Deliverable

Project Acronym: FERTIMANURE

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Grant Agreement No. 862849

D2.5. BBFs production and characterisation vs. time (list, average composition, and composition variability)

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Preface

This deliverable is part of the European project FERTIMANURE funded by the H2020 program (project number 862849). The project FERTIMANURE focuses on the formulation and evaluation of bio-based fertilisers (BBFs) produced at five pilot nutrient recovery installations and tailor-made fertilisers (TMFs) as blends of BBFs and (synthetic) mineral fertilisers to meet the soil-crop specific requirements.

In FERTIMANURE work package (WP) 2 (*Nutrient recovery from animal manure*), five different on-farm pilots have been implemented during the first year of the project and have been operated and optimised from month (M) 13 to M42. During the demonstration period, the inflow and outflow streams for each on-farm pilot were monitored, and the quality of the produced BBFs was determined and optimised within Task 2.2 (Inflow and outflow stream characterisation, M13-M42). Analytical ad hoc protocols were proposed for each BBF to assess its fertiliser properties (chemical, physical, biological, and functional fertiliser properties), physic-chemical properties, and the presence of toxic chemical elements or molecules.

Deliverable 2.5 (BBFs production and characterisation vs. time – list, average composition, and composition variability) aims to provide information about the BBFs characteristics obtained after the optimisation of the five FERTIMANURE pilots. This deliverable will complement the information provided within D2.6 (Mass and energy balance of the on-farm pilots).

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Summary

The FERTIMANURE concept integrates a set of innovative treatment schemes to efficiently valorise animal manure and to obtain fertilizing products with high added value. FERTIMANURE pursues the improvement of several technologies that are either currently under development or that have been successfully used for similar applications and proposes innovative integrated solutions to finally reach a zero-waste manure management approach. In WP 2, five different on-farm pilots were implemented during the first year of the project, and they have been operated and optimised from M13 to M42. WP2 activities were implemented by partners having different profiles: scientific partners (UVIC, LEITAT, WENR, RITTMO, UGENT, FHR), farmers (APF, CPV, APCA), public bodies (DARP, APCA) and technology providers (DORSET).

During the demonstration period the inflow and outflow streams for each on-farm pilot were monitored, and the quality of the BBFs was determined and optimised within Task 2.2. Analytical ad hoc protocols were proposed for each BBF to assess its quality in terms of physical, physic-chemical properties, and the presence of toxic chemical elements and molecules, and pathogens. Results from Task 2.2 were necessary for the activities of other FERTIMANURE WPs as follows:

- WP3 (Production of tailor-made fertilisers and quality assessment): BBFs characteristics are needed to allow the formulation of tailor-made fertilisers (TMFs).
- WP4 (Demonstration of the end-products performance: incubation, pot-tests, and field trials): BBFs characteristics are needed to set up experimental assessment of BBFs and TMFs.

Deliverable 2.5 aims to provide information about the characterisation of BBFs produced in the five FERTIMANURE pilots after optimisation. This deliverable is divided into six subsequent chapters. First, a brief introduction describes the context in which the research is carried out. The second chapter summarizes the array of chemical analyses to be used to characterize the BBFs. Then, the third chapter reports the results from the BBFs characterisation that was carried out during the operation of the pilot plants. The following chapter describes the optimisation work performed during the operation of some pilot plants to obtain BBFs of higher quality. Afterwards, chapter 5 will compare the obtained BBFs characteristics with what is stated in literature. Lastly, the report ends with a general evaluation of Task 2.2 at M48.

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List of Abbreviations

BBF	Bio-based Fertiliser
TMF	Tailor-made Fertiliser
AA	Amino acid
ALGE	ALGAENERGY
APCA	French Chamber of Agriculture
APF	Arjan Prinsen Farm
CPV	Cooperativa Plana de Vic
D	Deliverable
DARP	Catalan Department of Agriculture
DM	Dry matter
DORSET	Dorset Group
FHR	Fraunhofer UMSICHT
LEITAT	LEITAT Technological Center
LF	Liquid fraction
LHV	Lower heating value
MAP	Mono-ammonium phosphate
OM	Organic matter
PAH	Polycyclic Aromatic Hydrocarbons
PTR	Project Technical Report
RITTMO	RITTMO Agroenvironment
S/L	Solid/liquid
SDS-PAGE	Sodium dodecyl sulphate - polyacrylamide gel electrophoresis
SF	Solid fraction
TCR	Thermo-catalytic reforming
TK	Total potassium
TN	Total nitrogen
TP	Total phosphorus
UGENT	Ghent University
UMIL	University of Milano
UVIC	University of Vic – Central University of Catalonia
VS	Volatile solids

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WENR	Wageningen Environmental Research
WP	Work package

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1. Introduction

The world's population continues to rise, with a medium-variant forecast predicting that by 2050, the global population will have surpassed 10 billion people (United Nations, 2019). As a result, there is a need to increase food production to meet the world's expanding population as well as to meet the Sustainable Development Goals (2: Zero Hunger, 9: Industry, innovation and infrastructure, 11: Sustainable cities and communities, 12: Responsible consumption and production), which was defined by the United Nations in 2015 (United Nations, 2020). Since agriculture is the primary source of food (FAO, 2020a), improving crop yields is a key concern. Agriculture productivity has mostly been improved via the use of fertilisers in recent decades, and global demand for nitrogen, phosphate, and potassium for fertiliser usage is predicted to grow by nearly 10% from 2016 to 2022 (FAO, 2020b). The growing need for fertilisers raises major concerns, which are mostly about their production and the environmental impacts related to their production and use. Among the most used synthetic fertilisers, N and P are frequently obtained from non-renewable resources that use high-cost methods (Cherkasov et al., 2015; Günther et al., 2018), and the environmental concerns associated with their usage are well documented (e.g., eutrophication, gaseous emissions) (Khan et al., 2014).

Biobased fertilisers (BBFs) have improved the sustainability of agriculture by reducing the use of non-renewable resources and the impact of agriculture on the environment (Wang et al., 2018). Animal manure might provide a sustainable supply of BBFs, which would be low-cost and ecologically beneficial.

In this context, the activities of the FERTIMANURE project are of particular relevance in order to extend the knowledge in the field of innovative recovery of nutrients and production of high-added value fertilisers from animal manure. Indeed, results of WP1 (*FERTIMANURE framework*) showed that animal manure may represent a valuable source of nutrients and that the transformation of animal manure in concentrated BBFs is mandatory to maximize their benefits. To achieve this goal, in WP2 (*Nutrient recovery from animal manure*), five different pilots were implemented to test innovative technologies for nutrients recovery from animal manure (Table 1). The aims of Task 2.2 (Inflow and outflow stream characterisation) are the following:

- Monitoring the inflows/outflows streams during the demonstration period for each on-farm experimental pilot and determining/optimising the quality of the products. Special attention will be given to the variation in composition of the products over time due to changes in feedstock.
- Setting-up analytical ad hoc protocols for each BBF to assess its fertiliser properties (chemical, physical biological and functional fertiliser properties), physical and chemical-physical properties, the presence of toxic element or molecules and the presence of pathogens. Protocols will take into consideration, also, the proposal of fertilisers regulation within EU (Proposal for a regulation of the European parliament and of the council laying down rules on the making available on the market of CE marked fertilizing products and amending Regulations (EC)No 1069/2009 and (EC) No 1107/2009).

The present deliverable reports information about planning of chemical analysis for BBFs characterisation and final information about the five FERTIMANURE pilots and BBFs.

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Table 1-1. The five pilot plants of FERTIMANURE

On-farm pilot	Integrated Technologies	Main feedstock	Treatment capacity (m^3/d)	Bio-based fertilisers obtained
Spain	Membrane contractors Freeze concentration Micro-algae reactor Thermo-enzymatic reactor Biodrying + thermal treatment	Pig slurry and poultry manure	3	Nutrient-rich concentrate (ES-NC), Biodried solid fraction (ES-DSC), Phosphorous (ashes) (ES-PA), Ammonium sulphate (ES-AS), AA-based biostimulants (ES-AA)
Netherlands	Biological acidification Phosphorus precipitation Nitrogen stripping Acid scrubbing	Liquid cattle slurry	8	Ammonium sulphate solution (NL-AS), Liquid potassium fertiliser (NL-LK), Organic soil conditioner (NL-SC), Wet organic phosphorus rich fertiliser (NL-WP), Dried organic P-rich fertiliser (NL-DP)
Germany	Thermo-catalytic reforming Selective NH_3-removal reaction	Solid cattle manure	2.7	Biochar (DE-BC), Ammonium phosphate on perlite (DE-AP)
Belgium	N-stripping Acid scrubbing Vacuum dryer + condenser	Pig slurry (95%) and cattle manure (5%)	10	Ammonium nitrate (BE-AN), Ammonium sulphate (BE-AS), Ammonium water (BE-AW)
France	Mobile pyrolysis Mobile N-stripping	All feedstock (pig, cattle, poultry)	0.1-0.2	Biochar (FR-BC), Ammonium sulphate (FR-AS), Liquid K-fertiliser (FR-LK)

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2. BBFs assessment protocol

Definition of unique analytical protocols for BBFs assessment was expected from Task 2.2 (Inflow and outflow stream characterisation). However, since different types of BBFs are going to be produced from the five pilot plants, it was not possible to define only one analytical protocol. It was decided to propose *ad hoc* analytical protocols that take in consideration physic-chemical properties, agronomic parameters, and the presence of toxic chemical elements or molecules according to BBFs type (mineral BBFs, organic amendments and biostimulants), production process (i.e., S/L separation, thermo-chemical processes), and fertilizing regulation (EU Regulation 2019/1009).

Among all the selected parameters, some of them can be considered useful to check pilots' performances and will be analysed at a higher frequency (at least 10-12 sampling points during the pilot run). The other parameters, needed to assess BBFs quality in respect of EU Regulation 2019/1009, will be analysed at a lower frequency (4 sampling points).

Analytical parameters to be assessed can be grouped in the following categories:

- Chemical-physical parameters: pH, electrical conductivity, density, dry matter, organic matter and organic carbon
- Agronomic parameters: macronutrients (N, P, K) content and speciation, mesonutrients and micro-nutrients content
- Pollutants content

In addition to the agreed and common parameters introduced below, additional parameters (i.e., other metals, biological pathogens) can be analysed for complementing BBFs quality information. In addition, for those parameters that are found below detection limit (DL), they are indicated as <DL, where DL value are specified.

2.1.1 Mineral BBFs

Mineral BBFs are those that contain nutrients mainly in mineral form. The analyses selected to characterize each mineral BBFs produced by the FERTIMANURE project are listed in Table 2.1. Within the produced mineral fertilisers, some of them are based on a single macronutrient (N, P, K) while others contain more than one macronutrient therefore the parameters that need to be analysed for each one of them slightly differ as they have been personalised according to the specific characteristics of each type of BBF.

