control	50	0	0	19	26.5
Test 1	50	1	50	23.5	33
Test 2	50	2.5	125	25	35
Test 3	50	5	250	27.5	38
Test 4	50	10	500	30	39
Test 5	50	20	1000	31	40
Test 6	50	50	2500	31	42

The dosage of the phytase enzyme at 5 to 10 µg/g DS to the diluted paste resulted in the highest liberation of ortho-P. However, the increase of PO_4^{3-}-P remained limited (from 19 to 30 mg/L) and a corresponding increase of soluble P (from 26.5 to 39 mg/L) was measured.

The analytical data of the wastewater and solid fodder also clearly showed that the majority of phosphorus recovery potential is allocated with the wastewater flow and not the solid fodder. So the main focus will be on the wastewater related flow's rather then the solid fodder.

At this stage the a test was included to see if inducing K-struvite formation would be an option worthwhile investigating. Recovering K at level would be a major achievement but little research on K recovery has been done so far.

The aerobic effluent (low in PO_4^{3-}-P do to ferric addition in treatment plant on site) was spiked with PO4-P in order to see if formation of K-struvite could be an alternative approach. The aerobic effluent was spiked form the initial 3,4 mg PO4-P up to 105 mg PO4-P/l. Adding the appropriate amount of MgCl2 resulted in a decrease to 45 mg PO4-P/l. Microscopic examination of the settle residue showed some crystalline matter was produced (Figure 6), which is most likely K-struvite. Further tests will need to confirm this or not.



Figure 6: Crystalline matter produced aerobic effluent spike test

4. CULTIVATION OF FUNGI AND/OR YEASTS FOR IN VIVO PHYTASE PRODUCTION

4.1. Literature scan in vivo phytase enzyme production

Based on literature (B. Singh et al., 2014 and A. Vohra et al., 2001 S. Gargova et al., 1997), 2 fungal strains were selected for testing. It concerned *Pichia anomala* (or *Wickerhamomyces anomalus* - ATCC 8168 sequenced) and *Aspergillus ficuum* (ATCC 66876). Both strains were ordered at a Belgian culture collection but since the *Aspergillus ficuum* strain was not directly available, it took almost half a year before the strain was delivered at Avecom (end of March 2019). Hence, so far, only cultivation tests were performed with *Pichia anomala*.

For the cultivation of *Pichia anomala*, synthetic agar phytase screening medium was used, containing (g/L): D-glucose - 15.0; phytic acid sodium salt hydrate $C_6H_{18}O_{21}P_6.xNa.yH_20$ - 3.7 as sole P-source; ($NH_4.NO_3$ - 5.0; $MgSO_4.7H_2O$ - 0.5; KCl - 0.5; $FeSO_4.7H_2O$ - 0.01; $MnSO_4.4H_2O$ - 0.01. The pH was adjusted to 4.7 using 1 M HCl before autoclaving at 120°C for 20 min. After autoclaving, the pH amounted to 4.8

The cultivation in the liquid medium was performed in 100 mL Erlenmeyer flasks containing 30 mL nutrient medium, each inoculated with 5 colonies of *Pichia anomala*, previously grown on potato dextrose agar plates (PDA). The flasks were placed on a shaker at a temperature, ranging between 30 and 37 °C. Growth and phytase activity of *Pichia anomala* was followed in time. Growth of the appropriate strain was confirmed by plating on PDA.

4.2. In vivo induced phytase production by Pichia anomala

4.2.1. Batch in vivo cultivation

Analyses of ortho-P at the start demonstrated that part of the bound P in the phytic acid sodium salt was liberated because of the heat treatment (autoclaving). Therefore, the **batchtest** was repeated and the growth medium without phytic acid was previously autoclaved. Within a period of 2 to 7 days, no important increase of ortho-P was detected in both batch experiments, as demonstrated in Table 6.

Table 6: Evolution of phosphorus concentrations in the cultivation batchtests with Pichia anomala

Autoclaving of total medium		**start**	**2 days**	**5 days**
P total	**mg/L**	1390	-	-
P soluble	**mg/L**	-	917	1350
PO_4^{3-}-P	**mg/L**	317	347	325

Autoclaving of medium without phytic acid		**start**	**4 days**	**6 days**	**7 days**
P total	**mg/L**	999	976	984	1050
P soluble	**mg/L**	-	-	966	957
PO_4^{3-}-P	**mg/L**	39	18	28	28

-: not measured

In none of the tests, an important increase of ortho-P, demonstrating phytase activity of the cultivated *Pichia anomala*, could be experimentally detected.

4.2.2. Continuous in vivo cultivation

In a further approach, it was examined whether phytase activity of *Pichia anomala* could be induced in a more continuous cultivation test, with regular additions of C-source (D-glucose) and/or N-source (NH_4NO_3). Since P is also needed for biomass growth and since the only P-source in the cultivation medium was phytic acid, it was assumed that decrease of soluble P (part of the P is incorporated in the newly grown biomass) and/or increase of ortho-P would occur, both indicating phytase activity. The cultivation test was started and was followed for almost one month. On the basis of the soluble COD analyses, D-glucose was added 3 times in the course of the test. At the start, the COD and N-concentrations amounted to respectively about 15 g/L and 1.75 g/L. In total, 50 g COD/L and 2.5 g N/L were extra added and were mostly converted. After each batchwise feeding, the soluble COD concentration decreased to below 5 g COD/L within a few days, clearly indicating growth of *Pichia anomala*. However, as shown in Table 7, the latter was not accompanied with a clear liberation of ortho-P from phytic acid.

Table 7: Evolution of phosphorus concentrations in the cultivation continuous test with Pichia anomala

		start	6 days	9 days	12 days	13 days*	20 days	27 days
P total	**mg/L**	997	1060	-	-	514*	456	342
P soluble	**mg/L**	-	933	873	762	338	364	246
Psol/Ptot	**%**	-	88	-	-	66	80	72
PO_4^{3-}-P	**mg/L**	30	155	31	17	10	14	20

*Lower P concentrations due to enlargement of the active reactor volume (dilution with water)
-: Not measured

Because of frequent sampling for analyses, the reactor volume was enlarged on day 13 by adding tap-water (dilution). To the latter, extra C- and N-source and also trace elements were supplied. Except for the PO_4^{3-}-P measurement after 6 days of cultivation, there was no important increase of free PO_4^{3-}-P in the cultivation medium. As the test progressed, there was a gradual decrease of soluble P. Maybe the latter was due to conversion of phytic acid into free ortho-P and immediate take-up for biomass growth. However, since also the total P concentration decreased significantly at the end of the test, the soluble P decrease could not clearly be attributed to phytase activity.

No further tests with wastewater or solid waste were performed with this cultivated fungal medium. The cultivation tests will be repeated with another fungi strain, namely *Aspergillus ficuum*. The trials with this organism were not performed yet due to late delivery of the strain.

5. IN VIVO PHYTASE PRODUCTION BY ASPERGILLUS FICUUM

5.1. Species identification

The first attempt to produce in vivo phytase by cultivating a specific selected yeast (ic. *Pichia anomala)* did result in a noticed increased conversion of phytic acid into free PO_4^{3-}P. (Paragraph 4).

A second set of trials with another selected organism, fungus *Aspergillus ficuum* ATCC 66876 (BSL-1), was initiated to see if this would result in effective in vivo phytase activity or confirm the earlier results with yeast *Pichia anomala.*

5.2. Growth medium

The cultivation tests were repeated with another fungi strain, namely *Aspergillus ficuum* ATCC 66876 (BSL-1). The filamentous ascomycete fungi *Aspergillus ficuum* is identified as a promising candidate for phytic acid conversion as it produces large amounts of extracellular phytases. Grain components of barley, rice, wheat and maize can however inhibit the activity of *A. ficuum* phytase. This 3-phytase producing fungus grows optimally at 24-26°C in aerobic conditions and at around neutral pH. Further, the strain is known to produce acid phosphatase and glucoamylase. For the cultivation of *Aspergillus ficuum*, the same synthetic agar phytase screening medium as described for *Pichia anomala* was used. Analyses, performed on this medium, are shown in Table 8.

Table 8: Main characteristics of the growth medium for the cultivation of A. ficuum

Parameter	Unit	Value
P total	**mg/L**	802
PO_4^{3-}P	**mg/L**	23
COD total	**mg/L**	15480
pH		4.16

According to Gargova et al. 1997, the pH of the liquid medium should be dropped to 5.5 with 1N HCl. However, as the pH of the medium was already lower, this was not done. Neither was the pH increased as *A. ficuum* produces acid phosphatases (with phytase activity) with optimum pH ranging from 2.5 to 6.0. The medium was sterilized by microwave boiling. Phytic acid couldn't be directly measured but was indirectly quantified as total-P (in solution) minus PO_4^{3-}-P, considering that the defined growth medium didn't contain other phosphorus sources.

Different cultivation tests of *A. ficuum* were performed. It concerned batch experiments with a different inoculum at the start (spores or mycelia of *A. ficuum*) and with or without pH adjustments.

5.3. Batch Trial results

5.3.1. Batch cultivation test 1: Inoculation with spores

200 mL of the cultivation medium, containing phytic acid as sole source of phosphorus, was inoculated with spores of *A. ficuum* (harvested from PDA plate). The fungus grew as spherical shaped mycelia that covered the entire volume within an incubation period of 2.0-2.5 days at 32°C and with gentle agitation. In the course of the batch cultivation experiment, P analyses were performed (see Table 9).

Table 9: Phosphorus and COD concentrations in the first cultivation test with A. ficuum

Parameter	Unit	start	3 days	6 days
P total	**mg/L**	802	-	769
PO_4^{3-}-P	**mg/L**	23	322	40
CODsol	**mg/L**	15480	10230	2400

Note that some of the P was incorporated into the biomass though this could not be accurately measured as the biomass was not homogeneous suspended in the liquid culture but instead was present as spherical units which cannot be easily pipetted. After 6 days of cultivation, almost all COD was converted and dense biomass was formed. All accessible P was metabolised but no new phosphate was released.

Conclusion: Most of the phytic acid was converted to phosphate early in the *Aspergillus* growth curve. During exponential phase, the available phosphate was consumed.

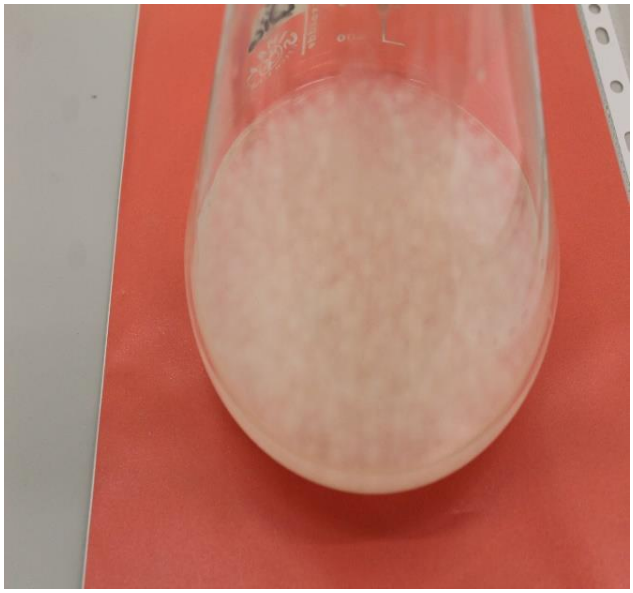


Figure 7: Aspergillus ficuum culture after 2 days of incubation at 32°C, growing on phytic acid as sole P-source

5.3.2. Batch cultivation test 2: Inoculation with a dense culture of A. ficuum

The second cultivation test was started with about 200 mL fresh phytic acid medium and 10 mL (5 v/v %) inoculum (dense culture of previous test). Presumably, the addition of 10 mL of culture (pH 2.4) further decreased the pH of the medium (pH 4.1-4.2). The results of the analyses, performed in the course of the cultivation test, are summarized in Table 10.

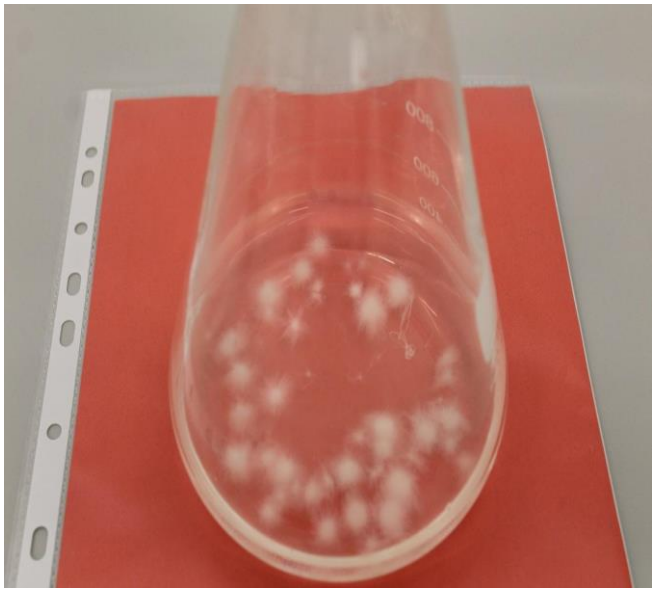


Figure 8: Fresh phytic acid medium with 5% inoculum from a 6 days A. ficuum culture (test 1)

5.4. Continuous incubation trials

Next to the batch trials a number of incubation trials on a continuous operation were performed. This allowed to further optimize conditions for enzyme production and determine most sensitive process parameters.

Table 10: Evolution of phosphorus and COD concentrations in the second cultivation test with A. ficuum

Parameter	Unit	start	1 day	2 days	3 days
P soluble	**mg/L**	768	733	810	774
PO_4^{3-}-P	**mg/L**	44	35	104	228
CODsol	**mg/L**	-	12800	22000	19950
pH				2.4	2.1

After inoculation with a fresh culture of *A. ficuum,* the existing mycelia increased in size but no new mycelia were formed. When the culture was started from spores, mycelia were formed during the first two days of incubation. When the culture was started from mycelia (cross-inoculation), no new mycelia were created but the "old" mycelia extended in size. After 2 and 3 days of cultivation, an important increase of soluble COD was measured. The COD increase and concomitant pH decrease might be caused by citric, oxalic acid or gluconic acid production together with the lysis of old hyphen (liberating soluble organic compounds). The dense mycelia biomass was separated from the liquid using sieving (yielding 12.2 g wet weight from a 200 mL culture) with a dry matter content of 5.4%.

5.4.1. Batch cultivation test 3: Inoculation with spores and higher pH at the start

Two Erlenmeyers were filled with 100 mL medium and the pH was increased to 5.01 (culture 3.1) and 4.69 (culture 3.2) using 0.1N NaOH. Subsequently the medium was inoculated with spores from a PDA plate. The results of the analyses, performed in the course of both cultivation tests, are shown in Table 11.

Table 11: Evolution of phosphorus and COD concentrations in the first cultivation test with A. ficuum - Cultivation test 3

		Test 3.1			Test 3.2		
Parameter	**Unit**	**start**	**2 days**	**4 days**	**start**	**2 days**	**4 days**
P soluble	mg/L	-	797	-	-	651	-
PO_4^{3-}-P	mg/L	-	21	-	-	21	-
CODsol	mg/L	-	16105	-	-	15845	-

pH		4.82	2.54	1.13	4.65	2.05	1.17

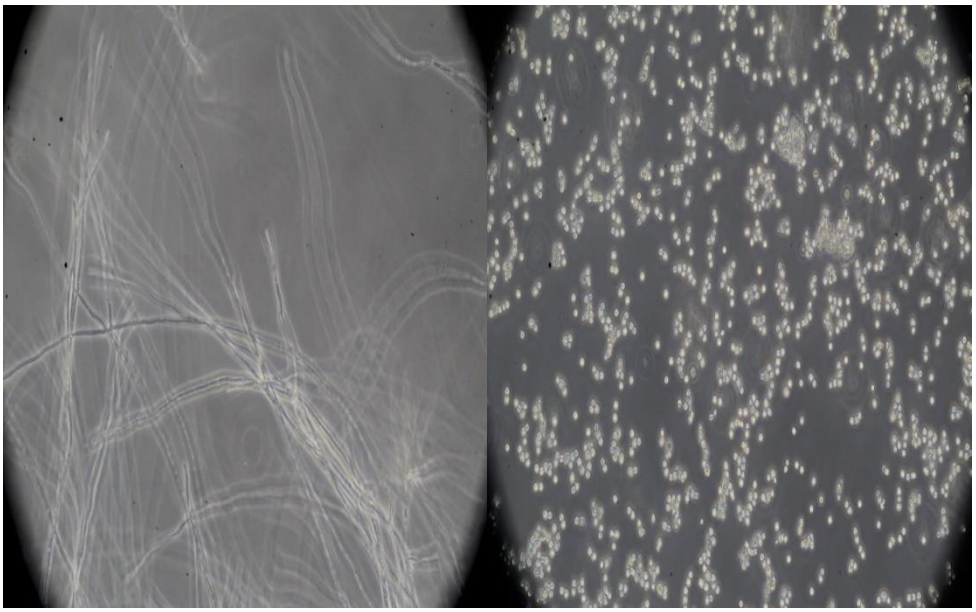


Figure 9: Microscopic image (400X, phase contrast) of the medium of culture 3.1 (LEFT) containing spores in the fluid and (RIGHT) the periphery of a mycelium sphere

5.4.2. Batch cultivation test 4: Inoculation with spores and regular pH adjustment

A fourth experiment was carried out using the knowledge gained in the previous experiments: starting from spores, adjusting pH and administering glucose when needed.

1 L of the medium was inoculated with spores of *Aspergillus ficuum* and incubated at 32°C with gentle agitation. The concentrations of phosphorus and soluble COD were frequently measured (see Table 12). After 2 days, a large quantity of mycelia spheres was observed. After 4 days, a dense culture of *A. ficuum* covered the entire volume.

In this experiment, the pH of the cultivation medium was regularly increased up to 3.4 à 3.9 by the addition of an NH_3-solution (nitrogen source). As can be derived from the results in Table 10, this pH adjustment resulted in an increased phytase activity: almost all P in solution was present as free phosphate-P after 4 days of cultivation (meaning a near complete conversion of phytic acid). To confirm this activity, extra phytic acid was added on day 5. Three days later, almost all P was again present under the form of phosphate-P.

Table 12: Evolution of phosphorus concentrations continuous cultivation test with A. ficuum

		start	**2 days**	**4 days**	**5 days**	**5 days Extra PA°**	**6 days**	**8 days**
P soluble	**mg/L**	822*	820	665	773	1460	1600	1500
PO_4^{3-}-P	**mg/L**	29	85	663	699	731	1230	1470
COD sol	**mg/L**	15960	16180	9970***	12010	-	9760	6980
pH		4.12	2.55↑3.7**	2.65↑3.9	3.62	-	3.42	3.36
NH_4^+-N	**mg/L**	-	-	784	1080	-	-	-

*At the start, P total and P soluble were equal
**pH was increased using about 1.1 mL 28-30% NH3-aq. to pH = 3.7
***Extra glucose addition (0.25 g/L)
°Extra addition of phytic acid

In this cultivation test with *A. ficuum*, a clear phytase activity could be demonstrated.

5.5. Extraction of phytase enriched cultivation fluid

In an extra batch experiment, it was tested whether the phytase enzyme, produced by *A. ficuum*, was present in the bulk solution. Hence, *A. ficuum* produces extracellular and soluble phytase enzymes. The fluid was collected from the functional culture (after 5 days of cultivation) and filtered to obtain the enzyme-rich filtrate. Phytic acid was added to a final concentration of about 3.7 g/L. It should be noted that fungal growth was possible because the supernatant was rich in soluble COD and ammonia and fungal hyphae/spores could pass the filter. Analyses were performed at the start and after 16 h of incubation at 35°C (see Table 13).

Table 13: Evolution of phosphorus concentrations in a batch test with filtrate of the A. ficuum culture of Test 3

Parameter	Unit	start	16 hours
P soluble	mg/L	1860	1870
PO_4^{3-}-P	mg/L	816	1180

The P analyses indicated phytase activity. However, within 1 day of incubation, the medium became turbid because of the growth of contaminating yeast (which was confirmed by microscopy and plating). Therefore, it couldn't be concluded from this experiment that phytase enzyme, produced during the cultivation of *A. ficuum*, was present in solution.

5.5.1. Batch cultivation test 5 and testing of phytase activity on a water sample, containing phytic acid:

In order to test the phytase activity of *A. ficuum* on a phytic acid containing water sample, a new cultivation test was started. The same procedure as in the previous cultivation test was applied, except for the pH adjustment. 900 mL of the medium was poured in a 5 L Erlenmeyer and *A. ficuum* spores were added. The Erlenmeyer was incubated at 35°C with gentle agitation. An overview of the analyses, carried out in the course of the cultivation test, are presented in Table 14.

Table 14: Evolution of phosphorus concentrations in the cultivation test with A. ficuum

		start	2 days	4 days	5 days	6 days
P soluble	**mg/L**	820*	842	777	804	742
PO_4^{3-}-P	**mg/L**	33	56	480	682	712
COD sol	**mg/L**	15600	16010	11060	8750	4800
pH		5.0	2.4	2.4	2.7	2.5

*At the start, P total and P soluble were equal

As shown by the increase of phosphate-P, a clear phytase activity was again obtained with the *A. ficuum* culture. After 6 days of cultivation, tests with a water sample, containing phytic acid, were started. This water sample had a pH of 4.4, a total P concentration of 140 mg/L and a phosphate-P concentration of 52 mg/L (37 % of total P).

Most of the batch experiments were performed with the filtrate (Whatman filter, 8 µm) of the culture as source of extracellular phytase in solution. In an extra test, mycelia of the *A. ficuum* culture were added. In Table 15, an overview of the batch experiments is shown. At the start and after 5 h of reaction time (at room temperature), samples were taken for the P analyses. Afterwards, the batch reactors were placed in a temperature-controlled room of 33°C and samples were taken after a longer reaction time, namely after 22 h. In Table 16, an overview of the P measurements is presented.

Table 15: Overview of the batch experiments with the water sample, containing phytic acid

	Parts water sample	Parts filtrate A.ficuum culture	Calculated P total (mg/L)*	Calculated PO_4^{3-}-P (mg/L)*
Control	100		140	52
Test 1	99	1	146	60
Test 2	95	5	173	90
Test 3	90	10	207	128
Test 4	75	25	307	241

Test 5	50	50	474	430
Test 6	100	Mycelia	140	52

*Calculated P concentrations, based on the P measurements in the filtrate of the A. ficuum culture and in the water sample

Table 16: Evolution of the P concentrations in the batch tests with the water sample and the filtrate or mycelia of the A. ficuum culture

	START	**START**	**After 5 h**	**After 22 h**
	P total (mg/L)*	**PO_4^{3-}-P (mg/L)***	**PO_4^{3-}-P (mg/L)**	**PO_4^{3-}-P (mg/L)**
Control	144	72	74 (2)	83 (11)
Test 1	149	76	80 (4)	95 (19)
Test 2	177	104	108 (4)	128 (24)
Test 3	210	136	142 (6)	159 (23)
Test 4	297	224	242 (18)	249 (25)
Test 5	438	380	398 (18)	391 (11)
Test 6	147	74	79 (5)	102 (28)

(): increase in mg/L, compared to the start phosphate-P concentration
*Measured values

The results of the batch experiments indicate that some liberation of phosphate-P from phytic acid, present in the water sample, occurred. However, the latter was rather limited. In all the tests with the filtrate of the *A. ficuum* culture, the increase of phosphate-P was higher than in the control test, indicating that there was some phytase activity. After 22 hours of reaction time, the maximum extra increase of phosphate-P amounted to only 14 mg P/L. This was more or less the same for the test with addition of mycelia (Test 6).

After 22 h, the release of phosphate-P was very similar in the batch tests with 5 % up to 25 % (vol) addition of filtrate of the *A. ficuum* culture (Tests 2 to 4). Hence, after this reaction time, a higher dosage of filtrate (or extracellular phytase) didn't result in a higher increase of phosphate-P.

Compared to the test results with the commercial phytase product, the experiments with *A. ficuum* were less satisfying.

6. STRUVITE FORMATION TESTS WITH ANAEROBIC SAMPLES OF SOYBEAN PROCESSING

6.1. Introduction general struvite approach

NuReSys has developed a standardized struvite formation trial batch test which under controlled conditions of pH and molar ratio Mg/ortho-P does define the potential for struvite formation. On the anaerobic effluent sample of delivery 2 (Table 1) and with an extra sample (delivery 3), the standardized struvite trial was preformed to see if this technology had any potential on the particular wastewater.

6.2. Specific storage observation

Immediately after delivery of the samples, the P analyses were performed. The struvite tests started after storage of these samples at 4°C for some days. This storage period gave rise to a pH increase and to a concomitant decrease of ortho-P. The pH of the anaerobic effluents was further increased by the addition of NaOH and in some experiments, also $MgCl_2$ was extra added under the form of a 7.5 % solution. An overview of the process conditions and results of the struvite tests is presented in Table 17 (sample delivery 2) and Table 18 (sample delivery 3).

The results of these tests show that storage of an anaerobic effluent sample and/or mixing of the sample did result in a significant increase of the pH accompanied with a clear PO4-P decrease. This observation in is concurrence with the elevated levels of magnesium present in the wastewater. These elevated levels of Mg are encountered in all types of wastewater examined (raw influent, anaerobic stage effluent and aerobic stage effluent). The relatively anaerobic stage effluent of 7,0 is also an important feature to be taken into account in this matter. Apparently, a simple pH increase does

result in a simultaneous decrease of soluble PO_4^{3-}-P. The above conclusions were initiated by the observations by Avecom summarized below.

Table 17: Overview of the process conditions and results of struvite formation tests with the anaerobic effluent sample of soybean processing - Sample of delivery 2

Analyses	**Unit**	**Fresh sample**	**After storage**
pH		7.0	7.5
PO_4^{3-}-P	**mg/L**	80	40
NH_4^+-N	**mg/L**	360	-
Action 1	pH increase with NaOH		
pH		8.3	
PO_4^{3-}-P	**mg/L**	After centrifugation: 3 After 0.45 µm filtration: 10	
Action 2	Addition of $MgCl_2$ (equimolar to 80 mg ortho-P/L)		
PO_4^{3-}-P	**mg/L**	After centrifugation: 0.1 After 0.45 µm filtration: 2	

Table 18: Overview of the process conditions and results of struvite formation tests with the anaerobic effluent sample of soybean processing - Sample of delivery 3

Analyses	**Unit**	**Fresh sample**		
pH		6.9		
PO_4^{3-}-P	**mg/L (mM)**	47 (1.5)		
NH_4^+-N	**mg/L (mM)**	405 (29)		
Mg^{2+}	**mg/L (mM)**	120 (5)		
Action	pH increase with NaOH to different pH values and mixing for 1 hour			
		Only mixing	**Addition of 5 meq NaOH/L**	**Addition of 9 meq NaOH/L**
pH		7.7	7.8	8.1
PO_4^{3-}-P*	**mg/L (mM)**	4 (0.13)	5 (0.16)	3 (0.10)
NH_4^+-N	**mg/L (mM)**	386 (27.6)	386 (27.6)	368 (26.3)
Mg^{2+}	**mg/L (mM)**	88 (3.7)	72 (3.0)	63 (2.6)

*Analysis after 11 µm filtration

In the first test (Table 18), the pH of the anaerobic effluent sample was further increased up to 8.3, resulting in a decrease of ortho-P: depending on the separation method (centrifugation or 0.45 µm filtration), the residual ortho-P concentration ranged between 3 and 10 mg/L. Because of these low ortho-P concentrations, the effect of the extra $MgCl_2$ addition was limited.

In the second test, it was experimentally shown that stirring of the anaerobic effluent increased the pH and significantly lowered the ortho-P concentrations. The corresponding analyses of NH_4^+-N and Mg^{2+} demonstrate that pH increase gave rise to N-struvite formation since the 3 equimolar components of struvite (PO_4^{3-}-P, Mg and NH4-N) decreased with more or less 1.5 mM.

Control of the anaerobic sludge at the soybean processing plant did show the presence of struvite crystals (Figure 10). This does strongly suggest that in the anaerobic stage already natural struvite formation is occurring and servs as P-sink. This process will be mainly controlled by the reactor pH and Mg levels.

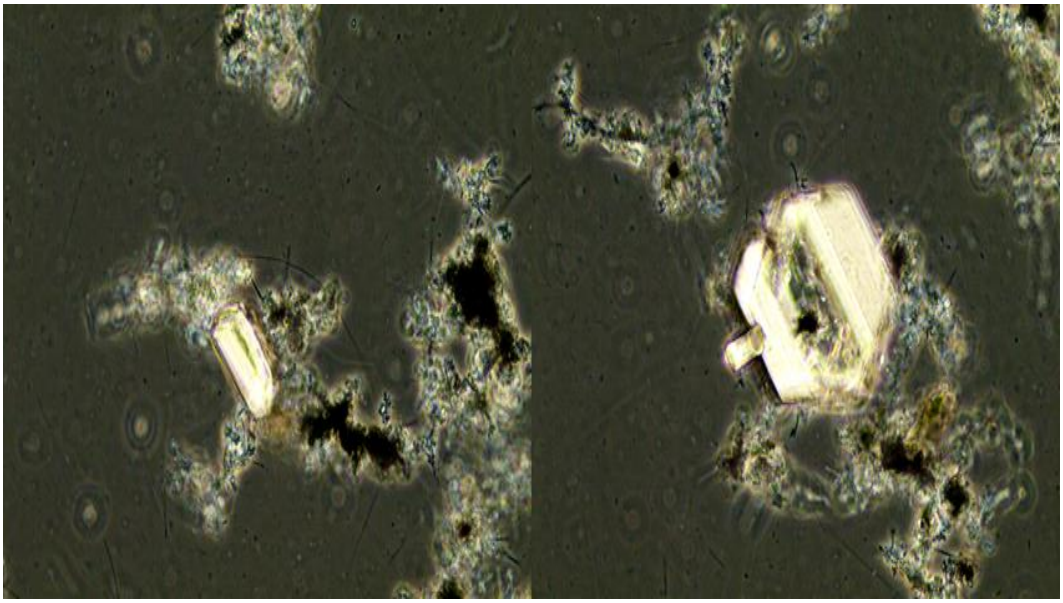


Figure 10: Crystalline struvite mater present in anaerobic sludge

6.3. Intermediate conclusion non-reagent struvite formation

In general, the struvite tests with the anaerobic effluent of soybean processing showed that increase of pH was sufficient for formation of N-struvite and for an important decrease of ortho-P. Both magnesium and ammonium were present in higher molar concentrations than PO_4^{3-}-P.