Mineral BBFs produced within FERTIMANURE can be classified as follows:

- N rich fertiliser: ES-AS, NL-AS, BE-AN, BE-AS, BE-AW, and FR-AS
- P rich fertiliser: ES-PA, NL-WP, and NL-DP
- K rich fertiliser: NL-LK and FR-LK
- NP rich fertiliser: DE-AP
- NPK rich fertiliser: ES-NC

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Table 2-1. List of the chemical analyses for the characterisation of mineral BBFs.

	Parameters	**Analytical method**	**N fertiliser**	**P fertiliser**	**K fertiliser**	**NP fertiliser**	**NPK fertiliser**
Chemical-physical	pH		x	x	x	x	x
	Electrical conductivity		x	x	x	x	x
	Density	Weighing a known volume	x	x	x	x	x
	Dry matter	Drying at 105°C	x	x	x	x	x
	Organic C	Dry combustion CN analyser	x	x	x	x	x
Nutrients	Total N	N-Kjeldahl	x	x	x	x	x
	Ammonium-N	Titration after distillation	x			x	x
	NO_3^--N	Devarda's alloy test	x[a]				
	Total P	Digestion with $HNO_3/HCl/H_2O_2$ + ICP-OES	x	x	x	x	x
	Total K		x		x		x
	S		x[b]	x	x	x	x
	Ca		x[c]	x[c]	x[c]	x[c]	x[c]
	Mg		x[c]	x[c]	x[c]	x[c]	x[c]
	Na		x[c]	x[c]	x[c]	x[c]	x[c]
Micronutrients	Fe		x[c]	x[c]	x[c]	x[c]	x[c]
	Cu		x[c]	x[c]	x[c]	x[c]	x[c]
	Zn		x[c]	x[c]	x[c]	x[c]	x[c]
	Mn		x[c]	x[c]	x[c]	x[c]	x[c]
Heavy metals	Cd		x[c]	x[c]	x[c]	x[c]	x[c]
	Ni		x[c]	x[c]	x[c]	x[c]	x[c]
	Pb		x[c]	x[c]	x[c]	x[c]	x[c]
	Cr		x[c]	x[c]	x[c]	x[c]	x[c]
	Hg		x[c]	x[c]	x[c]	x[c]	x[c]
	As		x[c]	x[c]	x[c]	x[c]	x[c]
	Al		x[c]	x[c]	x[c]	x[c]	x[c]

[a]Only relevant for ammonium nitrate
[b]Only relevant for ammonium sulphate, but useful for recommendations
[c]At least 4 sampling point

2.1.3 Organic amendments

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Table 2.2 reports the proposed analyses for the characterisation of the organic amendments. Organic amendments are BBFs composed mainly of organic matter (i.e., biochar, the solid fraction from solid-liquid separation of manure or soil conditioners). Differently from mineral BBFs, the analysis of ashes was included in the physic-chemical parameters for organic amendments.

The quantification of organic C and organic matter was expected for all organic amendments along with the quantification of macronutrients (total N, total P, total K, and total S). Ammonium-N analysis was avoided for the BFFs produced at high temperatures as high temperatures lead to the complete removal of ammonium-N.

As in the case of mineral BFFs, micronutrients and heavy metals content were also assessed. The analyses selected for the assessment of organic amendment BBFs will be adapted depending on specific characteristics, transformation processes, and origins of the BBFs.

Organic amendments produced within FERTIMANURE can be classified as follows:

- Biochar: DE-BC and FR-BC
- Biodried solid fraction and soil conditioner: ES-DSC and NL-SC

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Table 2-2 List of proposed chemical analyses for the characterisation of organic amendments BBFs.

	Parameters	Analytical method	Biochar	Biodried solid fraction and soil conditioner
Chemical-physical	pH		x	x
	Electrical conductivity		x	x
	Density	Weighing a known volume	x[a]	x[a]
	Dry matter	Drying at 105°C	x	x
	Organic matter	Loss of ignition (LoI)	x	x
Macro-nutrients	Total N	N-Kjeldahl	x	x
	Ammonium-N	Titration after distillation		x
	Total P	digestion with $HNO_3/HCl/H_2O_2$ + ICP-OES	x	x
	Total K		x	x
	Ca		x	x
	Mg		x	x
	Na		x	x
	S		x	x
Micronutrients	Cu		x[a]	x[a]
	Zn		x[a]	x[a]
	Fe		x[a]	x[a]
	Mn		x[a]	x[a]
Heavy metals	Cd		x[a]	x[a]
	Ni		x[a]	x[a]
	Pb		x[a]	x[a]
	Cr		x[a]	x[a]
	Hg		x[a]	x[a]
	As		x[a]	x[a]

[a]At least 4 samples

2.1.3 Biostimulants

Biostimulants are fertilising products which function is to stimulate plant nutrition processes by improving one or more of the following characteristics of plant or plant rhizosphere: (a) nutrient use efficiency, (b) tolerance to abiotic stress, (c) quality traits, or (d) availability of confined nutrients in the soil or rhizosphere.

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Analysis for biostimulants were selected for the only biostimulant produced from FERTIMANURE pilot plants, specifically, the amino acid-based (AA-based) biostimulant produced by the Spanish pilot (ES-AA). The analytical characterisation will be carried out for each production cycle that includes the required time for microalgae growth, harvest, concentration of biomass density through centrifugation and biomass hydrolysis to obtain the AA-based biostimulant.

Table 2-3. List of proposed chemical analyses for the characterisation of biostimulants BBFs

	Parameters	Analytical method	Biostimulants
Chemical-physical	pH		x
	Electrical conductivity		x
	Dry matter content	Drying at 105°C	x
	Density	Weighting a known volume	x
	Organic matter	Weight loss after ignition	x
Macro-nutrients	Total N	N-Kjeldahl	x
	Ammonium-N	Titration after distillation	x
	Free amino acids		x
	Total P	Digestion with $HNO_3/HCl/H_2O_2$ + ICP-OES	x
	Total K		x
	Ca		x
	Mg		x
	Na		x
Micronutrients	Cu		x
	Zn		x
	Fe		x
	Mn		x
Heavy metals	Cd		x
	Ni		x
	Pb		x
	Cr		x
	Hg		x
	As		x

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3. BBFs characterisation

In the following subsections of this D2.5, the results of the full characterisation of BFFs from each pilot plant are reported. In addition, the estimated production amounts of each BBF and the product form (solid, liquid) are also reported in order to provide data for WP3's tool, aimed to provide a decision support system for farmers regarding the use of raw manure and FERTIMANURE BBFs depending on the soil conditions and the targeted crop. These results will be used in section 4 for comparing FERTIMANURE BBFs with those reported in literature as well as the current regulation (WP3) for assessing the marketability (WP6).

Table 3-1.reports the summarised physic-chemical characteristics of all the 18 BBFs obtained in the project. A more detailed analysis of the characteristics is done within the sections specifically addressing each of the 5 pilots.

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Table 3-1 Summarised physic-chemical characteristics of all the 18 BBFs obtained

Code	Description	Estimated amount (tonne/y)	N samples	pH	Density (kg/L)	Dry matter (g/kg)	Organic matter (g/kg)	Organic Carbon (g/kg)	TN (g/kg)	Ammonium-N (g/kg)	TP (g/kg)	TK (g/kg)	S (g/kg)	Ca (g/kg)	Mg (g/kg)	Na (g/kg)
NL - AS	Ammonium sulphate solution	40	14	5.3	1.1	327	334	0.89	65.3	61.5	<0.03	<0.4	73.2	0.2	0.04	<0.05
NL - LK	Liquid K-fertiliser	1,703	14	8.3	0.99	42	25	15.7	3.1	1.7	0.44	5.1	0.5	0.9	0.5	0.7
NL - SC	Soil conditioner	179	14	8.4	0.5	260	205	-	6.5	2.4	2.4	5	1.4	4.4	2.1	0.8
NL - WP	Wet organic P-rich fertiliser	10	12	8.1	1.3	302	82	-	6.2	3.5	3.1	4.6	1.5	14.1	2.8	0.5
NL - DP	*90% dried organic P rich fertiliser (calc)*	*1*	*calculated*	-	-	900	245	-	18.4	10.4	9.3	13.6	4.5	42	8.2	1.5
ES - NC	Nutrient-rich concentrate (from MF and RO retentates, 1.5:1 v:v)	6.7	3	8.03	1	41.0	24.7	13.8	3.8	2.8	0.58	2.21	0.36	1.1	0.4	1.1
ES - DSC	Biodried solid fraction (from SF of pig slurry)	0.6	5	7.2	0.38	487.4	433.9	243.0	11.5	2.7	2.7	5.0	5.2	10.6	2.7	2.1
ES - DSC	Biodried solid fraction (from poultry manure)	0.6	2	8.6	-	665.3	556.1	331.4	22.0	4.3	3.6	16.1	4.1	13.7	4.3	2.4
ES - PA	Phosphorous (ashes)	0.02	1	11.9	-	1000	-	-	-	-	68.0	73.8	10.0	149.6	36.2	35.0
ES - AM	Ammonium salts	0.7	1	5.5	0.96	235	-	-	44	44	<1	< 1	62.9	<1	<1	<1
ES - AA	AA-based biostimulants	0.01	2	7.7	0.99	65.4	60.4	25.2	4.5	-	40.9	1.2	0.37	<0.1	<1	0.33
DE - BC	Biochar (solid)	65	8	12.3	0.5	996	522	393	10.06	0.14	30.4	95	2.5	22.9	6.7	8.2
DE - AP	Ammonium phosphate on perlite (solid)	8	3	4.0	1.8	990	<1.0	0	122	122	198	0	0	0	0	0
BE - AN	Ammonium nitrate	285	5	6	1.3	390.8	<1	0.12	153.1	76.2	0.06	0.55	0.37	0.37	0.08	0.58

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BE - AS	Ammonium sulphate	285-85,300	15	5.6	1.2	308.5	<1	0.82	74.2	74.1	0.05	0.68	81.3	0.56	0.04	0.78
BE - AW	Ammonium water	724	12	10.3	1.1	-	-	0.56	158.2	154.9	0.03	0.89	0.55	0.30	0.07	0.41
FR - BC	Biochar (from poultry manure)	4.2	5	11.8	0.21	979	700	350	25.3	< 1	24.8	76.7	7.1	35.7	16.7	13.4
FR - BC	Biochar (from solid digestate)	7.2	5	10.3	0.13	955.5	621.6	359.8	15.9	< 0.1	17.7	38.9	9.9	35.8	17.6	-
FR - AS	Ammonium sulphate (from pig manure)	0.188	12	4.75	1.12	302.9	< 1	<1	48.8	48.8	<1	<1	130.6	13.7	<0.002	-
FR - LK	Liquid K-fertiliser (from pig manure)	11.7	5	10.9	1.01	25.2	8.5	4.25	1.92	<1.1	0.26	2.23	0.22	0.43	0.05	6.75