The above preliminary results will be double checked using similar samples form a second industrial soybean processing unit.

7. K-STRUVITE TRIALS LABORATORY

7.1. Introduction K-struvite treatment option

In order the confirm earlier obtained results, spiking aerobic effluent form the soybean processing plant with PO4-P to produce K-struvite, trials were repeated under more strict conditions. The same sample of aerobic effluent was spiked with Na_2(HPO4). Initial pH was 7.88 and was not controlled further during the trial. The molar K^+/PO4-P ratio was around 7 and MgCl2 was added at molar 1/1 level (Mg^{2+}/PO4-P). Mg^{2+} was dosed as MgCl2 and spiked as one dose (with 32% vol m/m) at begin of trial. Samples were continuously stirrer with magnetic stirrer. Samples were taken at indicated intervals and filter immediately over a 0.45 µm syringe filter prior to analysis of PO4-P levels. Results of decreasing PO4-P levels are shown in Figure 11.

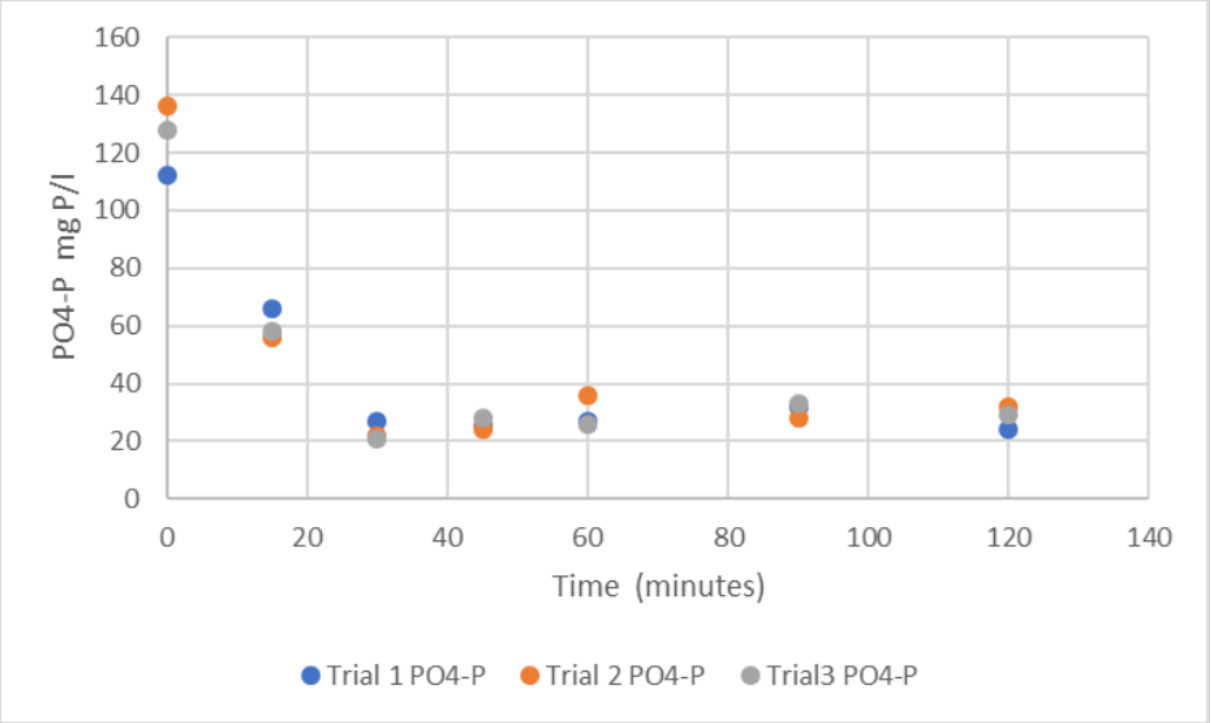


Figure 11: Phosphate levels at batch trials K-struvite trials

Results indicate a sharp decline form the initial levels (average 128 mg PO_4^{3-}P/l) within 30 minutes down to levels between 20 to 26 mg PO_4^{3-}-P/l or a 81% reduction. No significant further decreased is noted in the subsequent period from 30 to 120 minutes of reaction time.

The rapid decline in PO_4^{3-}P/l levels needs to be put in the correct perspective given this is a batch trial with addition of the entire MgCl2 dosage at time zero. This does creature a high momentous saturation resulting in sharp decline of initial concentration of PO_4^{3-}-P. This also explains why within 30 minutes final PO_4^{3-}-P already attained. These former observations are additionally confirmed by the yield of a large amount of very small native K-struvite crystals exhibiting the typical orthorhombic-pyramidal shape.

7.2. Product control

A microscopic view of these accumulated K-struvite crystals is shown in Figure 12.

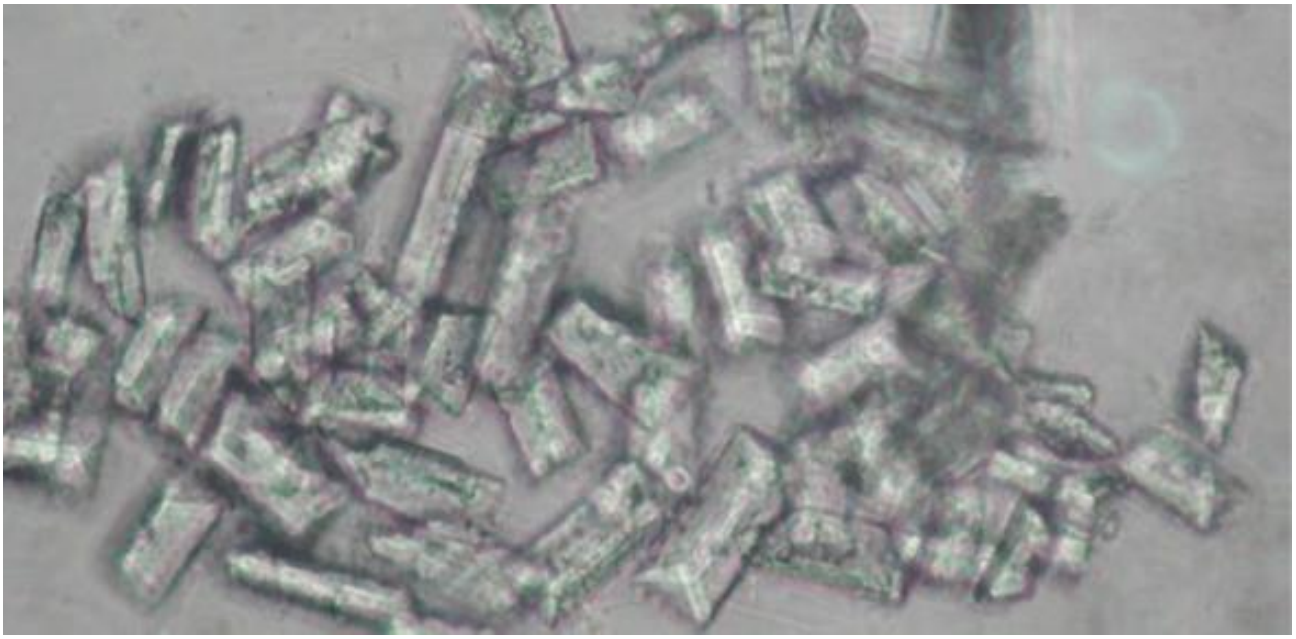


Figure 12: Typical orthorhombic-pyramidal K-struvite crystals batch trials

The most important major conclusions of these trials are:

a. confirmation of possibility to produce K-struvite
b. reaction kinetics are comparable to NH4-struvite

The latter indicate that continuous operation of the pilot will need to taken into account a long enough retention time and precise MgCl2 dosing to exclude high saturation levels avoiding yield of smaller crystal fines. The crystal fines need to be avoided since the increase the risk of crystal wash-out reducing recovery yield.

In particular the high K levels are responsible for this process conditions. Also to be taken into account is that with the natural levels of PO4-P present (or potentially present after phytase treatment) the decrease in K levels present in the aerobic effluent will hardly be noticeable. The main goal at this stage is produce K-struvite as an added value product containing both recovered K and PO4-P based on the limiting phosphorus levels.

8. STRUVITE FORMATION COMBINATIONS

The lab-scale trials performed, taken into account the data gathered form real industrial soybean processing unit, did eventually result in a number of viable options to be further investigated with the pilot plant. Figure 13 shows the selected options that will be investigated in further detail to finalize the project and to be able to draw the correct conclusions and make appropriate recommendations to combine efficient wastewater treatment with valuable resource recovery.

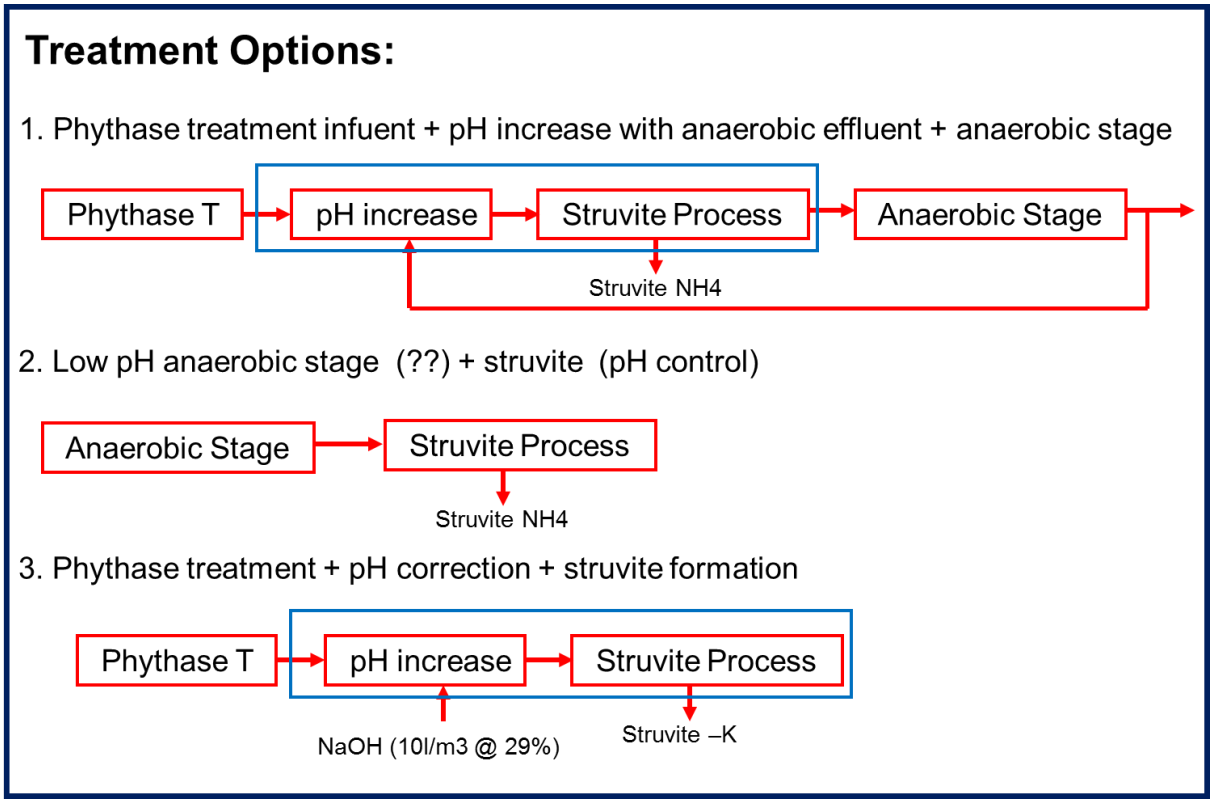


Figure 13: Treatment Options to be include in pilot unit

Option 1: aligns strictly with the initial concept of liberating PO_4^{3-}P form phytic acid to increase the recovery potential by the most common form of struvite formation being the NH4-struvite.

Option 2: is an alternative approach based on option 1 where using the specific composition of soybean wastewater (especially the ratio Mg/ PO_4^{3-}) obtained after applying option1. The perspective of producing a valuable P-recovered product not needing any reagent to be added opens a very promising perspective to implement this approach.

Option 3: also this option was not initially in the scope but due to analytical data providing deeper insight in the K levels compared to P levels in raw soybean wastewater opened a unique opportunity to include now all three major nutrients NPK in a recovery process.

Since struvite technology is an add-on feature the most appropriate selection of which option to choose can also take into account the very specific conditions prevailing on existing plants.

9. PILOT UNIT DESIGN

The basic schematic for the pilot unit had already been established during the early stage of the project (Figure 14). Taking into account the observations made and conclusions drawn during the performed laboratory trials this initial design can largely be retained and executed thus as such. So the pilot plant to be constructed will follow the initial design, containing still the two major basic compounds being the phosphate release reactor (T02) and the actual crystallization reactor (T03). The soybean waste hopper will also remain as infuent buffer for liquid wastewater flows. The strain press becomes less critical since the major focus will be on the liquid waste flows not needing this strain press. It has become obvious

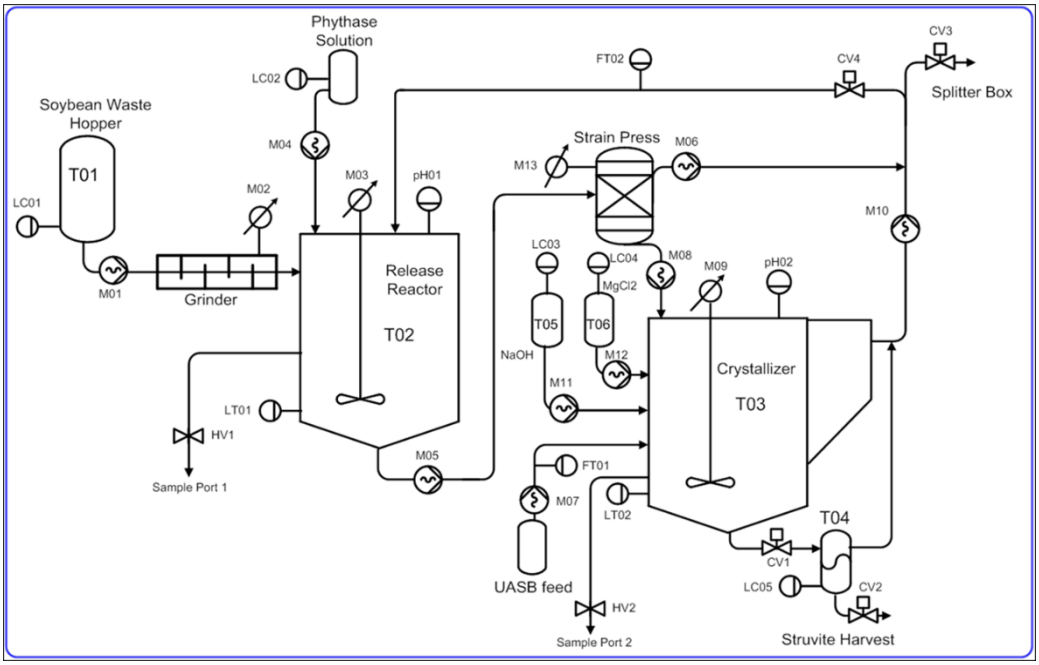


Figure 14: Basic schematic pilot unit

10. PILOT CONSTRUCTION

Figure 14 gave a basic principal schematic process flow of the pilot. The pilot full PI&D has been elaborated to technical level providing all needed technical features to perform the different treatment scenarios on either anaerobic effluent, aerobic effluent, enzyme treatment influent or all other possible combinations. A Detailed PI&D shown in Figure 15.