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Code	Description	N samples	Cu (mg/kg TS)	Zn (mg/kg TS)	Fe (mg/kg TS)	Mn (mg/kgTS)	Cd (mg/kgTS)	Ni (mg/kgTS)	Pb (mg/kgTS)	Cr (mg/kgTS)	Cr VI (kg/kg TS)	Hg (mg/kgTS)	As (mg/kgTS)	Salmonella spp. (unit/25g)	*E.coli* (CFU/g)	*Enterococcaceae* (CFU/g)	PAH (mg/kg TS)	Cl (g/kg)
NL - AS	Ammonium sulphate solution	14	<50	<250	11	<50	<0.2	<2.39	<2.39	<2.39	-	<0.024	<0.44	0	<3	<3	-	-
NL - LK	Liquid K-fertiliser	14	174	683	3400	884	<0.4	8.3	<5	9.4	-	0.053	2	0	25	11000	-	-
NL - SC	Soil conditioner	14	154	277	2391	521	<0.4	5.2	<5	6.6	-	<0.05	<1	0	9500	11000	-	-
NL - WP	Wet organic P-rich fertiliser	12	<103	<510	5664	833	<0.4	24	<5	17.2	-	<0.05	1.1	0	<3	<3	-	-
NL - DP	*90% dried organic P rich fertiliser (calc)*	*calculated*	<308	<1519	16878	2482	<1.2	71.7	<15	51.2	-	<0.15	3.3	-	-	-	-	-
ES - NC	Nutrient-rich concentrate (from MF and RO retentates, 1.5:1 v:v)	3	195	838	-	-	<0.5	10	<5	<10	<0.5	<0.4	2.5	0	<10	600	-	-
ES - DSC	Biodried solid fraction (from SF of pig slurry)	5	62.1	719.9	-	-	<0.5	4.8	3.8	<10	<0.5	<0.4	<2	0	<10	3700	0.2	1.8
ES - DSC	Biodried solid fraction (from	2	78.7	455.3	-	-	25	5.4	21.5	<10	<0.5	<0.4	<2	0	<10	<100	5.8	0.72

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	poultry manure)																	
ES - PA	Phosphorous (ashes)	1	770	2000	-	-	0.25	67	0.25	92.0	3.4	0.2	1	-	-	-	-	-
ES - AM	Ammonium salts	1	<0.1	< 0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<1	0	0	0	-	-
ES - AA	AA-based biostimulants	2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-	<0.1	<0.1	-	-	-	-	-
DE - BC	Biochar (solid)	8	51.3	358.5	3402	403	0.06	6.90	1.93	11.02	0.05	0.02	0.47	0	<10	-	2.99	17
DE - AP	Ammonium phosphate on perlite (solid)	3	0	0	0	0	0	0	0	0	0	0	0	-	-	-	0	0
BE - AN	Ammonium nitrate	5	1.2	3.4	12.3	0.4	< 0.028	0.23	0.1	0.13	-	<0.003	<0.1	-	-	-	-	-
BE - AS	Ammonium sulphate	8	2.2	5.5	18.2	1.3	< 0.028	15.2	0.1	3.52	-	<0.003	<0.1	-	-	-	-	-
BE - AW	Ammonium water	4	3.4	8.6	22.8	1.2	< 0.028	0.32	0.9	0.26	-	-	-	-	-	-	-	-
FR - BC	Biochar (from poultry manure)	1-4	157.4	897.6	2020	-	<0.19	67.5	<3.2	0.2	<0.32	<0.13	<1.3	-	<10	<23	2.25	22.1
FR - BC	Biochar (from solid digestate)	1-2	38.7	164.7	5050	430	0.15	12.7	2.4	2.7	<0.5	<0.14	0.94	0	<10	<23	1272	10.3
FR - AS	Ammonium sulphate (from pig manure)	1-2	<1	<1	21.8	<2	<0.25	<0.29	<0.76	<0.29	<0.1	<0.025	<0.245	0	<10	<10	<0.05	<0.01
FR - LK	Liquid K-fertiliser (from pig manure)	1-4	85.1	172.1	<0.0001	-	<0.43	4.26	<10.5	3.34	<2	<0.43	2.47	<100	1344	0	<1.26	<23.7

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3.1 BBFs from the Spanish pilot plant

3.1.1 The pilot plant in Spain

The Spanish pilot combines two separate treatment trains for the treatment of either solid or liquid streams derived from raw pig slurry. As a first pre-treatment step, a solid/liquid separation unit is installed to obtain different flows from the raw material. From this unit, a solid fraction is obtained and valorised either as organic amendment or as phosphorous-rich ash while the liquid fraction is valorised as nutrient-rich concentrate, ammonium sulphate solution and biostimulant. Reclaimed water is also generated as high added value by-product.

The solid fraction of pig slurry feeds the biodrying reactor (or trench) to remove part of the moisture contained in the stream by biological heat and using forced aeration. A biodried solid fraction (**ES-DSC**) is obtained from the biodrying trench which can be applied as an organic amendment to the soil or be used as a biofuel in a biomass boiler. After the combustion of the biodried solid fraction in the boiler, phosphorous-rich ashes (**ES-PA**) are generated and valorised to produce phosphoric acid using acidic extraction.

The liquid fraction of pig slurry is firstly treated through three subsequent membrane systems (MF – MC – RO) which are successively fed with the permeate of the previous membrane system. The train of technologies starts with a microfiltration with membrane pore size of 400 nm, aimed to remove suspended solids while assuring a proper performance of subsequent treatment steps. Afterwards, the membrane-assisted stripping process (MC) is fed with the permeate from the MF and ammonium sulphate (**ES-AS**) solution is obtained as product. It is worth to mention that initially, gaseous emissions were intended to be collected from the biodrying reactor towards the membrane contactor unit to be valorised as ammonium salts in this unit. However, ammonia emissions occurred as diffused emission and they are highly diluted with low pressure. Collecting and leading such ammonia emissions towards the MC unit was not feasible as it would lead most likely to low recovery efficiencies, thus, this valorisation pathway was discarded. The last filtration step is a RO that produces a nutrient-rich retentate which is further concentrated through freeze concentration technology together with the retentate from the MF. The crystallizer produces ice crystals which are then filtered to separate and to obtain reclaimed water and a nutrient-rich concentrate (**ES-NC**). Lastly, the permeate obtained from the membrane systems is used as growth media to cultivate microalgae, which are then enzymatically hydrolysed after harvesting and centrifugation of algal biomass to obtain biostimulants (**ES-AA**), while the supernatant obtained in the harvesting could be also used as reclaimed water.

During the optimisation period, different membrane-systems and freeze concentration coupling strategies were assessed. Such optimisation trials and the characteristics of BBFs obtained in each configuration are better described in section 4.1 of this report.

After two years of optimisation and running of the Spanish pilot, a variable readiness level was achieved due to the variety of the units and their treatment capacity. Most of the units of the pilot plant were consistently operated directly on farm and reached at least TRL 7 as they were implemented on-farm. In the case of micro-algae reactor and enzymatic hydrolysis unit, although they worked remotely, the TRL7 was achieved with works with upscaled systems while valorising the samples produced in the biorefinery (permeate from membrane-systems, and *Scenedesmus spp*. algae paste produced in the photobioreactor). Only the step of acidic extraction of phosphorus from ash reached a lower TRL 4 as it was only implemented at laboratory scale.

Considering the nature of the biorefinery, the treatment capacity demonstrated would lead to reconsider the type of farm where the system would be feasible to be implemented. In fact, various options arise: first of all, the separation unit and the treatment capacity for the solid fraction could be easily implemented in farm once biodrying unit is properly dimensioned for the flow of the solid fraction produced. Secondly, the scenarios for the treatment train for liquid fraction would be differing depending on the system implemented: (1) membrane-based systems could be dimensioned to treat the liquid fraction produced in a small group of nearby family

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farms or in a medium scale farm (~1000 heads), but (2) valorising RO permeate for microalgae production and biostimulant production by enzymatic hydrolysis, only could be feasible in a centralised facility receiving liquid fraction from multiple farms (>40,000 heads).

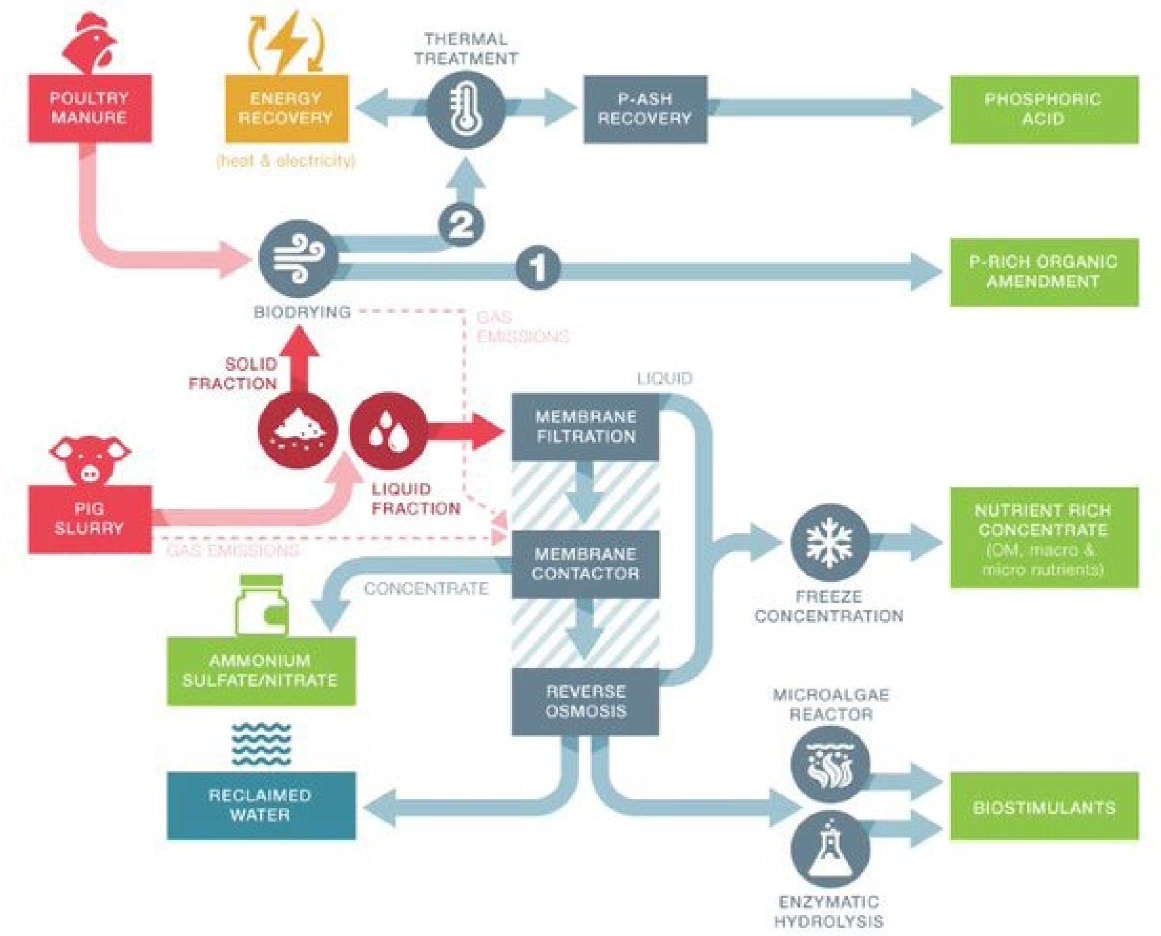

Figure 3.1. Spanish pilot infographic, including feedstocks and technologies

3.1.2 BBFs characterisation

The Spanish pilot was expected to produce five different BBFs, including mineral BBFs (**ES-PA**, and **ES-AS**), organo-mineral (**ES-NC**), solid soil amendment (**ES-DSC**), and liquid biostimulant (**ES-AA**) from microalgae hydrolysis. Table 3-2 reports the estimated production capacity of the Spanish pilot plant considering the updated BBF production yields. The updated values result in a lower production capacity given the operating timings required by the installed equipment, modifications done in experimental equipment due to the variability of feedstock characteristics and the updated mass balances obtained after 1 year operation of variable input material. The longer assessment period allowed better-estimating the production capacity of the biorefinery proposed. After the operational period, microfiltration step was identified as the main bottleneck in the production train and therefore, the production capacity was re-calculated in reference to such bottleneck.

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Therefore, the treatment capacity of Spanish biorefinery was calculated to be of 60t/y which accounts for the 3% of the overall yearly generation of slurry in the farm. In addition, it should be clarified that 50 L of biostimulant are produced from one complete microalgae production cycle, which requires roughly 3m^3 of RO permeate. Thus, up to 160 L of biostimulant could be produced yearly.

Table 3-2. Definitions of the BBFs from the Spanish pilot plant and their estimated production amounts

#BBF	Description	Type	Product form	Estimated production
ES-NC	Nutrient-rich concentrate	NPK	Liquid	6.7 tonne/year
ES-DSC	Biodried solid fraction	Soil amendment	Solid	0.6 tonne/year
ES-PA	Phosphorous (ashes)	P	Solid	0.02 tonne/year
ES-AS	Ammonium salts	N	Liquid	0.7 tonne/year
ES-AA	AA-based biostimulants	Biostimulant	Liquid	0.01 tonne/year

Although the biodrying process was improved for enhancing moisture removal efficiency, the process efficiency reported high seasonality (58 – 63% of initial moisture content removal as general terms), resulting in highly variable production rate and composition of the biodried solid fraction (**ES-DSC**). In average, organic amendment achieved a dry matter content of 487 ±175 g DM/kg. The high standard deviation reflects the high seasonal variability in the performance of biodrying. In all cases, biodried product was partially stabilised, achieving DRI values of 2.13± 0.01 (n=2) and AT4 values of 168.2± 9.9 (n=2), meaning that excessive carbon mineralization was prevented. The LHV for this product varied between 5 and 13 MJ/kg of product, which is comparable to conventional biomass fuels obtained from agricultural wastes (Quiroga et al., 2010, Winkler et al., 2013). Additionally, two trials of poultry manure biodrying were conducted at bench scale (100L working volume) obtaining a promising biodried product in terms of dry matter 665±60 g DM/kg.

In the case of the ammonium sulphate (**ES-AS**) solution, the optimisation process resulted in higher concentrations of N in the product, thanks to more recirculating liquid fraction through the membranes, reaching a level that is competitive with commercial products. In addition, the sulfuric acid dose was adjusted properly to avoid extra salinity of final product by adjusting product pH (5.5 ± 0.3) through the ammonia extraction itself. Therefore, reported characterisation is regarding the product obtained after process optimisation. The evolution of product characterisation is detailed in section 4.1.

AA-based biostimulants (**ES-AA**) characterisation were obtained from the processing of commercial *Scenedesmus* microalgae as ALGAENERGY left FERTIMANURE consortium due to company bankruptcy. Enzymatic hydrolysis optimisation tests were performed by using manure-sourced water as growth media and results are presented and discussed in section 4.1.

Regarding the nutrient-rich effluent, different membrane systems and freeze concentration configurations allowed to achieve various nutrient-rich concentrates with variable characteristics. The following tables report the data for the most promising nutrient-rich concentrate (**ES-NC**) obtained via freeze concentration of retentates from microfiltration and reverse osmosis mixed according to their production ratios (1.5 to 1, respectively) (**ES-NC-MFRO**).

The main physic-chemical characteristics of Spain BBFs are reported in Table 3-3.

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Table 3-3. Physic-chemical characterisation of the BBFs produced by the Spanish pilot plant

Parameter	Unit	ES-NC-MFRO	ES-DSC (solid fraction of pig slurry)	ES-DSC (poultry manure)	ES-PA	ES-AS	ES-AA
pH	-	8.03±0.7 (n=3)	7.2±0.2 (n=5)	8.6±0.6 (n=2)	11.9 (N=1)	5.5 (n=1)	7.7±0.2 (n=2)
CE	mS/cm	26.9±6.8 (n=3)	2.8±0.8 (n=5)	5.6± 4.1 (n=2)		56.6 (n=1)	n.a
Density	kg/L	1.0	0.38 (n=1)	n.a	n.a	0.96 (n=1)	0.99±0.01 (n=2)
DM	g/kg	41.0±13.5 (n=3)	487.4±175.2 (n=5)	665.3±59.9 (n=2)	1000	235 (n=1)	65.4. ± 0.1 (n=2)
Organic Matter	g/kg	24.7±3.9 (n=3)	433.9±159.3 (n=5)	556.1± 36.9 (n=2)	**n.a**	**n.a**	60.4 ± 1.2 (n=2)
Organic C	g/kg	13.8±2.2 (n=3)	243.0±89.2 (n=5)	311.4±20.6 (n=2)	**n.a**	n.a	25.2 ± 0.2 (n=2)**-**

-: not applicable or unknown

Macro- and micro- nutrients contents of Spain BBFs are reported in Table 3-4.

In the case of the ammonium sulphate solution, a significant effect of the temperature was observed on the extraction efficiency as the ammonium – ammonia equilibrium pKa is temperature dependent. A better extraction performance is achieved in summer (pKa 8.9 at 35ºC) than in winter (pKa 9.7 at 5ºC). After optimisation, it was achieved an ammonium sulphate solution with 44 g/L N-NH_4 and a product pH of 5.7 by acid neutralization using the stripped ammonia, so not further chemicals addition was required. Therefore, the obtained product could be potentially used directly on soil.

The nutrient-rich effluent was expected to concentrate nutrients of the liquid fraction in a small volume. Here, the configuration with most promising results achieved the concentration of 74% of nitrogen, 57% of phosphorus and 80% of potassium in 22% of the initial liquid fraction volume treated. The nutrient content in nutrient-rich product, however, is below the contents required for an organic liquid fertiliser. Therefore, the process should be further improved to concentrate up to 8.6-fold the nutrients in the influent by improving the concentration efficiency of membranes and more particularly the reverse osmosis. Optimisation of all the treatment train is still ongoing and improved concentration capacity and quality of the nutrient-rich concentrate are expected.

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Table 3-4. Macro- and micro- nutrients content of the BBFs produced by the Spanish pilot plant

Parameter	Unit	ES-NC-MFRO	ES-DSC (pig slurry SF)	ES-DSC (poultry manure)	ES-PA	ES-AS	ES-AA
Total N (NTK)	g/kg	3.8±0.1 (n=3)	11.5±3.8 (n=5)	22.0±3.5 (n=2)	n.a	44 (n=1)	4.5 ±0.3 (n=2)
Ammonium -N	g/kg	2.8±0.1 (n=3)	2.7±2.8 (n=4)	4.3 (n=1)	n.a	44 (n=1)	n.a
N-NO_3	g/kg	n.a	n.a	n.a	n.a	n.a	n.a
Total P	g/kg	0.58±0.14 (n=3)	2.7±0.8 (n=5)	3.6±1.4 (n=2)	68.0±6.9 (n=3)	< 1	40.9 ±0.1 (n=2)
Total K	g/kg	2.21±0.66 (n=3)	5.0±1.6 (n=5)	16.1±0.1 (n=2)	73.82 (n=3)	< 1	1.2 ±0.1 (n=2)
S	g/kg	0.36±0.00 (n=2)	5.2±2.4 (n=4)	4.1 (n=1)	10.0 (n=1)	62.9 (n=1)	0.37 ±0.03 (n=2)
Ca	g/kg	1.1±0.2 (n=2)	10.6±2.3(n=4)	13.7 (n=1)	149.6 (n=1)	< 1	<0.1 (n=2)
Mg	g/kg	0.4±0.1 (n=2)	2.7±0.7 (n=4)	4.3 (n=1)	36.2 (n=1)	< 1	< 1
Na	g/kg	1.1±0.5 (n=2)	2.1±0.8 (n=4)	2.4 (n=1)	35.0 (n=1)	<1	0.33 ±0.03 (n=2)
Cu	mg/kg DM	195±74 (n=3)	62.1±3.4 (n=4)	78.7±57.0 (n=2)	770 (n=1)	<0.1 (n=1)	<0.1 (n=2)
Zn	mg/kg DM	838±272 (n=3)	719.9±489.4 (n=4)	455.3±182. 0 (n=2)	2,000 (n=1)	<0.1 (n=1)	<0.1 (n=2)
Fe	mg/kg DM	n.a	n.a	n.a	n.a	<0.1 (n=1)	<0.1 (n=2)
Mn	mg/kg DM	n.a	n.a	n.a	n.a	<0.1 (n=1)	<0.1 (n=2)
Protein content	g/kg	n.a	n.a	n.a	n.a	n.a	28.3 ±1.8 (n=2)
Total free amino acids	g/kg	n.a	n.a	n.a	n.a	n.a	13.6 ±1.2 (n=2)

-: not applicable or unknown

The content of pollutants and pathogens in the BBFs produced are reported in Table 3-5.

It is important to note that it is expected to obtain a highly purity ammonium salt solution containing ammonium sulphate with no excess of sulphate ions nor protons. Although no other micronutrients and pollutants are expected to be found, analysis to detect contamination will be still carried out periodically. The most problematic heavy metals in both organic amendments obtained after biodrying process are cadmium and zinc. Most probably zinc is from veterinary origin (additives in feed or pharmaceuticals), however, more should

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be investigated regarding the origin of cadmium. Besides, although *Salmonella spp*. and *E. coli* presence meets the requirements stated for organic fertilisers and soil improvers (Regulation 2019/1009), presence of *Enterococcaceae spp*. and their colony forming units are currently not meeting the criteria established. Sanitisation of these products must be guaranteed and improvement of the product in this regard will be assessed in coming months.