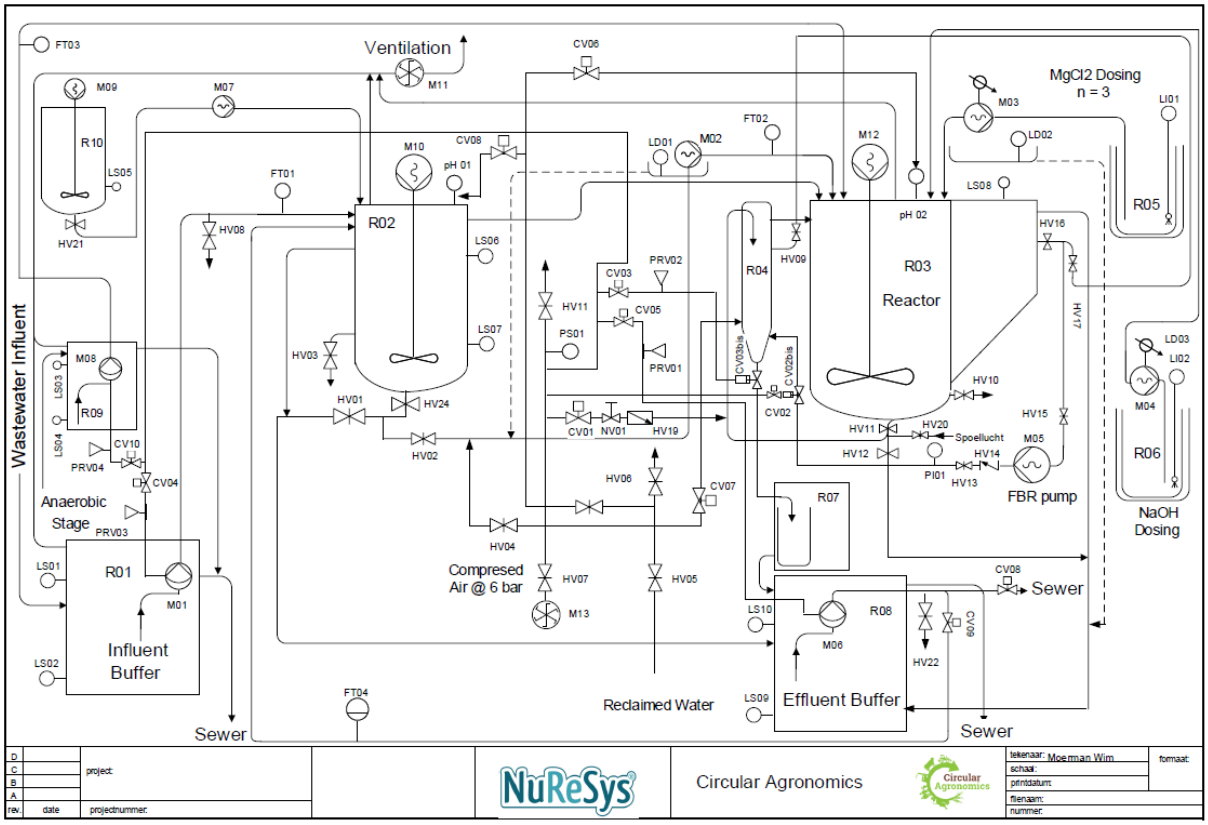


Figure 15: Detailled PI&D Flow-Scheme

The entire pilot is mounted in an isolated and ventilated mobile container. Next to the needed pilot equipment the unit was assembled considering the safety prescriptions. The unit can run fully automatically and is controlled by a separate PLC that also lists any occurring malfunctioning and alarms, also allows remote control and operation. Once construction was finalized all testing of all sensors/equipment is currently being performed so a Factory Acceptancy Test (FAT) can be finalized. Also, an independent contractor verified and approved the electrical cabinet. Critical equipment was also placed in redundant configuration. Pilot is completely automatized with a separate and individual PLC for process control and off-site supervision.

Following picture series given a overview of constructed pilot:



Figure 16: Several pneumatic feed pumps (left) & Harvest column struvite (right)



Figure 17: Flow meters different incoming and internal flows



Figure 18: General view entry side pilot (left) & Purge valve struvite wascher (right)



Figure 19: Dosing pumps reagents MgCl2 (n+1) and NaOH



Figure 20: Release and crystallization reactor tanks impellers



Figure 21: Internal recycle flow struvite harvest

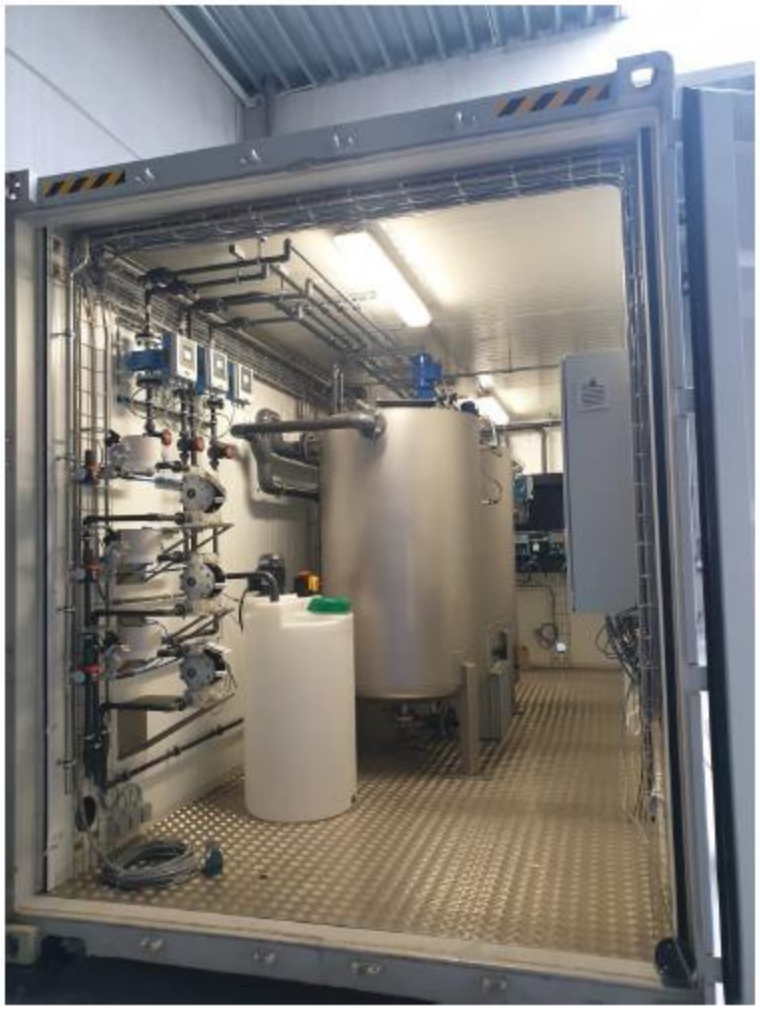


Figure 22: Finalize and FAT approved pilot ready for transport

11. PILOT START-UP

11.1. Covid contingency plan

The initial start-up of the pilot had already been postponed by a major merge of the location of soybean processing plant (US owned company) with another US based company. These needed a second procedural check by all legal-entities and coming to a separated renewed NDA. Following this first delay Covid-19 kick-in. All access to the soybean plant restricted from 23/03 onwards until further notice. In addition, a near to general lock-down in Belgium as imposed by the government in the first terms until 5th of April and in meanwhile extended until 19th of April (with strong indication that a further extension until 3th of May).

Since uncertainty with regard to start-date for pilot an alternative location was sought with similar options to run mobile pilots' trials as initially programmed. There are only very limited large soy bean processing units at disposal for trials. So, the alternative trials with phytase induced phosphate release has been pushed backwards and main focus is at this stage on the continuous production of K-struvite. An alternative location was found producing at this stage a ammonium free aerobic effluent containing similar level of K and free PO_4^{3-}-P levels between 60 to 90 mg PO_4^{3-}-P /l. The plant processes a combination of manure and digestate. The aerobically treated wastewater is filtrate of an MBR (Membrane Bio Reactor) producing a solids free effluent.

11.2. Pilot plant trial options

Based on the findings of the laboratory trials done the pilot will be operated to validate these obtained results under longer period and thus taking into consideration the specific fluctuations that can occur in this particular type of wastewater. A number of confirmation trials could be validated on the alternative flows thus also demonstrating that these approaches could possibly also be implemented on other types of wastewater. The incorporation of the pilot at this particular location is given in Figure 23. In addition, this new location is less than 20 km form the soy bean processing plant.

This alternative was discussed with both WP Leader KWB and overall project manager IRTA.

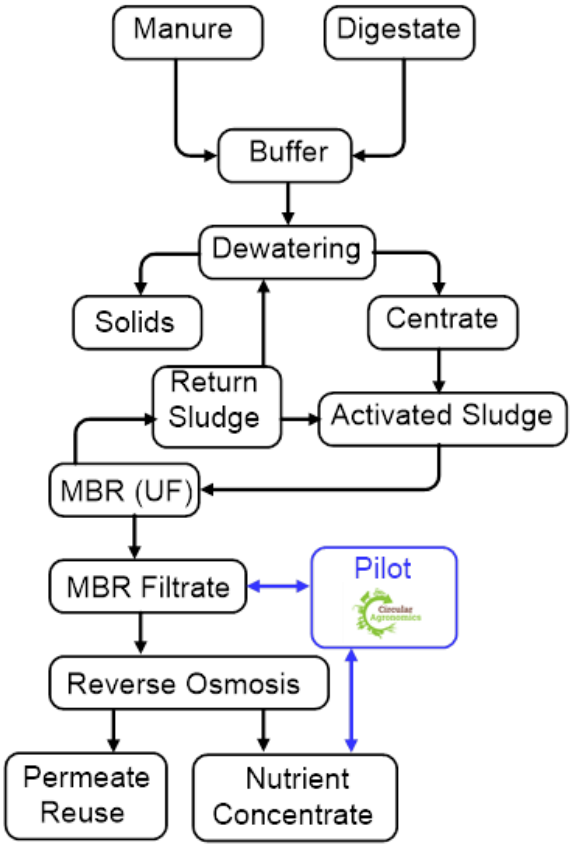


Figure 23: Flow schematic alternative location and pilot incorporation

12. PILOT PLANT OPERATION

12.1. Introduction general pilot operation

These objectives were achieved as will be elaborated in next paragraph section. In addition, based on observations made during the lab scale and pilot trials the approach of inducing phosphorus recovery without any chemical reagent addition was examined. The wastewater composition (with elevated levels of magnesium) render this approach very interesting. Wastewater to be processed will have to be transported form soy-bean processing plant towards the alternative pilot location.

The pilot unit was finalized and after submitted to functional test control as well as “wet test” with clean water prior to shipment towards the alternative location. The alternative location was selected based on its vicinity of the so bean processing plant (12 km) and the fact this location has the needed permits to process external wastewater flows. The treatment on site does consist of an aerobic stage using Dissolved Air Flotation (DAF) for sludge/water separation (Figure 24). The DAF effluent can in addition be passed through a Reverse Osmosis (RO) which makes it possible to work with concentrated nutrient levels.

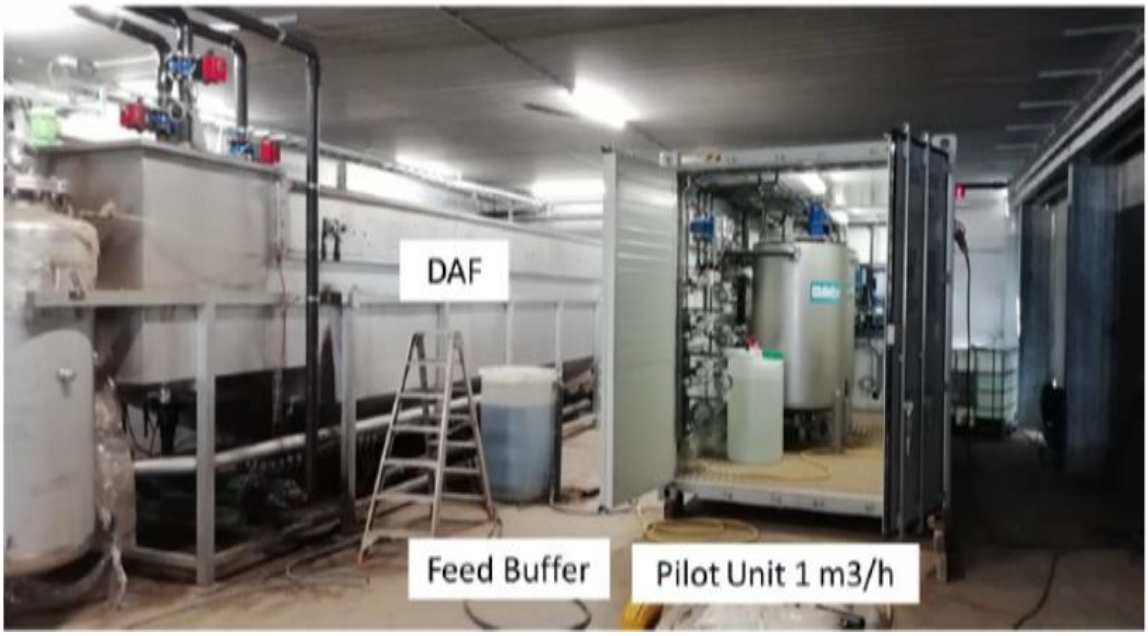


Figure 24: Pilot plant installed on site

The pilot was installed on site and connected in a flexible way so both DAF effluent or RO concentrate can be processed. A series of trials run were performed to check stable and error free operation of the pilot plant. The pilot plant was also equipped with remote sensing so it could be monitored on-line form our main office location.