Table 3-5. Content of heavy metals and pathogens in the BBFs produced by the Spanish pilot plant

Parameter	Unit	ES-NC-MFRO	ES-DSC (solid fraction of pig slurry)	ES-DSC (poultry manure)	ES-PA	ES-AS	ES-AA
Cd	mg/kg DM	< 0.5 (n=1)	<0.5 (n=3)	25 (n=1)	0.25 (n=1)	<0.1 (n=1)	<0.1-(n=2)
Ni	mg/kg DM	10 (n=1)	4.8±2.0 (n=3)	5.4 (n=1)	67 (n=1)	<0.1 (n=1)	-<0.1 (n=2)
Pb	mg/kg DM	< 5 (n=1)	3.8±2.2 (n=3)	21.5 (n=1)	0.25 (n=1)	<0.1 (n=1)	-<0.1 (n=2)
Cr	mg/kg DM	< 10 (n=1)	<10 (n=3)	< 10 (n=1)	92.0 (n=1)	<0.1 (n=1)	<0.1-(n=2)
Cr VI*	mg/kg DM	< 0.5 (n=1)	<0.5 (n=3)	< 0.5 (n=1)	3.4 (n=1)	<0.1 (n=1)	n.a
Hg	mg/kg DM	< 0.4 (n=1)	<0.4 (n=3)	< 0.4 (n=1)	0.2 (n=1)	<0.1 (n=1)	<0.1 (n=2)-
As	mg/kg DM	2.5 (n=1)	<2 (n=3)	< 2 (n=1)	1 (n=1)	<1 (n=1)	<0.1 (n=2)-
Salmonella spp.*	unit/25g	0 (n=1)	0.0±0.0 (n=3)	0 (n=1)	n.a	0 (n=1)	n.a
Escherichia coli*	CFU/g	< 10 (n=1)	<10 (n=3)	< 10 (n=1)	n.a	0 (n=1)	n.a
Enterococcaceae*	CFU/g	600 (n=1)	3700 (n=3)	<100 (n=1)	n.a	0 (n=1)	n.a
PAH*	mg/kgDM	n.a	0.20±0.01 (n=2)	5.8 (n=1)	n.a	n.a	n.a
Cl*	g/kg	n.a	1.8±1.4 (n=3)	0.72 (n=1)	n.a	n.a	n.a

-: not applicable or unknown

*: optional characterisation parameters

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3.1.3 Resulting upgrading from the original manure and specific storage needs for the recovered BBFs

Raw manure is composed by a complex and highly organic mixture with relevant content of macro- and micro-nutrients that makes difficult its use for precise fertilisation techniques. Due to its highly organic nature, manure is highly variable over the time significantly varying the species in which nutrients are present with the variable age of manure. Besides, it contains several pollutants (i.e. heavy metals, microorganisms) that limit its use as fertiliser without causing harm to soil quality. The Spanish pilot plant treats raw manure to obtain stable and upgraded fertilisers that would allow precise fertilisation strategies and accomplish with European regulation on fertilising products, enabling its deployment for technical agriculture. Table 3-6 summarises and compared the main physic-chemical characteristics of raw pig slurry and poultry manure with the obtained products in the framework of the Spanish pilot.

In the case of ES-AS, it can be observed that the product reports a concentration up to 4% N m/m, high purity, with no presence of metallic pollutants, neither other macro- or micro- nutrients. Thus, it is a suitable alternative to be used whether as a fertilising product or a secondary raw material in fertiliser industry.

Compared to the original raw slurry, the nutrient rich concentrate obtained (ES-NC-MFRO) shows similar characteristics except for the organic matter and carbon content which report half of the concentration found in raw slurry. 74% of the total nitrogen in the concentrate is present in the form of ammoniacal nitrogen, lower than the 83% found in raw slurry. The concentrations of nitrogen and phosphorus are 15% and 42% lower respectively in the nutrient rich concentrate compared with the raw slurry. Considering its characteristics, this product could be used and applied following the guidelines set for the use of pig slurry, however, according to the mass balances (see Deliverable 2.6), half of the arable land available would be needed for that.

Regarding the organic amendments obtained, both products derived from pig slurry and poultry manure improve their characteristics particularly in terms of organic matter and nutrients. Organic matter is almost 9 times higher in the biodried fraction of pig slurry (ES-DSC from pig slurry) while nitrogen, phosphorus and potassium are concentrated more than twice compared to the pig slurry. The concentration effect of biodrying in poultry manure (ES-DSC from poultry manure) is not as important as in pig slurry, probably because of the difference in the first solid-liquid separation step in pig slurry. In the case of poultry manure, organic matter, phosphorus and potassium are slightly concentrated (between 1.3 and 1.6 times). In both cases, nutrients are concentrated in a smaller volume of material which would allow to improve the logistics of their application, allowing their transportation to further arable lands. When the biodried solid fraction of pig slurry is combusted, phosphorus and potassium are 25 times and 15 times concentrated, respectively. However, Copper and Zinc are also equally concentrated (19 and 4 times, respectively). When comparison is done to the original raw slurry, phosphorus and potassium are 68 and 31 times concentrated, respectively, in a 2% of the original mass (see Deliverable 2.6).

In the case of ES-AA, it can be observed that the nutrient profile differs substantially in comparison with raw manure. Even though both products show similar dry matter, ES-AA reports higher organic matter with total nitrogen concertation up to 4500 mg/L and total phosphorous content up to 4009 mg/Kg in comparison with raw manure with only 5mg/L and /Kg of nitrogen and phosphorous, respectively. Besides, no presence of metallic pollutants is detected. It must be highlighted that ES-AA is an enzymatic hydrolysate produced from microalgae cultivated in manure-derived permeate. Microalgae cultivation allowed the bioconversion of initial nutrients present in manure into microalgae biomass, rich in nutrients such as nitrogen and phosphorus. The hydrolysis of this microalgae biomass allows the release of these nutrients into the final hydrolysate. In addition, enzymatic hydrolysis produces free-AA (up to 14g/Kg). Altogether, ES-AA is an added value alternative to be used as biostimulant.

Finally, in case that it is not feasible to implement an on-farm photobioreactor for microalgae cultivation, the obtained RO permeate could be also used as reclaimed water as its quality accomplishes with what is stated in both national and European regulations. All in all, the concept of the biorefinery allows a fairly high volume

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of water recovery, meaning that the nutrients are concentrated in the half of the original volume, improving the overall logistics of their management as half of the arable land available would be needed to apply the same amount of nitrogen.

In terms of storage requirements, several recommendations should be given according to the nature of the BBFs themselves. As the ammonium sulphate (ES-AS) obtained is mainly an inorganic solution, with no organic content, it does not need special conditions to be effectively and safely stored. Same consideration should be taken for phosphoric acid when acidic extraction of phosphorus is done from the combustion ashes. Even though phosphoric acid was produced at rather low scale, if no purification step is included in the treatment, partial crystallisation, probably of impurities, can occur. In addition, if phosphorus rich ashes (ES-PA) will be used as BBFs, then they can be directly stored, even long-term storage could be possible. Regarding organic BBFs, biodried organic amendments (ES-DSC) would not need any special requirement of storage rather than keeping it in dry conditions and away from the sunlight, at room temperature. Nutrient rich concentrates (ES-NC) should be kept in cold chambers and applied shortly after its purchase due to its highly organic condition. Chemical characteristics of the product might change easily even in cold conditions and even generation of mold can happen. Finally, the produced biostimulant (ES-AA) is a liquid product, it could be used as liquid form. In this case acidification up to pH 3 and cool storage is advised. In turn, liquid product could be dehydrated (by spray drying for instance) and stored at room temperature.

Although the fresh RO permeate does not contain microbial contaminants, if it will be stored, it is recommended to perform a disinfection process (i.e. UV, ozone) prior its use.

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Table 3-6 Summary of main physic-chemical characteristics in raw materials and BBFs obtained in the Spanish pilot

Parameter	Raw pig slurry*	Raw poultry manure	ES-NC-MFRO	ES-DSC (solid fraction of pig slurry)	ES-DSC (poultry manure)	ES-PA	ES-AS	ES-AA	ES - Water
pH	6.87±0.43 (n=7)	7.5±0.22 (n=2)	8.03±0.7 (n=3)	7.2±0.2 (n=5)	8.6±0.6 (n=2)	11.9 n=1)	5.5 (n=1)	7.7±0.2 (n=2)	7.79±0.85 (n=8)
Electrical conductivity (mS/cm)	26.43±3.86 (n=7)	5.2±2.7 (n=2)	26.9±6.8 (n=3)	26.9±6.8 (n=3)	2.8±0.8 (n=5)	5.6± 4.1 (n=2)		-	0.15±0.98 (n=8)
Dry matter (g/kg)	64.69±18.48 (n=7)	418.5±27.6 (n=2)	41.0±13.5 (n=3)	487.4±175.2 (n=5)	665.3±59.9 (n=2)	1000	235 (n=1)	65.4. ± 0.1 (n=2)	0.50±0.08 (n=8)
Organic C (g/kg)	27.59±8.45 (n=7)	205±13.4 (n=2)	13.8±2.2 (n=3)	243.0±89.2 (n=5)	311.4±20.6 (n=2)	-	-	25.2 ± 0.2 (n=2)	0.26±0.04(n=8)
Organic matter (g/kg)	49.26±15.09 (n=7)	366.1±23.9 (n=2)	24.7±3.9 (n=3)	433.9±159.3 (n=5)	556.1± 36.9 (n=2)	-	-	60.4 ± 1.2 (n=2)	-
Total N (g/L or g/kg)	4.45±0.43 (n=6)	22.0±10.2 (n=2)	3.8±0.1 (n=3)	11.5±3.8 (n=5)	22.0±3.5 (n=2)	-	44 (n=1)	4.5 ±0.3 (n=2)	0.14E10-3±0.02E10-3 (n=8)
Ammonium-N (g/kg)	3.71±0.34 (n=5)	3.9±0.5 (n=2)	2.8±0.1 (n=3)	2.7±2.8 (n=4)	4.3 (n=1)	-	44 (n=1)	-	-
Organic-N (g/kg)	1.79±1.04 (n=6)	18.3±10.7 (n=2)	0.95±0.2 (n=3)	6.47±5.99 (n=5)	15.16 (n=1)	-	-	-	
TP (mg/kg)	996.45±298.83 (n=7)	2793.5±4.9 (n=2)	580.3±143 (n=3)	2729.6±747.2 (n=5)	3647±1296 (n=2)	68020±6908 (n=3)	< 1	4009 ±100 (n=2)	$(3.40±0.51)·10^{-4}$ (n=8)
TK (mg/kg)	2369.79±510.59 (n=7)	12,879±3777 (n=2)	2213.7±660.2 (n=3)	5031±1634 (n=5)	16052±101(n=2)	73848±2189 (n=3)	< 1	1200±.100 (n=2)	0.09±0.01 (n=8)
Cu (mg/kg)	5.49±1.5 (n=3)	27.6 (n=1)	7.63±1.1 (n=3)	39.68±13.33 (n=5)	44.73±41.6 (n=2)	770 (n=1)	<0.1 (n=1)	<0.1 (n=2)	<0.1 (n=8)
Zn (mg/kg)	39.83±11.63 (n=3)	166.7 (n=1)	31.02±3.3 (n=3)	492.5±253.9 (n=5)	247±165 (n=2)	2,000 (n=1)	<0.1 (n=1)	<0.1 (n=2)	<0.1 (n=8)

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*data included only comprise the operational period after the modification of the mesh size (April 2022)

3.2 BBFs from the Dutch pilot plant

3.2.1 The pilot plant in The Netherlands

The process flow diagram of the Dutch pilot plant Animal Prinsen Farm (APF) is shown in Figure 3.2. A more detailed description can be found in deliverable 2.6. Cattle slurry produced in the stable is collected in a continuously mixed cellar with a storage capacity of about 400 m^3. The anaerobic digester is fed from this cellar by pumps every 140 minutes. The added co-substrates (feed residues and grass, in the past beet tips) are mixed in a separate biological acidification tank together with some digestate. The feed residues are the parts of the grass, hay, maize, and wheat that the cattle did not eat. The liquor acidified by fermentation is pumped to the anaerobic digester in order to increase the biogas production due the easily decomposable material. The annual biogas production is about of 42,863 Nm^3/year with 23,575 Nm^3 methane and 904 GJ total calorific value.

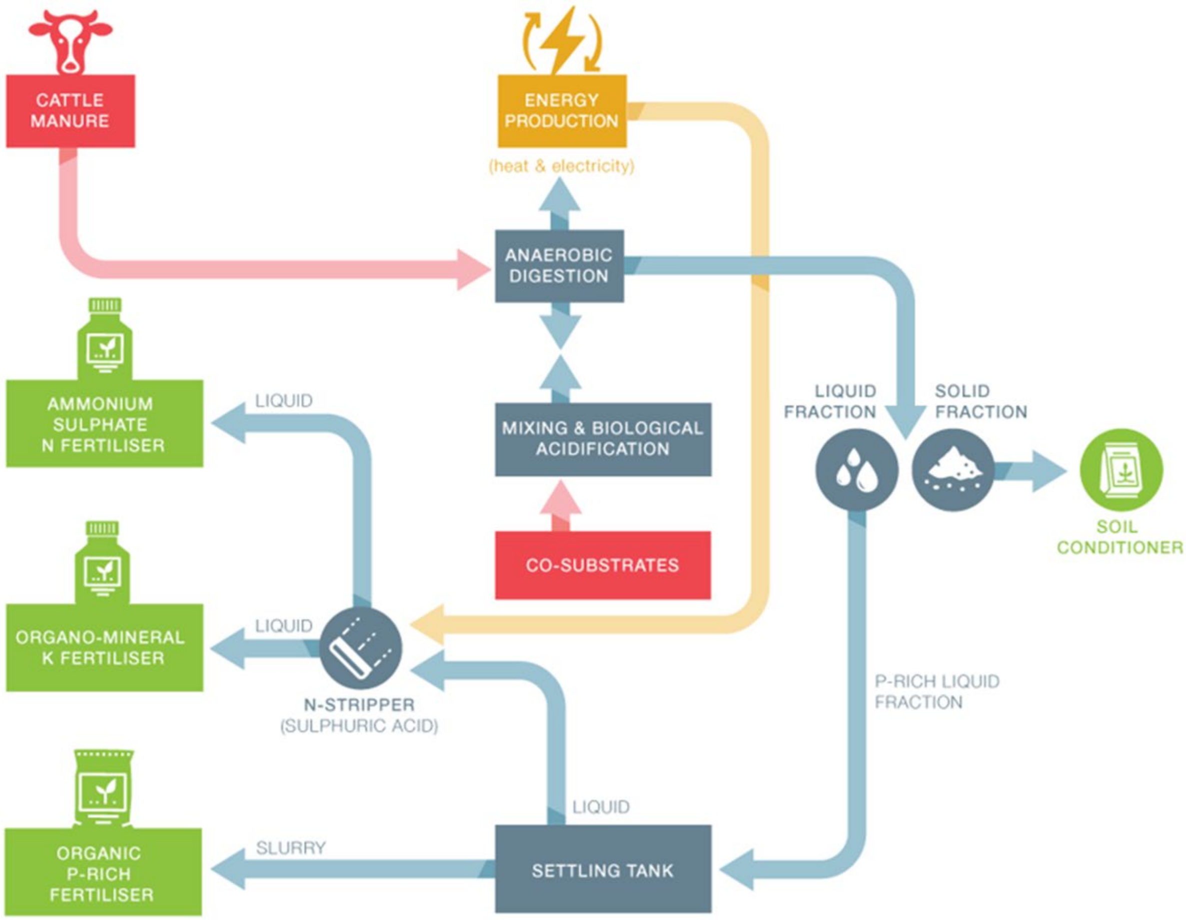

Figure 3.2. Dutch pilot infographic, including feedstocks and technologies

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The produced digestate (average 1,780 tonne/year) flows into a buffer tank. From there it is fed batch wise to a screw press with a 500 µm pore size filter to separate the digestate in a solid and a liquid fraction. The solid fraction is rich in fibres and it is therefore used as an organic soil conditioner (**NL-SC**) or could be used for biobased materials/products. Part of this solid fraction, which contains most of the surplus phosphorus (P), is sold directly from the farm to customers (hobby gardeners) and is used at own farm or can be used by neighboring farmers.

The liquid fraction of digestate is pumped to a settling (reactor) tank, with an effective volume of 17 m^3, to remove part of the remaining particles before it is treated in the nitrogen stripper-scrubber installation. The settling (reactor) tank operates in batches and a base (MgOH or CaOH) be added to raise the pH of its content in such way a part of the P in solution precipitates settling as a sludge. This sludge can be collected at the bottom of its conus as a wet organic P rich fertiliser (**NL-WP**). The P recovery (**NL-WP**) can be further developed and tested, for better recovery rates and if possible dried to obtain a 90% dried organic phosphorus rich fertiliser product (**NL-DP**). The remaining liquid fraction is further treated in the N stripper that operates in batches of six hours, where ammonia is stripped to the gas phase and subsequently dissolved again in the attached scrubber with sulfuric acid. Thereby a concentrated ammonium sulphate solution (**NL-AS**) and a liquid potassium (K) fertiliser (**NL-LK**) are produced.

It is worth mentioning that the Dutch pilot is treating all the manure that Arjan Prinsen farm is generating. Moreover, this pilot will remain operating in the farm and has supposed a positive business case for Arjan Prinsen. In addition, the scheme proposed is currently being replicated in several farms nearby demonstrating the role of Arjan Prinsen as front runner in the improved management of the manure that their farm generate.

3.2.2 BBFs characterisation

Table 3-7 describes the BBFs from the pilot in The Netherlands providing typology, product form and expected amount (tonne/year). Five BBFs are formally reported, although two of them are the same BBF type but in a wet and a dried form (NL-WP and NL-DP). The other BBFs are mineral BBFs NL-AS and NL-LK, and the NL-SC as organic BBF. Results of priority characterisation of the dried organic P-rich fertiliser are calculations assuming an up-concentration of 90%.

Table 3-7. Definitions of the BBFs from the Dutch pilot plant APF and their estimated production quantities

#BBF	Description	Type	Product form	Estimated production
NL-AS	Ammonium sulphate solution	N	Liquid	40 tonne/year
NL-LK	Liquid potassium fertiliser	K	Liquid	1,703 tonne/year
NL-SC	Organic soil conditioner	Soil amendment	Solid	179 tonne/year
NL-WP	Wet organic phosphorus rich fertiliser	P	Semi-solid (sludge)	10 tonne/year
NL-DP	90% dried organic phosphorus rich fertiliser (calc.)	P	Solid	1 tonne/year

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The main chemical-physical characteristics, macro- and meso- nutrients content as well as the content of micronutrients, heavy metals, and some pathogens pollutants of the BBFs produced by the Dutch pilot plant are reported in Table 3-8, Table 3-9 and Table 3-10, respectively. In total there were 14 sampling rounds of the flows of the system and end-products in the period 2020-2022. Not every parameter was analysed in every round for minimizing costs and there were also some sample errors (see sample sizes in the tables). Generally standard parameters like pH, dry matter, organic matter, most macro and meso nutrients and some micronutrients/heavy metals (copper and zinc) were sampled (almost) every round. Micronutrients and heavy metals were sampled at least 4 times. Some pathogens were analyses for one round in the context of the EU FPR and RENURE criteria compliance, showing results in line with what is expected for these products after anaerobic digestion.

The ammonium sulphate (NL-AS) has a low pH (acid) of about 5.3 (can be set) compared to all other products which have a pH around 8. AS is rich in total nitrogen with 6.3% w/w, almost completely in the form of ammonium. Apart from sulphur, it does not contain significant amounts of other elements/nutrients, as is the goal of this product. The liquid potassium rich fertiliser (NL-LK) is relatively rich in K, especially compared to P and N, but contains also many other nutrients and still contains some organic matter. The organic soil conditioner (NL-SC) product is rich in organic matter with about 20% OM, but contains also significant levels of P, K and other nutrients. The P rich sludge (NL-WP) had a high density and is relatively rich in P and since passive precipitation is in placer contains also some organic matter and relatively high levels of Ca, Mg and Fe, probably in the form of precipitates in combination with phosphate. Measured heavy metal concentrations and pathogen occurrence are in line what could be found in cattle and other livestock manure.

Table 3-8 Chemical-physical characterisation of the BBFs produced by the Dutch pilot plant APF on fresh matter basis

Parameter	Unit	NL-AS	NL-LK	NL-SC	NL-WP	NL-DP (calculated) [a]
pH	-	5.3 ± 1.2 (n=14)	8.3 ± 0.2 (n=14)	8.4 ± 0.5 (n=14)	8.1 ± 0.2 (n=12)	-
EC	mS/cm	66.6 ± 96 (n=10)	2.6 ± 0.3 (n=14)	2.2 ± 0.5 (n=12)	2.4 ± 0.5 (n=12)	-
Density	kg/L	1.1 ± 0.03 (n=11)	0.99 ± 0.03 (n=9)	0.5 ± 0.2 (n=14)	1.3 ± 0.2 (n=7)	-
DM	g/kg	323 ± 60 (n=13)	42 ± 8 (n=14)	260 (± 84) (n=14)	302 ± 118 (n=12)	900 ± 45
Organic Matter	g/kg	334 ± 69 [b] (n=9)	25 ± 7 (n=14)	205 ± 61 (n=14)	82 ± 12 (n=12)	245 ± 37
Organic C	g/kg	0.89 ± 0.17 (n=9)	15.7 ± 3.8 (n=2)	-	-	-

-: Not applicable or unknown
[a] Same sample size as NL-WP, since calculated based on those samples
[b] Value deemed unreliable, likely due to evaporation of NH_3 during analysis as determined by an external lab, the alternative parameter of (total) organic carbon (TOC) is accurate

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Table 3-9 Macro- and micro- nutrient content of the BBFs produced by the Dutch pilot plant