Once pilot functionality was established the planned trials were performed; enzymatic conversion of phytic acid by phytase addition, K-struvite formation on both spiked aerobic effluent and RO concentrate.

Based on earlier observations of spontaneous decrease of PO_4^{3-}-P the option of inducing phosphorus recovery without reagent addition was also tested.

12.2. Secondary semi-pilot unit

Based on the results of both the K-struvite trials and non-reagent adding P recovery a smaller pilot was configurate to optimize both approaches (Figure 25). This smaller pilot unit will be operated on collected soybean wastewater. Once on-site operation is allowed both pilots can be combined.



Figure 25: Second pilot setup

12.3. Pilot plant adjustments

An alternative location was selected to operate the pilot unit given the fact no non-essential access was not allowed to the soybean processing plant. This was agreed upon with KWB and IRTA as otherwise the pilot trails would be delayed. The location was acceptable alternative since it has flows similar composition on site. The first thing done after installing the pilot on site was testing the unit over a longer period of time to check operation and automated features such as: chemical dosing, crystal retention and crystal harvesting and automated operation including alarm messaging and finally remote control. After a few days' issues arose with compressed air supply (essential since major pumps used are compressed air driven membrane pumps). This was related to the local air supply which did not have enough capacity. To avoid further problems a separate compressor/air dryer was installed (Figure 26). No more similar issues occurred ever since.



Figure 26: Air dryer/compressor

12.4. Pilot plant wet trial control period

The pilot was operated over period of 9 weeks in full automated mode and performed as expected. Incoming phosphate levels varied from 66 to 118 mg PO_4^{3-}-P /l (which is comparable to soybean wastewater). Final effluent levels of PO4-P were constantly below 20 mg PO_4^{3-}-P /l (given 1 exception due to shortage of MgCl2). A summary is given in Figure 27.

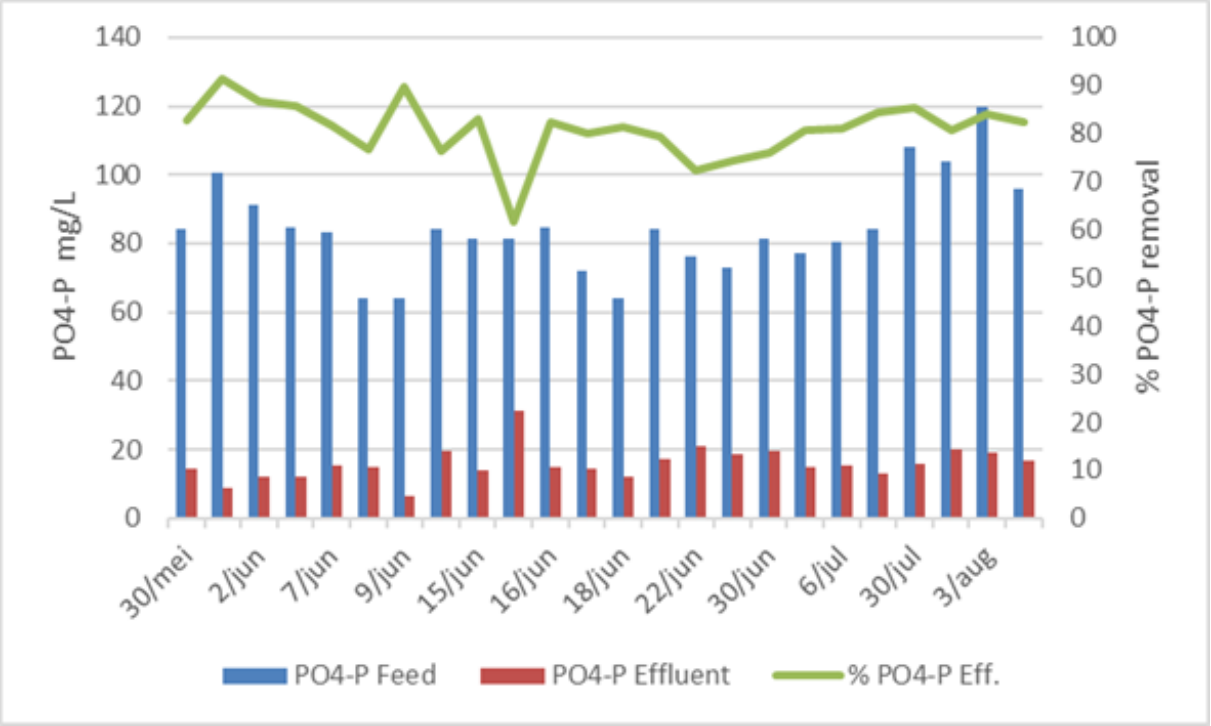


Figure 27: Results trial period pilot plant

12.5. Phytase addition trial runs

The second trial was related to the actual conversion of phytic acid by addition of the selected phytase enzyme. The earlier lab results clearly showed that adding the phytase enzyme had more potential as compared to in vivo fermentation by selected organisms with induced enzyme production. Not only would this render implementation easier (adding water-based enzyme solution versus additional bioreactor controlling selected organism growth conditions) the phytase enzyme is a readily available and cheap bulk commodity product (produced in large quantities as feed additive). In addition, dosing quantities needed are very low (5 g/ton of COD added). The pilot trial at this stage were performed on two sets of raw soybean waste with major components listed in Table 19.

Table 19: Samples phytase addition trials

Parameter	Unit	Sample 1	Sample 2
pH	**-**	3,15	4,08
Total P	**mg/L**	164	152
PO4-P	**mg/L**	42	62
PO4-P/Total P	**%**	25,6	40,8
COD	**mg/L**	11342	13336

As can be seen, there is already some free PO4-P present (25 to 41%) related to the total P content. However still a significant portion of the total P is available to be converted into PO_4^{3-}-P (the chemical species needed for P recovery). Samples were incubated over a 4-hour period (continuous mixing) of time with an initial phytase addition – so processing occurred as a batch operation. Figure 28 gives the according PO_4^{3-}-P profiles of both trials. The actual conversion quantities are listed in Table 20. In can be noted that although initial PO_4^{3-}-P for sample 2 was higher as compared to sample 1 the final percentage of PO4-P on the total P content present was in a much closer range (53.7 to 60.5 %). This is likely related to the phytic acid substrate affinity of the enzyme used which is indicative for achievable final levels. This also indicates that when even higher initial total P content would be processed similar finale levels of residual phytic aced would be left. The enzyme (given low dosage) was specially developed to be added in soybean-based feed. It does it also resistant to the relatively low incoming pH (3 to 4) of the raw wastewater. Processing time can possible still be shortened to 2 at 2.5 hrs given the PO_4^{3-}-P profiles. In addition, the raw wastewater on site would be at 40-45°C (trials at 20-25 °C range) show most likely enzyme kinetics will be more performant at higher temperature further reducing needed process time.

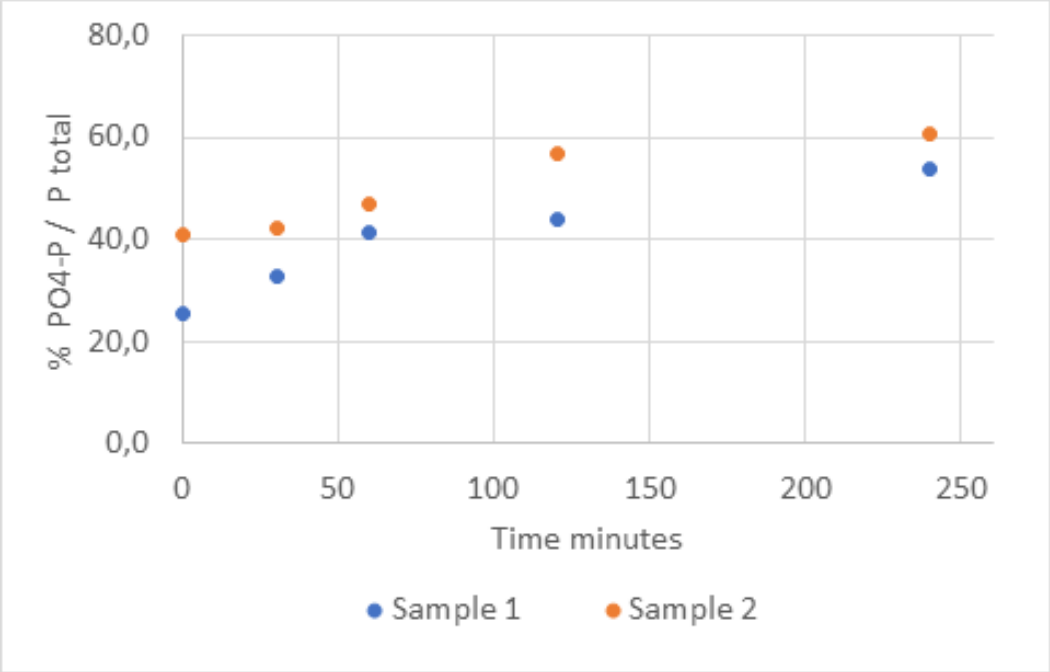


Figure 28: PO4-P profile phytase treatment tests

Table 20: Conversion % of total P to PO43--P

	Sample 1		Sample 2	
	PO4-P	**PO4-P/Total P**	**PO4-P**	**PO4-P/Total P**
Minutes	**mg/L**	**%**	**mg/L**	**%**
0	42	25,6	62	40,8
30	54	32,9	64	42,1
60	68	41,5	71	46,7
120	72	43,9	86	56,6
240	88	53,7	92	60,5

12.6. K-struvite trials runs

The third trial session was related to investigate the possibilities to produce K-struvite after the aerobic treatment stage but prior to the tertiary phosphate removal. To simulate this PO_4^{3-}-P (as Na(H2PO4)) was added together with a corresponding MgCl2 dosing (Mg/PO4-P of 1). It should be noted that also teh aerobic stage still does contain elevated levels of Mg (close to 100 ppm). The pH during the trials was adjusted in the upper range of 8.8 to 9.0. This is typical for K-struvite that requires higher pH values as compared to NH4-struvite to precipitate. As could be expected and shown in Figure 29 a significant (83 to 88%) of the added PO4-P was noticed. However, K recovery would have been minimal in terms of absolute concentration change.

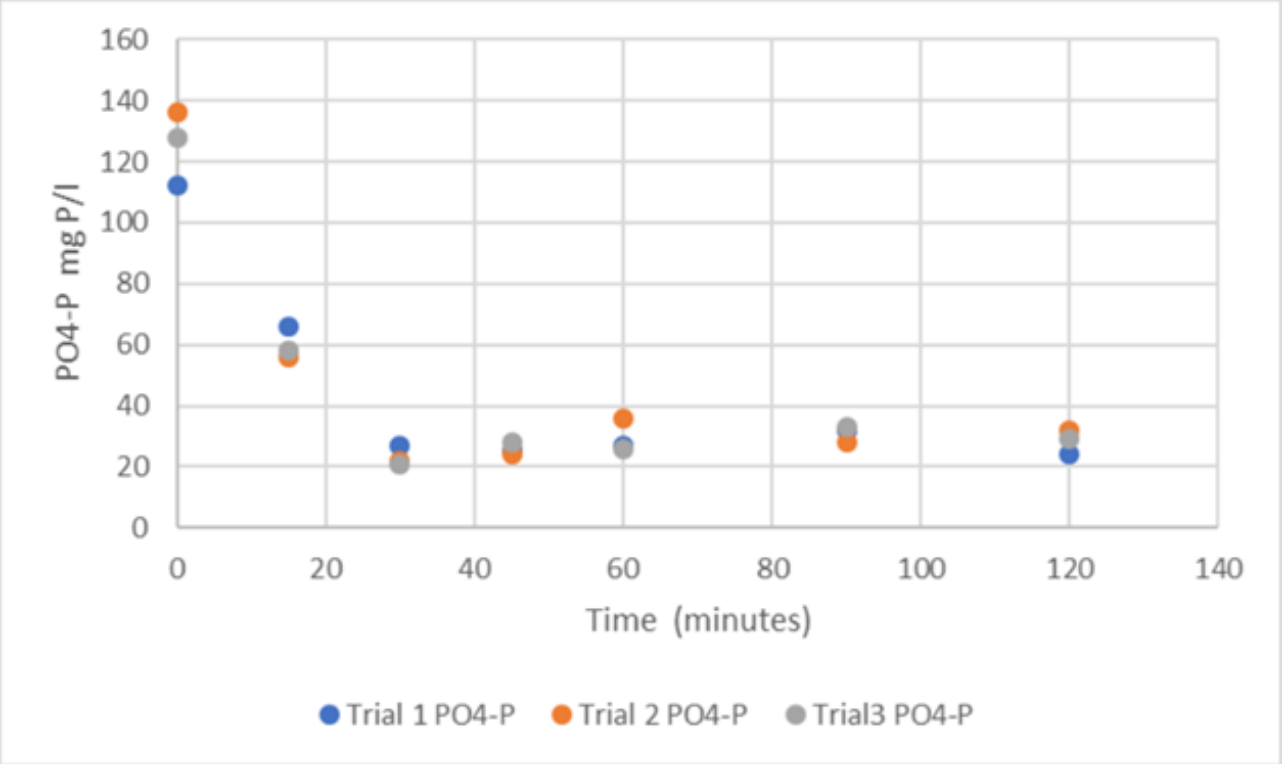


Figure 29: PO4-P profiles K-struvite aerobic stage effluent

These trials results were repeated on site using the concentrate of the RO unit, was had high initial PO_4^{3-}-P and very high initial K values of respectively 108 to 126 mg PO_4^{3-}-P /l and 6500-9000 mg K/l (high range exces). Another typical feature was of course the high salinity that can influence process results. The samples were incubated with a molar Mg/PO4-P ration of 1 and pH was kept between 8.8 to 9.2 over a 3-hour batch trial time frame. The following results were obtained, repeating the trial at 3 different occasions:

- Trial 1: 118 mg PO4-P/l to 10 mg PO_4^{3-}-P/l or 91,5 % conversion
- Trial 2: 126 mg PO4-P/l to 12 mg PO_4^{3-}-P/l or 90,4 % conversion
- Trial 3: 108 mg PO4-P/l to 16 mg PO_4^{3-}-P/l or 85,2 % conversion

Again, the focus is only on the option of P-recovery the reduction of K is marginal. The trials did however indicate that for example the use of RO in water reuse approach can result in concentrated flows which are very interesting for recovery purposes both in terms of elevated concentrations and reduced volume to be treated. However, the trials also indicated that due to the high K levels saturation levels are quickly achieved and can occur very locally in the vicinity of reagent injection points resulting in fines production (< 0.2 mm) where locally high saturation levels might occur. This will have been additionally strengthened by the batch operation mode (Figure 30) but will remain an important point of attention.