Parameter	Unit	NL-AS	NL-LK	NL-SC	NL-WP	NL-DP (calculated) [a]
Total N	g/kg	65.3 ± 11.3 (n=13)	3.1 ± 1 (n=14)	6.5 ± 1.4 (n=14)	6.2 ± 0.7 (n=12)	18.4 ± 0.3
Ammonium-N	g/kg	61.5 ± 9.8 (n=13)	1.7 ± 0.7(n=14)	2.4 ± 0.7 (n=14)	3.5 ± 0.4 (n=12)	10.4 ± 0.2
Total P	g/kg	<0.03 ± 0.01(n=10)	0.44 ± 0.11(n=14)	2.4 ± 0.5 (n=13)	3.1 ± 0.1 (n=12)	9.3 ± 0.4
Total K	g/kg	<0.4 ± 0.1 (n=10)	5.1 ± 0.3(n=14)	5 ± 0.5 (n=14)	4.6 ± 0.8 (n=12)	13.6 ± 0.3
S	g/kg	73.2 ± 12.2(n=14)	0.5 ± 0.1(n=14)	1.4 ± 0.4 (n=14)	1.5 ± 0.3 (n=12)	4.5 ± 0.1
Ca	g/kg	0.2 ± 0.1 (n=10)	0.9 ± 0.2(n=14)	4.4 ± 2 (n=14)	14.1 ± 3.7 (n=12)	42 ± 1.4
Mg	g/kg	0.04 ± 0.03 (n=9)	0.5 ± 0.1(n=14)	2.1 ± 0.7 (n=14)	2.8 ± 0.9 (n=12)	8.2 ± 0.3
Na	g/kg	<0.05 ± 0.003(n=9)	0.7 ± 0.1(n=10)	0.8 ± 0.1 (n=9)	0.5 ± 0.1 (n=8)	1.5 ± 0.03
Cu	mg/kg DM	<50 ± 0 (n=8)	174 ± 169 (n=14)	154 ± 362 (n=12)	<103 ± 6.5 (n=12)	<308 ± 2.5
Zn	mg/kg DM	<250 ± 0 (n=8)	683 ± 426(n=14)	277 ± 204(n=12)	<510 ± 34 (n=12)	<1,519 ± 13
Fe	mg/kg DM	11 ± 16.6 (n=5)	3,400 ± 812(n=12)	2,391 ± 1,002 (n=11)	5,664 ± 2,385 (n=11)	16,878 ± 913
Mn	mg/kg DM	<50 ± 0 (n=4)	884 ± 110 (n=7)	521 ± 235 (n=7)	833 ± 97.1 (n=7)	2,482 ± 37.1
Se	mg/kg DM	<0.3 ± 0 (n=3)	3.5 ± 0.9 (n=3)	1.4 ± 1.4 (n=4)	0.8 ± 0.02 (n=3)	2.2 ± 0.01
Mo	mg/kg DM	<0.5 ± 0 (n=2)	5.2 ± 0.9 (n= 2)	1.6 ± 0 (n=1)	1.2 ± 0 (n=1)	3.6 ± 0
Co	mg/kg DM	<0.5 ± 0 (n=4)	4.3 ± 0.3(n=7)	1.8 ± 0.9 (n=7)	2.3 ± 0.4 (n=7)	6.7 ± 0.1

-: not applicable or unknown

[a] Same sample size as NL-WP, since calculated based on those samples

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Table 3-10. Heavy metals and pathogens content of the BBFs produced by the Dutch pilot plant

Parameter	Unit	NL-AS	NL-LK	NL-SC	NL-WP	NL-DP (calculated) [a]
Cd	mg/kg DM	<0.2 ± 0.002 (n=5)	<0.4 ± 0.02 (n=7)	<0.4 ± 0 (n=7)	<0.4 ± 0 (n=7)	<1.2 ± 0
Ni	mg/kg DM	<2.39 ± 0.24 (n=5)	8.3 ± 1.4 (n=7)	5.2 ± 0.2 (n=7)	24 ± 8.6 (n=7)	71.7 ± 3.3
Pb	mg/kg DM	<2.39 ± 0.24 (n=5)	<5 ± 0 (n=7)	<5 ± 0 (n=7)	<5 ± 0.1 (n=7)	<15 ± 0.03
Cr	mg/kg DM	<2.39 ± 0.24 (n=5)	9.4 ± 2.4 (n=7)	6.6 ± 1.6 (n=7)	17.2 ± 3.8 (n=7)	51.2 ± 1.5
Hg	mg/kg DM	<0.024 ± 0.002 (n=5)	0.053 ± 0.007 (n=7)	<0.05 ± 0 (n=7)	<0.05 ± 0 (n=7)	<0.149 ± 0
As	mg/kg DM	<0.44 ± 0.14 (n=5)	2 ± 0.4 (n=7)	<1 ± 0.1 (n=7)	1.1 ± 0.05 (n=7)	3.3 ± 0.01
Tl	mg/kg DM	<0.13 ± 0.05 (n=5)	<0.25 ± 0.3 (n=7)	<0.25 ± 0 (n=7)	<0.25 ± 0 (n=7)	<0.7 ± 0
Salmonella spp. [c]	unit / 25g	Not established (n=1)	Not established (n=1)	Not established (n=1)	Not established (n=1)	-
Escherichia coli [c]	CFU/g [b]	< 3 (n=1)	25 (n=1)	9,500 (n=1)	< 3 (n=1)	-
Enterococcaceae [c]	CFU/g [b]	< 3 (n=1)	11,000 (n=1)	11,000 (n=1)	< 3 (n=1)	-

-: Not applicable or unknown
[a] Same sample size as NL-WP, since calculated based on those samples
[b] Colony-forming unit
[c] Optional characterisation parameters for RENURE criteria

3.2.3 Resulting upgrading from the original manure and specific storage needs for the recovered BBFs

The Dutch pilot plant treats raw cattle slurry from the stables (with on farm co-products) by anaerobic digestion delivering the digestate which is the input of the further nutrient recovery and reuse system with a screw press, passive precipitation and nitrogen stripping. The macro, meso and micro nutrients of the these two input flows are presented in Table 3-11, in a similar way to the biobased fertiliser products in the previous sections.

Along the APF process the pH starts with 7.4 with cattle slurry, raises slightly by anaerobic digestion to on average 7.8 and up to 8.2. The pH of the soil conditioner and liquid K rich fertiliser are similar and the ammonium sulphate is much lower with 5.3. Digestion lowers the organic matter content from 6.8 % for the slurry to 5.4 for the digestate and after further separation with the screw press to 20% in the organic soil conditioner. The N, P and K content of ingoing cattle slurry is slightly increased by AD in the digestate. In the biobased end products the N content of the K fertiliser and the P rich sludge are roughly two times higher compared to the cattle slurry. The P content of de soil conditioner and the P rich sludge are roughly 3 – 4 times higher compared with digestate. The K content of the biobased fertiliser end products are similar to what is found in the digestate.

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The most valued final end product ammonium sulphate at the NL pilot plant with 6.5% N and pH 5.3 (adjustable) would comply as a RENURE product according to the RENURE criteria at this moment. It has a Nmin/Ntotal ratio of 0.942 which is higher than the >0.9 criteria, and TOC/total-N ratio of 0.014 which is lower than the <3 criteria. In addition Cu and Zn requirements are also met, with for cupper measured <50 compared to criteria ≤ 300 mg/kg TS and zinc measured <250 compared to criteria ≤ 800 mg/kg TS.

Heavy metal contents of the Dutch BBFs are often below official laboratory detection limits, with acceptable presence and risks in comparison with organic fertilisers. Ammonium sulphate has relatively low contents of heavy metals and pathogens, compared to the other BBFs. But generally speaking, BBFs are made out of processes that have selection separation effects and more importantly create up concentration. The risk of having higher absolute levels of heavy metals or introduction of other pathogens is important to monitor and take into account for the production and also application limits of BBFs based on manure.

Looking to the market potential and application techniques of the BBFs, the fit in what famers know and what application techniques are ready for. The liquid ammonium sulphate product is comparable with synthetic AS product from nitrogen strippers, with a much higher N content than N water from stable scrubbers. The products is already known by Dutch farmers and can be applied in arable farming or grassland with a row injector or spoke wheel injector for liquid fertilisers or with a manure slurry injector. These application techniques are commonly used in the Netherlands and injection is obligatory to minimize ammonia and odour emissions. The K rich fertiliser can be used for arable farming and grassland with a slurry injector. The organic soil improver can be used in arable farming or natural grassland, spread on land with an above ground solid organic fertiliser applicator as commonly used for compost or solid digestate, but there is a legal obligation to incorporate it into the soil directly afterwards. For manure and manure-based products this is obligatory, again to minimize ammonia and odour emissions.

Regarding storage, all Dutch BBFs are safe, relatively stable (not like digestate) but have a risk for N emissions and especially ammonia emissions. To minimize environmental impact the storage should be close, as cold as possible and if possible with lowest pH possible. Liquid products can be stored in closed silo’s or cellars, solid products can be stored in closed building, silo’s or heaps that are covered (e.g. with canvas).

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Table 3-11 Summary of the main physic-chemical characteristics of the raw manure and digestate with the BBFs obtained in the Dutch pilot

Parameter	Unit	Cattle slurry manure	Cattle slurry digestate
pH	-	7.42 ± 0.24 (n=13)	7.79 ± 0.43 (n=14)
EC	mS/cm	2.81 ± 0.37 (n=14)	3.15 ± 0.32 (n=13)
Density	kg/L	0.97 ± 0.02 (n=8)	0.98 ± 0.02 (n=8)
DM	g/kg	89.9 ± 12.1 (n=14)	78.7 ± 17.3 (n=14)
Organic Matter	g/kg	67.8 ± 10.9 (n=14)	54.3 ± 12.2 (n=14)
Organic C	g/kg	-	-
Total N	g/kg	3.87 ± 0.60 (n=14)	3.87 ± 0.60 (n=14)
Ammonium-N	g/kg	1.84 ± 0.49 (n=14)	2.59 ± 0.52 (n=14)
Total P	g/kg	0.61 ± 0.07 (n=14)	0.71 ± 0.18 (n=14)
Total K	g/kg	4.63 ± 0.52 (n=14)	5.05 ± 0.39 (n=14)
S	g/kg	0.58 ± 0.17 (n=14)	0.54 ± 0.12 (n=14)
Ca	g/kg	1.07 ± 0.18 (n=14)	1.39 ± 0.40 (n=14)
Mg	g/kg	0.72 ± 0.12 (n=14)	0.77 ± 0.19 (n=14)
Na	g/kg	0.61 ± 0.08 (n=8)	0.78 ± 0.15 (n=9)
Cu	mg/kg DM	< 100 (n=14)	< 100 (n=13)
Zn	mg/kg DM	< 500 (n=14)	< 500 (n=14)
Fe	mg/kg DM	1324 ± 204 (n=13)	2262 ± 938 (n=11)
Mn	mg/kg DM	323 ± 56.5 (n=7)	450 ± 58.3 (n=5)
Se	mg/kg DM	1.05 ± 0.89 (n=5)	2.28 ± 2.21 (n=4)
Mo	mg/kg DM	-	-
Co	mg/kg DM	-	-
Cd	mg/kg DM	< 0.40 (n=7)	< 0.40 (n=7)
Ni	mg/kg DM	< 5.00 (n=7)	< 5.00 (n=7)
Pb	mg/kg DM	< 5.00 (n=7)	< 5.00 (n=7)
Cr	mg/kg DM	< 5.00 (n=7)	6.59 ± 1.31 (n=7)
Hg	mg/kg DM	< 0.05 (n=7)	< 0.05 (n=7)
As	mg/kg DM	< 1.00 (n=14)	< 1.00 (n=7)
Tl	mg/kg DM	-	-

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3.3 BBFs from the German pilot plant

3.3.1 The pilot plant in Germany

Figure 3.3 schematically reports the pilot plant process for BBFs production from cattle dung in the German pilot. The process can be divided in three main steps: cattle dung pre-treatment, thermochemical conversion and mono-ammonium phosphate (MAP) production.