Figure 30: Fines formation K-struvite trials

12.7. Non-reagent trials runs

The final trial did explore the noted fact that for this particular wastewater not Mg but PO_4^{3-}-P/l is the limiting parameter for struvite formation, even at the noted levels of 60 – 80 ppm PO_4^{3-}-P/l after the anaerobic lagoon treatment. In addition, it should be noted that due the anaerobic lagoon treatment the final effluent is below or at maximum 7. This is within the lower range of anaerobic processing (6.5 to 7) but also in a range where only limited struvite formation will occur even when respective levels of NH4, PO_4^{3-}-P/l and Mg2+ are present. On average the following values range can be observed after the anaerobic processing step: 280 to 400 mg NH4-N/l, 60 to 80 mg PO_4^{3-}-P/l /l and 85 to 120 mg Mg2+/l at pH 6.5 – 7.2. It will mainly be the pH that governs the residual Mg2+ level. There are two possible ways to increase the pH and thus initiate struvite formation: adding an alkaline reagent (NaOH) or inducing CO2 stripping. The latter has the preference since it does not need any reagent to be added nor handled. Creating turbulence by coarse bubble aeration is a known technique to induce the desired pH increase. Figure 31 shows the result of such a turbulence induced pH increase. Initial PO_4^{3-}-P/l levels drops consistently with increasing pH values. The pH increase goes from 6.95 to 8.15 and corresponding PO4-P drops form 114 down to 38 mg PO4-P/l. The time frame of 90 minutes needs to be seen relative to intensity of turbulence which did depend on the reactor set-up. Additional process performance or importance are both molar NH4-N/PO4-P and Mg2+/PO4-P ratios of respectively 7.4 and 1.78 indicating clearly again that PO_4^{3-}-P/l is the limiting parameter for this particular type of wastewater.

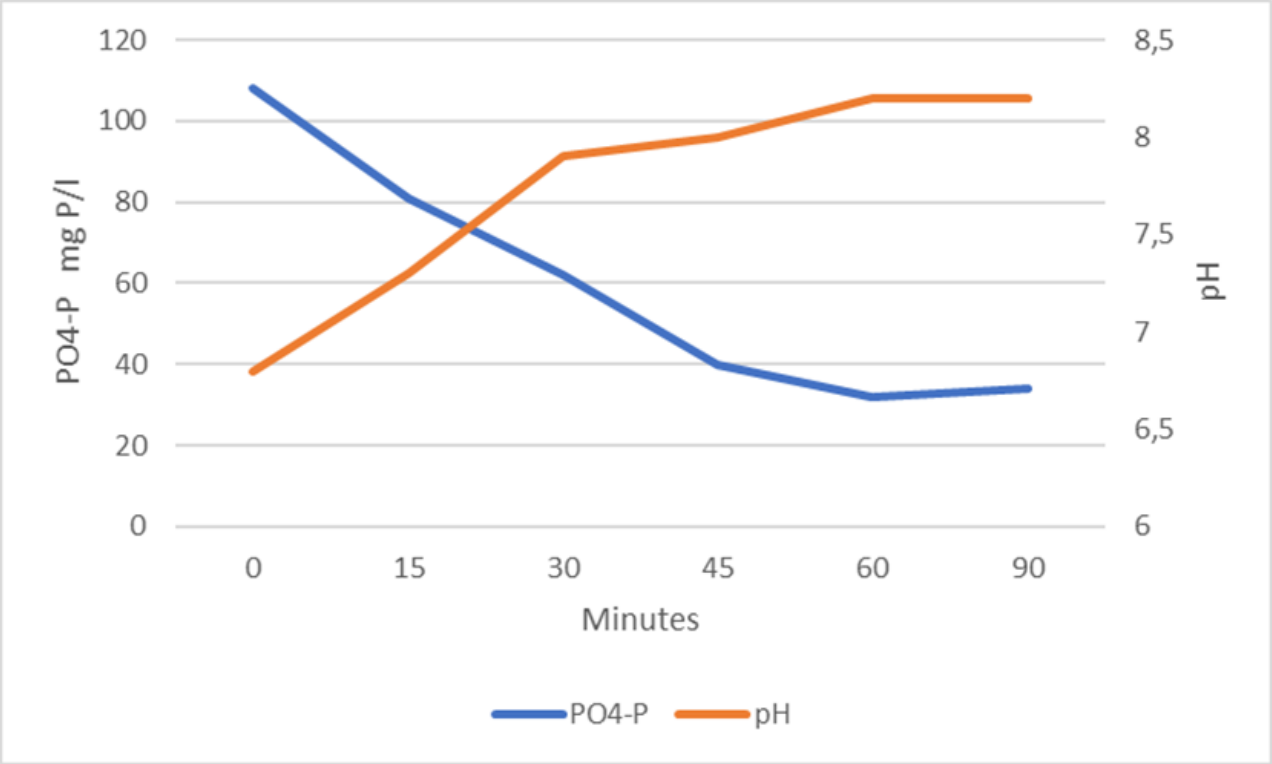


Figure 31: Non-reagent struvite formation

13. UPSCALING OPTIONS

13.1. Introduction treatment options

These objectives were achieved and will be elaborated in next paragraph section. The pilot plant showed enough flexibility to perform all pilot trials needed and could be adapted according to the type of trial or experiment to be done. As a technology provider we did focus mainly on process developments or improvements that can easily upscaled from the pilot trials preformed to industrial applications. These applications have shown not to be only applicable for soy bean processing but could also be used in other industrial sections of food and beverage. Finally, recovered products are in line with the new EU fertilizer regulation and can be part of EU Green Deal set goals.

13.2. Non-reagent struvite formation

An interesting finding of the initially preformed trials and tests was the struvite formation by simple pH control so excluding the need for Mg-reactant or NaOH to be added. Als already explored in laboratory trials this is a promising option for full-scale application. Producing struvite without addition of the basic chemicals Mg^{2+} and caustic (NaOH) is not only cheaper but also excludes handling these reagents (mainly NaOH being a corrosive hazardous substance). The latter is less of an issue for $MgCl_2$ but avoiding addition does reduces NaOH need ($MgCl_2$ is a slight acidic reactant) and prevents a rise in chloride content (which can cause corrosion issues). An important point of attention is that given the lagoon type of anaerobic treatment at the full-scale soybean processing plant there were little or no problems occurring with unwanted struvite formation due to pH increase in high turbulent reactor sections. Figure 32 shows what might occur under conditions were turbulence creates unwanted struvite scaling.



Figure 32: Turbulence induced struvite scaling food processing plant

Evaluating the boundary conditions to optimally use the specific property of soybean wastewater containing more Mg as compared to P was investigated in the pilot by processing batch wise provided soy-bean wastewater. A key parameter is the successful application of this approach remains the conversion of phytic acid to free PO_4^{3-}-P/l (needed for struvite formation). The excess of Mg^{2+} is already present as such in the acidic (pH 3.5 – 4.0) influent. This low pH is not only the result of spontaneous bacteriological acidification but also caused by use of HCl a major reactant in soybean processing. Successful application of non-reagent struvite formation will depend on the right combination of PO_4^{3-}-P/l released and pH increase by turbulence induced in separate reactor section. Two types of wastewater were provided: influent and anaerobically processed wastewater. Composition range of major compounds of interest are shown in Table 21.

Table 21: Main parameters wastewaters non reagent pilot trials

Parameter	Influent	Anaerobic effluent
pH	3,66- 4,21	6,64 - 6,89
Total P mg/l	128-164	94-116
PO4-P mg/l	88-102	61-90
NH4-N mg/l	22-42	286-338
Mg mg/l	144-172	82-96
COD total g/l	16,2-18,4	1,25-2,22

Measurements confirmed the earlier data of an excess of Mg^{2+} versus PO_4^{3-} of about 2.5 which is in terms of struvite formation a large enough excess to induced struvite formation when correct reaction conditions are met, more in particular $NH4^+$-N levels and pH. There is noticeable decrease of Mg^{2+} when comparing influent and anaerobic stage effluent (also seen for phosphorus). This indicates that some struvite is produced and accumulating in the anaerobic lagoon, but causing no operational problems given the lagoon layout. Other more modern type of reactor configuration such as UASB reactor would be more sensitive to this struvite scaling potential and need an adapted operational strategy. A first trial run (with 0,100 m3/h) was done using only anaerobic effluent to induce pH increase by injection of compressed air (impeller not running) in the lower reactor section increasing the pH in the first reactor and letting the reaction proceed further in the second stirred tank reactor. Target pH by aeration was manually controlled and kept between 7.80 – 8.15 by adjusting aeration intensity. Table 22 gives an overview of the results of 3 batch trials run over an 8 hr period.

Table 22: Results pilot trials non reagent struvite formation

	Trial Run 1		Trial Run 2		Trial Run 3	
Time hr	**pH**	**PO4-P**	**pH**	**PO4-P**	**pH**	**PO4-P**
0	6,64	86	6,89	61	6,72	90
1	7,82	47	8,15	36	7,99	42
2	7,92	52	8,17	32	8,15	34
3	8,04	38	8,06	44	8,04	34
4	7,77	42	7,85	46	7,88	44
5	8,09	36	8	33	7,74	48
6	8	40	7,77	38	8,08	37
7	7,95	38	7,89	34	8	32
8	8,12	33	8	38	7,94	36
		46		40		44

Final PO4-P values between 40 to 46 mg P/l were obtained taking. The data also show that when pH remains below 7 the PO4-P levels remain fairly high indicating that scaling is far less to occur under these conditions. Directly blending raw influent with anaerobically processed (at a ratio that would typically be 25% raw influent an 75% anaerobically treated wastewater on a UASB recycle flow) resulted in pH values of 5.2 to 6.0. At these levels little of no scaling will occur. This is an important design parameter to be considered for applying UASB reactors under the conditions specifically for this type of wastewater. Operating the UASB at pH levels of 6.50 -6.75 should prevent scaling but still opens the option for non-reagent struvite formation as shown in schematic below. Important is to keep in the pH below 6 to protect recycle pump and internal distribution grid. The surplus anaerobically processed wastewater passes to a CO2 stripper causing pH increase and resulting is struvite fines to be separated by hydro cyclone (Figure 26).

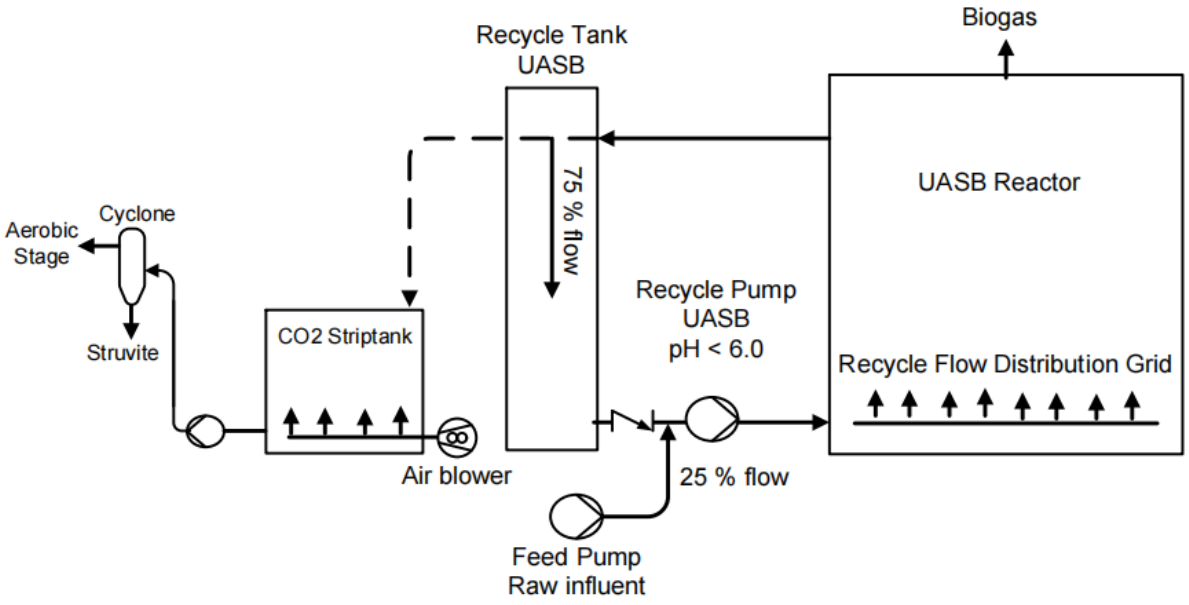


Figure 33: Adapted UASB process flow for non-reagent struvite recovery

13.3. K-struvite option

The alternative to make K-struvite after the aerobic stage has shown to be a valid approach given the high residual K levels (around 1000-1200 mg K/l). Currently excess of phosphate in the aerobic stage is removed by adding $FeCl_3$ to the activated sludge to co-precipitate the phosphorus in the sludge. However then most of it is lost for recovery. In addition, to

achieve final effluent discharge levels of 2 ppm P or even lower, will require a tertiary treatment. One option might be processing the aerobic effluent in a two-stage tertiary treatment of which the first part consists of K-struvite formation. This would also be an option not only to recover phosphorus but also the other major nutrient potassium. The full-scale plant use PAC tertiary treatment to remove aerobic stage levels form 5-10 ppm down to the 2 ppm discharge limit. Without the $FeCl_3$ dosing aerobic PO_4^{3-}-P/l levels would be in the order of 40 to 50 ppm PO_4^{3-}-P/l. If combining this with water reuse even higher levels of PO_4^{3-}-P/l (100 – 150 ppm) combined with also increased K-levels (3000 – 3600 ppm) can be available to run the process. The higher concentrations are beneficial to drive the process be means of the concentration gradient but also have a down side effect in resulting in high saturation levels increasing the production of crystal fines. This was also already observed in the laboratory trials and RO concentrate of alternative site tested. The formation of fines also largely depends on the actual concentration in the reactor vessel. When starting a batch trial, a high concentration is more likely to produced fine crystals. On the other hand, a continuous operating system, even with high incoming levels, is subjected to lower concentration of the limiting reactant since in a completely mixed system both final effluent concentration and reactor concentration are close to equal. To repeat the trial with soybean waste water, similar to the non-reagent pilot trials, aerobic effluent was collected to process through the pilot unit. The water was not processed through the RO unit and thus operated at lower K levels. The PO_4^{3-}-P/l levels of incoming water were 5-10 ppm PO4-P and were spiked with Na2(HPO4) to achieve PO_4^{3-}-P/l levels of 100 to 125 ppm PO_4^{3-}-P/l. Also, the absence of ammonium was controlled since residual levels of ammonium might adversely affect process efficiency. Again, it needs to noted that also the aerobic stage effluent is fairly rich in magnesium, this proportion of magnesium will also participate in the reaction. Since these trials are seen as proof of concept dosing MgCl2 to obtain the desired molar Mg^{2+}/PO4-P ratio was done based on the PO_4^{3-}-P/l level not considering Mg^{2+} already present. The trial was run over a week period using several batch of aerobic processed soybean wastewaters all of the collected wastewater was processed thus as one single batch. Incoming flow with 0.05 m3/h (low flow to minimize high saturation levels) was passed through stirred reactor 2 equipped with pH control (set at 8.80 Run 1 and set at 9.25 Run 2 / dosing 29% NaOH) and MgCl2 (32% w/w) which was done flow proportionally (the pilot PLC controller calculates and directs dosing according to incoming PO_4^{3-}-P/l level and desired molar Mg/PO4-P ratio). Incoming and outgoing PO_4^{3-}-P/l levels were monitored, produced product was collected that had accumulated over the entire trial period. Each day incoming water was samples once for analysis, during daily trial run finale effluent was sampled 4 times – average values of these effluent sample were taken effluent values in Figure 34 (Run 1) and Figure 35 (Run 2). Operational difference was reactor pH, since previous trials had shown that higher pH values not only gave lower final effluent values but also affected crystal morphology.

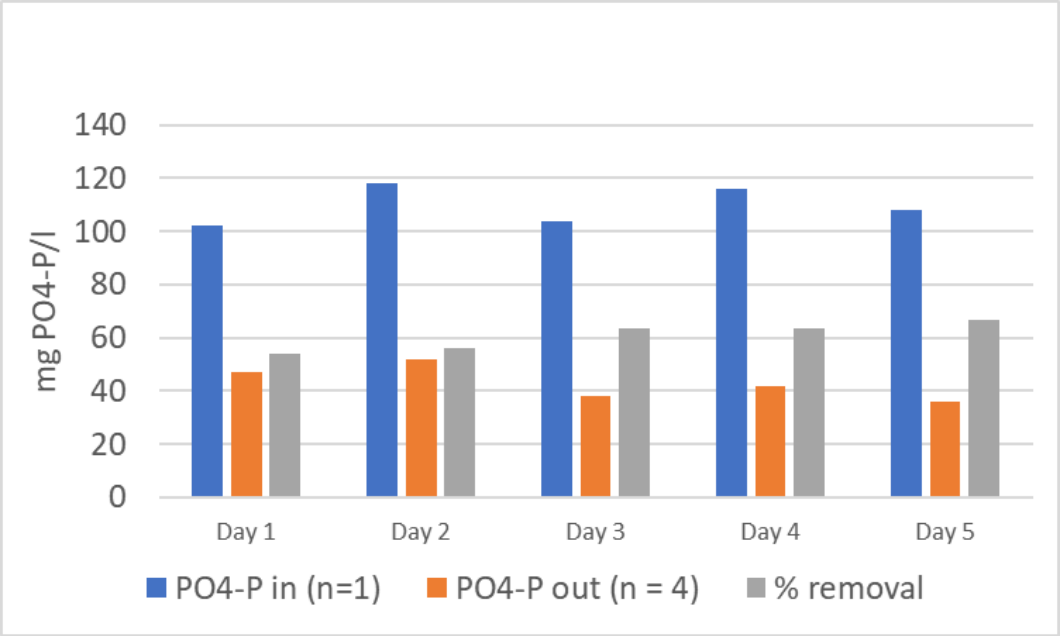


Figure 34: Phosphate levels K-struvite pilot unit Run 1 pH 8.80

Earlier observations of laboratory trials were confirmed since recovery efficiencies at pH 8.80 varied between 54 to 67% (with absolute levels left of 32 to 57 mg PO_4^{3-}-P/l /l). During Run 2 at higher pH of 9.25 recovery efficiencies varied between 74 to 83% (with absolute levels left of 18 to 32 mg PO4-P/l – Figure 35). Considering the final solubility of K-struvite the effluent values obtained at pH 9.25 are close to what is chemically possible. This means that anyway some further tertiary treatment will be needed to achieve final effluent values but it has been proven that K-struvite formation is a viable option.

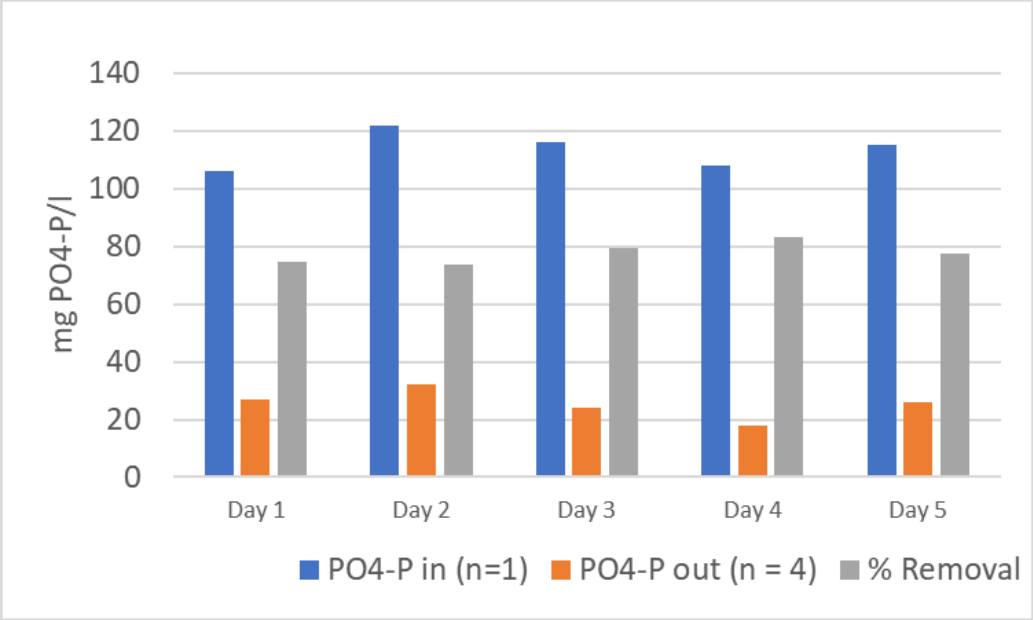


Figure 35: Phosphate levels K-struvite pilot unit Run 1 pH 9.25

At the end of the trial the reactor was drained slowly to recover produced product. The amount of wastewater processed did not allow to produce a large quantity of product (theoretical maximum was 1.80 kg of K-struvite). It was possible to collect sediment but not visually grain size was detectable. Only microscopic examination revealed the presence of crystalline material in the sediment (Figure 36). In can be noted that next to pristine crystals also some conglomeration is started to occur, being one of the initial stages of large crystal formation.

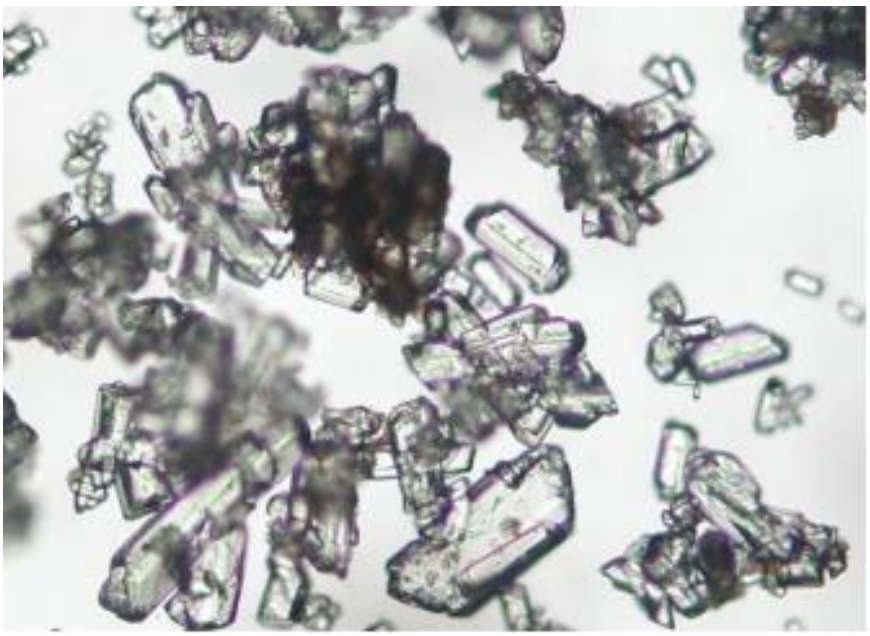


Figure 36: Microscopic image K-struvite trial Run 2

14. SOYBEAN WASTEWATER TREATMENT: INCORPORATION OPTIONS STRUVITE TECHNOLOGY

14.1. Conventional treatment process

Soybean wastewater contains high amount of phosphorus and soybean processing plants are in most cases relatively large generating significant volume of this phosphorus rich wastewater. As such this type of wastewater represents an opportunity to recovery significant amounts of phosphorus as fertilizer. However, the main challenge is that phosphorus is present as mainly phytic acid in the raw wastewater rendering it not suitable for recovery since PO4-P is needed to do so. Also, most treatment plants consist out of an anaerobic stage showing only limited phytic acid conversion to PO4-P. This means most of conversion of phytic acid to PO4-P happens in the aerobic stage resulting in high PO4-P levels needing chemical co-precipitation in the aerobic stage and in most cases still followed by a tertiary treatment to obtain final discharge limits. This co-precipitation and tertiary treatment do require chemical reactant addition and generate physico-chemical sludge that needs further processing and disposal. Due to the nature of the coagulants used (Fe/Al) the phosphorus gets tight in strongly so is now longer plant available (Fe/Al sludges) or is not allowed for land application (Al-sludges especially). Considering the above the main goal of subtask "D3.3 Design and performance of soya bean wastewater treatment with integrated struvite recovery" was to work out and test an alternative approach circumventing the issues mentioned occurring in the current wastewater treatment line combined with maximizing phosphorus recovery as a product with high fertilizer potential. The basic initial idea of adding an additional process step enhancing phytic acid conversion to PO4-P, to introduce the NH4-struvite recovery process, has been fully studied and was shown to be a viable alternative. However, elaborating this approach revealed some extra challenges (scaling control) and also eventually resulted in a second recovery route (K-struvite). So, in fact two alternatives were developed which both can recover phosphorus as fertilizer in line with the new EU fertilizer regulation but can be selected considering the case specific situation of existing treatment plants (retrofitting and incorporating P-recovery) or can be integrated in the early design stage of newly to build soybean wastewater treatment plants. Figure 37 shows the current type of processing soybean wastewater.

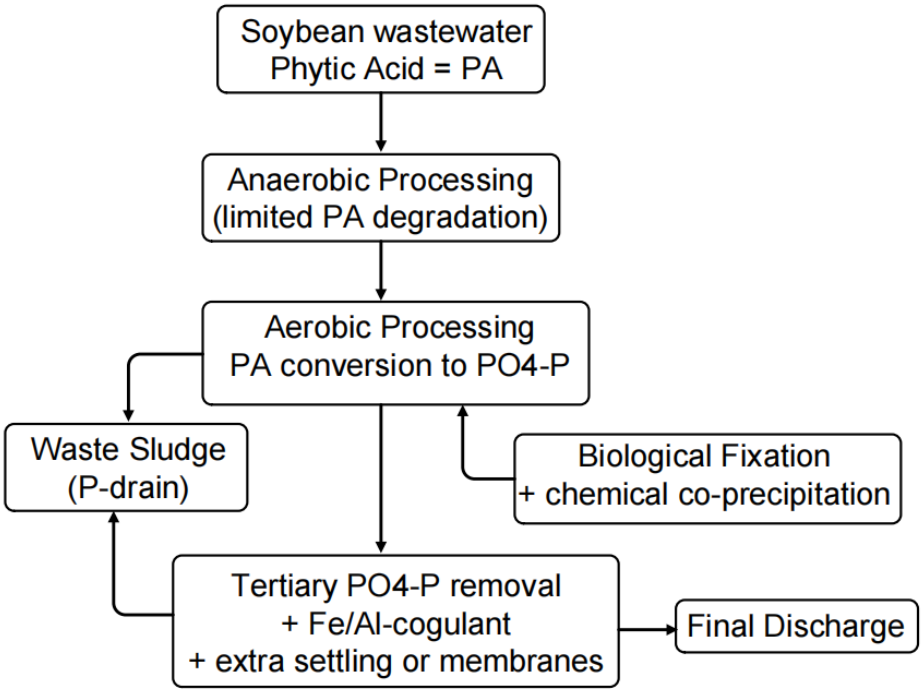


Figure 37: Current state-off-the art soybean wastewater processing

The raw wastewater passing an anaerobic stage processing where phytic acid is only limited converted into the PO4-P. This conversion is essential since PO4-P is needed to produce NH4-struvite, a premium recovered phosphorus fertilizer. Most of the phytic acid passes through the anaerobic stage and enters the aerobic stage. In the aerobic stage the phytic acid is readily converted into PO4-P. This increase the soluble PO4-P content in the processed wastewater. Phosphorus

can only be removed as a solid form wastewater, so it needs to be extracted as some kind or sludge. There are only 2 ways to do so, being either uptake as part of the biological waste sludge or co-precipitate with Fe/Al as a physical sludge type. The biological uptake can be enhanced by inducing P-luxury uptake but will still not be enough to attain final discharge levels. One has to consider, that compared to the raw wastewater, the anaerobic stage treatment has reduced the residual COD load by 80 to 90 % but nutrient levels of both N and P have remained fairly unchanged. This induces a surplus of N and P for the aerobic stage. The excess PO4-P not taken up by the biomass will be precipitated by co-precipitation in the aerobic stage by adding in most cases FeCl3. Addition of FeCl3 in the aerobic stage requires an overdose (or higher Fe/PO4-P molar ratio) to taken down the residual levels to 5-10 mg PO4-P/l. The co-precipitated Fe-P compounds become part of the overall sludge mass of the aerobic stage. Finally, to achieve the discharge limit of 2 mg PO4-P/l a tertiary stage coagulation is used. Applying this on clarified water reduces the use of coagulant (in most cases some type of Poly-aluminum-chloride, which is less acidic compared to FeCl3). Also, this step produces physico-chemical sludge that needs to be disposed by mostly processing it together with the aerobic stage sludge handling. This current processing according to Figure A results in almost of the ending up in a surplus sludge mass with the majority of the phosphorus retained as physico-chemical sludge with very or even no potential for reuse.

14.2. Combined phytase pretreatment and NH4-struvite

Figure 38 is the first alternative route to process soybean wastewater but incorporating NH4-struvite formation to recover phosphorus. As mentioned earlier the conversion of phytic acid to PO4-P is essential for NH4-struvite recovery. The latter is done by incorporating and additional step before the anaerobic stage process consisting out of enzyme (*ic. phytase enzyme*) induced conversion of phytic acid to PO4-P. Two possible ways to this by either adding commercially available phytase enzyme or introduced fungi induced phytase enzyme in-situ production. Both options were studied in detail in paragraph 4 The increase in PO4-P levels make NH4-struvite formation possible. The anaerobic stage treatment is still needed since also the TN needs to be converted into soluble NH4-N the other key ingredient of NH4-struvite. At this point a first challenge occurred since deeper analysis of the soybean wastewater revealed that for this type of wastewater not magnesium but phosphorus was the limiting parameter. The raw wastewater contains a molar surplus of magnesium to phosphorus form about 1.20. This might sound interesting but also entails a major risk. If process conditions, in more particularly pH, is not carefully monitored potential scaling will occur causing pipe/pump clogging eventually leading to a shutdown of the anaerobic stage process. The latter risk is also depending on the type of anaerobic reactor type involved. Large covered anaerobic lagoon will hardly be hampered but UASB reactor (with internal distribution grid / piping / pumps) are highly sensitive to this scaling issue. To counteract these scaling issue a variation on UASB operation was proposed so that this approach could still be applied. The extra effort of an adapted operation of the UASB operation is warranted since it does require additional reactants to be added such as MgCl2 and NaOH. This makes this approach next to the NH4-struvite route also being identified as the non-reactant adding option. By adapting the process to Figure 38 route still will require most likely the chemical co-precipitation in the aerobic stage and the tertiary treatment to achieve the final effluent goals, but the bulk quantity of the phosphorus will be removed/recovered as NH4-struvite resulting in a significant reduction of the physico-chemical sludge quantities. Also, by reducing the proportion of the physico-chemical sludge versus the biological quantity will improve the quality and reuse options of this type of sludge.

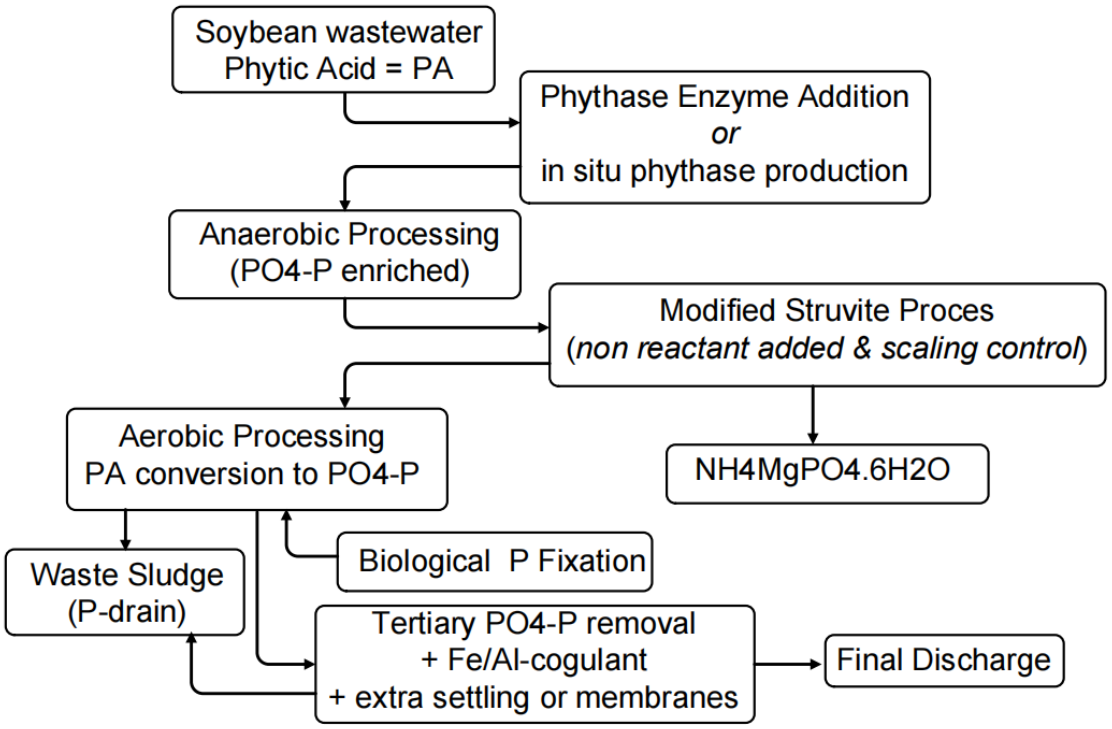


Figure 38: Alternative processing route NH4-struvite

14.3. Combined phytase pretreatment and K-struvite

The second alternative is defined as the K-struvite route as shown in Figure 39.

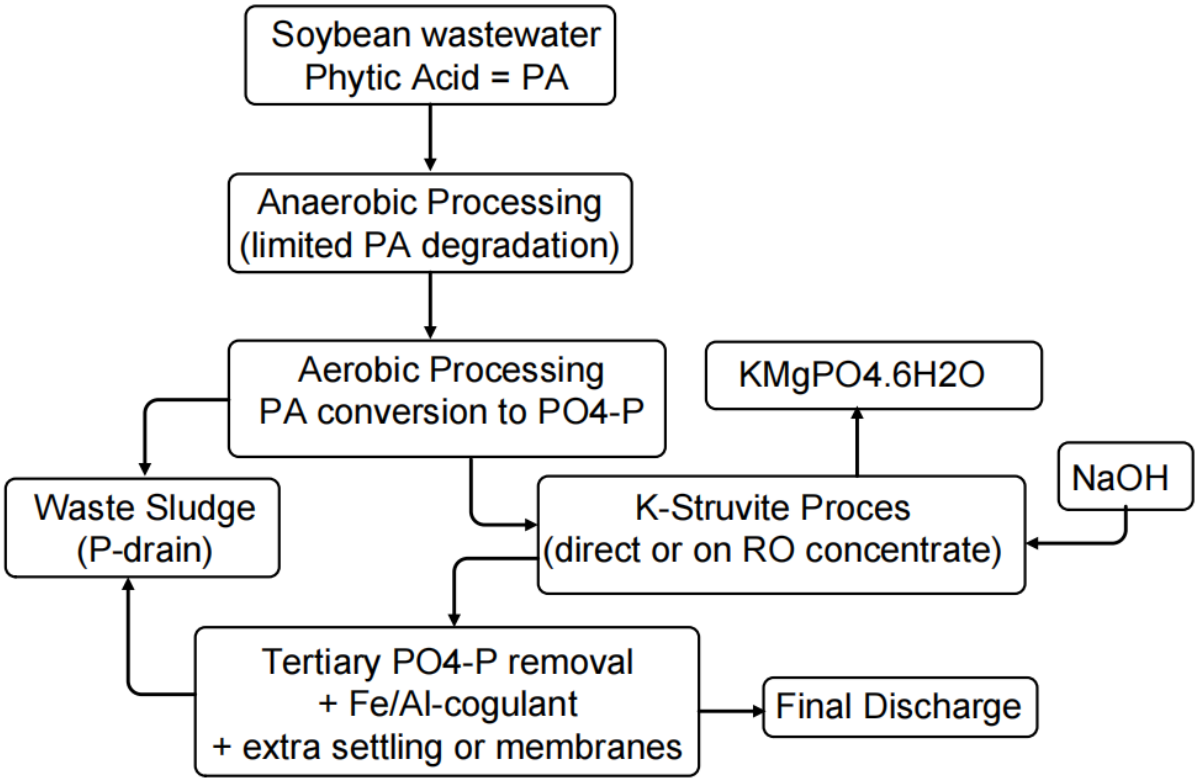


Figure 39: Alternative processing route K-struvite

The second route focuses run in a similar way as the standard treatment in which most of the PO4-P is released during the aerobic treatment stage. If differs in such that it does not use any co-precipitation in the aerobic stage. On the contrary

the goal is to maximize residual PO4-P levels in the aerobic processed wastewater. The aerobic treatment stage will also remove ammonia nitrogen thus excluding the formation of NH4-struvite. As a fact as long as ammonia nitrogen is present the formation of K-struvite is reduced since by preference ammonia nitrogen fits best the crystal structure. Only when ammonia nitrogen is no longer present the formation of K-struvite will occur. At that moment K^+ replaces NH4-N in the struvite molecule. Important to note also K-struvite can be commercialized as a fertilizer according to the new EU fertilizer directive. The aerobic effluent contains an overload of K^+ compared to liberated PO4-P and also relatively high levels of Mg^{2+} are still present. The option to produce K-struvite can either be done on the aerobic effluent directly or on RO concentrate flow originating from further upgrading of this aerobic effluent for reuse purposes. As compared to NH4-struvite the needed pH increase cannot be achieved by CO2 stripping and addition of some NaOH will be needed. One needs to see the K-struvite option as an alternative for the co-precipitation in the aerobic stage whit the advantage that a crystalline high added value product is produced instead of significant quantities of amorphous sludge that contains P in a non-reusable condition. Similar to the NH4-struvite route this also opens the full potential of the dewatered biosolids to be used as soil conditioner since no contamination with coagulants did occur.

14.4. Mass balance analysis treatment options

The two developed routes for enhanced phosphorus recovery all a clear distinct mass balance both in terms of reactants used or products/flow produced. A comparison for between these two alternatives compared to the current treatment process is shown in Table 23. As an example, a processing plant with 100 m3/h and with total P levels incoming at 150 mg P/l was taken as a reference. Another important assumption was incoming COD of 15 kg COD/m3 with an COD removal of 85% over the anaerobic stage leaving 2.25 kg COD/m3 for aerobic sludge growth (with a sludge yield of 0.4 and 3% P content in Bio WAS). Process efficiencies were either taken form trials test preformed. To outline differences similar quantities of P were either retained in the Bio WAS and effluent. As can be seen the alternatives routes are needed to process the excess of PO4-P that is not taken up by the Bio WAS (except for residual level in effluent with 4.8 kg P/day). The amount of PO4-P that could be captured as NH4-struvite is determined by the conversion of phytic acid into the free PO4-P (based on lab and pilot trials 2/3 of incoming P (minus need for Bio WAS) leaves about 100 kg PO4-P/day resulting in a 793 kg of NH4-struvite produced. The remaining 178.4 kg P still needs to be co-precipitated resulting in extra chemical waste activated sludge. For the K-struvite route all excess PO4-P is passed to the K-struvite recovery with a 242.2 kg P load having a potential K-struvite yield of 2 087 kg K-struvite. This is a major difference with the NH4-struvite since co-precipitation in involved more PO4-P can be directed towards recovery and an additional advantage is that no Chem WAS is produced. There is an increase in tertiary sludge production since the K-struvite is only taking down free PO4-P to 20 ppm, as compared to the conventional and NH4-struvite (both using coprecipitation) were residual levels are the aerobic stage were set a 5 ppm PO4-P. Figure 40 gives a view on the on the phosphorus balance for the possible treatment scenarios.

Also, of importance is that the different routes and the conventional treatment have each their specific sludge production (Bio WAS, Chem WAS and tertiary sludge) with on top either NH4-struvite or K-struvite. These quantities are also calculated under the same assumptions as for Table 23.

Table 23: Calculated sludge production different process routes (kg DM/day)

Amount DM/day	Conventional	NH4-struvite Route	K-struvite Route
Bio WAS	2160	2160	2160
Chem WAS	2227	1427	0
Total WAS	4387	3587	2160
NH4-struvite	0	794	0
K-struvite	0	0	2090
Tertiairy Sludge	96	96	384
Total WAS + Ter. Sl.	4483	3683	2544

Given the assumptions made al Bio Was levels are the same for all routes examined, which is normal since this is related to the biological conversions involved. The Chem WAS is clearly different showing a major contribution (51%) for the conventional route as compared to 40% in the NH4-struvite route. The K-struvite route, if maximally exploited, should generate no Chem WAS. However, one needs to consider following and exploiting to the max the K-route will need a pH correction on the final effluent. The latter will require to set a recovery goal with still might need some co-precipitation but nevertheless this route will produce significantly Chem WAS as compared to the other routes. This is subsequently also reflected in the quantities of either NH4-struvite or K-struvite that can be recovered, respectively calculated at nearly 800 kg NH4-struvite and 2000 kg K-struvite. Keep in mind that these are produced quantities not harvested ones. The actual harvested quantities can be set at 85-90 % of the produced levels. There is a clear difference in tertiary sludge production since the K-route will have higher residual PO4-P levels (20 ppm) compared to the conventional and NH4-struvite (5 ppm) to be captured and retained in the final effluent tertiary processing.

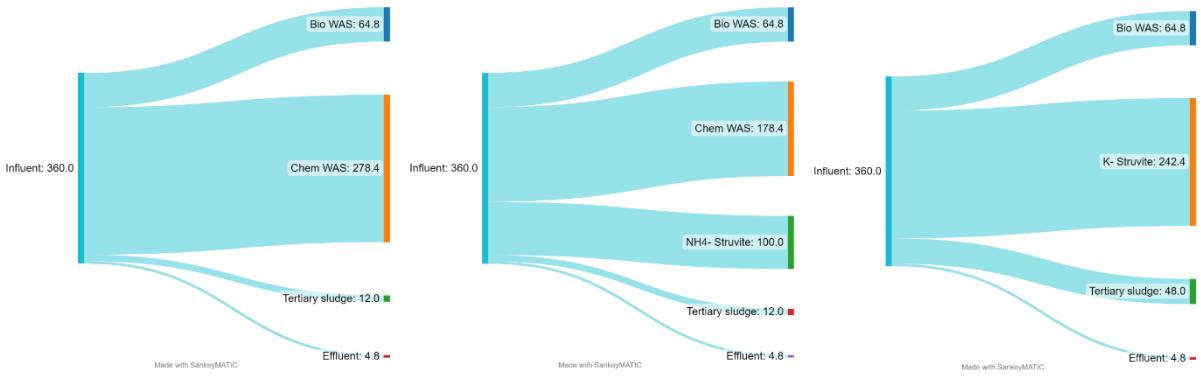


Figure 40: Mass flow P-balance for examined case example

15. GLOBAL SUMMARY

This global summary gives a condensed overview of the most important conclusions that could be made for the different stages during the entire project:

Goal of project

1. Select and upgrade technologies to highest TRL possible focused on nutrient management to ensure sustainable agronomics practice in EU as well as reduce dependency form current nutrient incoming flows
2. Select a case study within agro-industrial field to achieve above goal.
3. Adapt selected technologies to align and focus on to contribute to the EU Green Deal goals set

Agro-industrial sector selected

4. Sector finally selected was soybean processing. This represents a significant import of nutrients in EU with high GHG profile.
5. Soybean sector has a high recovery potential producing concentrated levels of NPK in wastewater + enriched with high magnesium levels. The latter opens a number of interesting opportunities for general NPK recovery
6. Challenge is occurrence of phosphorus as phytic acid. Specific pre-treatment is needed

Pretreatment options

7. Liberating bound phosphorus out of phytic acid was shown to be possible with both addition of commercially available phytase enzyme or in vivo enzyme production by selected yeasts or fungus. Enzyme addition was most effective and can be done a reasonable cost.
8. Wastewater analysis showed particular property of this type of wastewater to contain a surplus of magnesium compared to phosphorus. The presence of high levels of potassium can also be turned into advantage with regard to phosphorus recovery.

Selected Technologies

9. It was shown that phytase pretreatment did enhance PO4-P levels thus improving overall recovery efficiencies. The latter to be combined with anaerobic treatment.
10. A very interesting development was the possibility of producing recovered phosphorus as struvite not needing any additional chemical input based on CO2 stripping induced pH increase.
11. The process described under 10 did also point out the need for adapted anaerobic reactor design to prevent scaling issues that could cause otherwise frequent process shutdowns for maintenance purposes.
12. Next to most know NH4-struvite production it was also demonstrated that K-struvite production after the final aerobic stage. This is a very important finding since it opens the option for K recovery as a solid – a completely new concept on this industrial scale level.
13. An number of findings can also be implemented on other agro-industrial sector such as potato processing.

16. REFERENCES IN VIVO PHYTASE ENZYME PRODUCTION

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