The innovative key components are (i) the application of additives for binding NH_3 in the solid dung, (ii) the Thermo-Catalytic Reforming (TCR) unit for the conversion of the cattle dung, (iii) the carrier material impregnation (phosphoric acid on perlite or biochar) and (iv) the mono-ammonium phosphate reactor.

The targeted BBFs of the pilot plant are (i) the phosphorous rich biochar (**DE-BC**) produced from Thermo-Catalytic Reforming of dried cattle dung and (ii) the mono-ammonium phosphate on perlite (**DE-AP**) obtained from the MAP reactor.

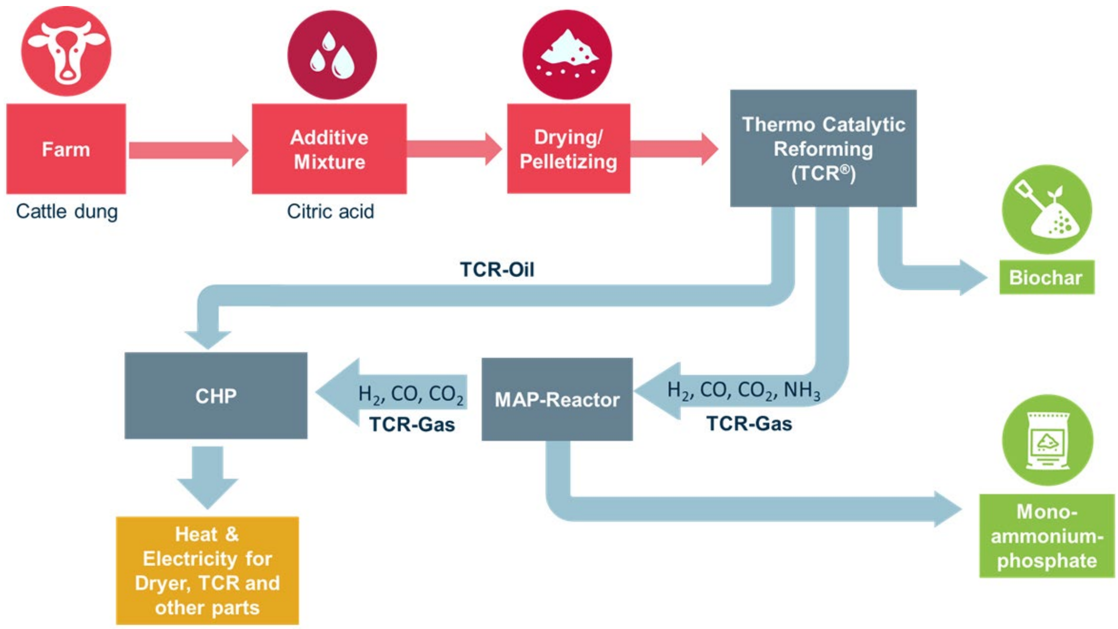

Figure 3.3. German pilot infographic, including feedstock and train of technologies

During the project, the pyrolysis plant of the German Pilot successfully reached Technology Readiness Level (TRL) 7, demonstrating the technology's capability under relevant environmental conditions. The testing of the cattle manure has been done in an operational environment that closely resembles the final intended setting. However, the recovery of ammonia from the pyrolysis gas through scrubbing was only tested and demonstrated on a smaller-scale plant. Due to this limited scale of testing, the ammonia recovery process did not reach the same level of maturity as the overall pyrolysis plant. As a result, this aspect of the project achieved a TRL of 4-5, indicating that while the concept was validated and tested in a lab or small pilot environment, it has not yet been fully demonstrated under operational conditions typical of a larger, integrated system.

To ensure that the products are compatible with products on the market the German pilot plant would be operated in a decentralized big scale capacity or in a centralised facility treating large volumes of cattle manure.

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This is mainly because of the high investment costs of the technology. Therefore, a large-scale operation is needed in areas with intense livestock production like North Rhine-Westphalia in Germany.

3.3.2 BBFs characterisation

Table 3-12 provides essential information on Germany BBFs (definition, type, product form, estimated production). The reported estimated amounts of BBFs are based on the installed capacity of the pilot plant assuming a yearly operation of 7000 h. The estimated production amounts of the BBFs in the PTR1 were based on a production batch of three weeks (considering 5 workdays as a week).

The German pilot was expected to produce a mineral BBF (DE-AP) and a soil amendment (DE-BC). It is important to note that the estimated production of ammonium phosphate on perlite is theoretical as the production process is still under optimisation (see section 4) and it is difficult to predict the yearly production. The value is obtained considering that during pyrolysis it is produced 30% of gas containing 2% of ammonia. Also, it was estimated 80% of recovery rate from the ammonia present in the TCR-gas.

Table 3-12. Definitions of the BBFs from the German pilot plant and their estimated production amounts

#BBF	Description	Type	Product form	Estimated production
DE-BC	Biochar	Soil amendment	Solid	65 tonne/year
DE-AP	Ammonium phosphate on perlite	NP	Solid	8 tonne/year

The main chemical-physical characteristics, macro- and micro- nutrients content as well as heavy metals and pathogens content of Germany BBFs are reported in Table 3-13, Table 3-14 and Table 3-15, respectively.

The mono-ammonium phosphate (MAP) product is characterised by light acidity (pH: ~4) while the biochar is basic (pH: ~12). The bulk density of the BBFs is in the range of 0.6 kg/L with a very high dry matter (DM) content because the reaction temperatures in the production processes are >400 °C therefore causing water evaporation. The low water content is due to resorption of humidity from the air.

Table 3-13. Chemical-physical characterisation of the BBFs produced by the German pilot plant

Parameter	Unit	DE-BC	DE-AP
pH	-	12.3 (n=8)	4.0 (n=3)
CE	mS/cm	16.08 ± 1.98 (n=8)	-
Density	kg/L	0.50 ± 0.14 (n=2)	1.8 ± 0.3 (n=3)
DM	g/kg	996 ± 1.7 (n=8)	990 (n=3)
Ash*	g/kg	464 ± 23 (n=7)	-
Organic Matter	g/kg	522 ± 24 (n=5)	< 1.0
Organic C	g/kg	393 ± 124 (n=3)	0

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The organic matter (OM) for the biochar is 522 g/kg when accounting the carbon content as organic matter. The MAP on perlite are mineral fertilisers with very low organic matter content <1 g/kg.

In the case of biochar, the main macro-nutrient is potassium followed by phosphate and nitrogen. Most of the nitrogen is bound in the carbon matrix of the char and only 0.14 g/kg is present in form of ammonia-nitrogen. Sulphur, calcium and magnesium are also present in relevant quantities in the biochar. This mineral content is directly correlated with the mineral content of the cattle dung used as feedstock. The micro-nutrients that have been measured in the biochar are copper and iron. Zinc is also available within the biochar with an average of 358 mg/kg. These levels are still below the upper limit set by the EBC for applications on soil or for use as building materials (limit value 400 mg/kg). For applications as a feed additive, the values are above the required 200 mg/kg.

In the case of MAP on perlite, the nutrient content from the mono-ammonium phosphate (N, P) can be differentiated from the minerals contained in the perlite (Ca, K, Fe). MAP is highly soluble in water and directly available for the crops after application. During the operation of the MAP reactor, it became apparent that adsorption of hydrocarbons and side reactions with phosphoric acid take place. Therefore, the ammonium content on the perlite was low to not detectable. To solve this problem, a second approach to isolate MAP was chosen. Within this scope, the use of perlite was completely dispensed and extraction from liquid phosphoric acid was targeted. Indeed, a colourless solid could be obtained from the process but only in small amounts. Up until now the maximum recovery rate of ammonia from the process was not higher than 6% leaving very small quantities for analysis. The parameters in Table 3.11 refer to the colourless solid obtained from liquid phase matching with theoretical values of pure MAP.

Table 3-14 Macro- and micro- nutrients content of the BBFs produced by the German pilot plant

Parameter	Unit	DE-BC	DE-AP
Total N	g/kg	10.06 ± 1.49 (n=5)	122
Ammonium-N	g/kg	0.14 ±0.09 (n=7)	122
N-NO_3	g/kg	0	0
Total P	g/kg	30.4 ± 2.58 (n=8)	198
Total K	g/kg	95 ± 23.4 (n=8)	0
S	g/kg	2.5 ± 0.14 (n=5)	0.0
Ca	g/kg	22.9 ± 1.5 (n=2)	0.05
Mg	g/kg	6.7 ± 0.3 (n=2)	3.57
Na	g/kg	8.2 ± 0.7 (n=5)	0
Cu	mg/kg DM	51.30	0.0
Zn	mg/kg DM	358.50	0.0
Fe	mg/kg DM	3,402	0.0
Mn	mg/kg DM	403	0.0

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Table 3-15. Heavy metals and pathogens content of the BBFs produced by the German pilot plant

Parameter	Unit	DE-BC	DE-AP
Cd	mg/kg DM	0.06	0.0
Ni	mg/kg DM	6.90	0.0
Pb	mg/kg DM	1.93	0.0
Cr	mg/kg DM	11.02	0.0
Cr VI*	mg/kg DM	0.05	0.0
Hg	mg/kg DM	0.02	0.0
As	mg/kg DM	0.47	0.0
Mo	mg/kg DM	<5.0	0.0
Salmonella spp.*	unit/25g	-	0.0
Escherichia coli*	CFU/g	<10	0.0
PAH*	mg/kgDM	2.99	0.0
Cl*	g/kg	17	0.0

*: optional characterisation parameters

3.3.3 Resulting upgrading from the original manure and specific storage needs for the recovered BBFs

The transformation of cattle manure into biochar presents several important advantages for both environmental sustainability and agricultural productivity. The characteristics of the original cattle manure pellets are reported in Table 3-16. One of the most significant improvements is the stabilization of carbon. The biochar produced contains carbon that is highly resistant to degradation, creating a long-term carbon sink. This persistent carbon can remain in soils for centuries, contributing to carbon sequestration efforts and reducing greenhouse gas emissions from manure decomposition (Lehmann et al., 2006).

Pyrolysis also leads to a substantial reduction in volume, making the biochar easier to store, transport, and apply. The stability of biochar allows for flexible application schedules, ensuring it can be used year-round without the risk of decomposition or nutrient loss (Kammann et al., 2017). Additionally, the production of biochar lowers the nitrogen content compared to raw manure, which helps farmers comply with nitrogen regulations, reducing the risk of nitrate leaching and water contamination (Steiner et al., 2008).

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Table 3-16 Summary of the main physic-chemical characteristics of the raw manure and digestate with the BBFs obtained in the German pilot

Parameter	Cattle manure pellets
pH	9.2 ± 0.42 (n = 4)
Electrical conductivity (mS/cm)	5.35 ± 0.65 (n = 4)
Dry matter (g/kg)	85.1 ± 1.87 (n = 4)
Organic C	331.75 ± 8.22 (n = 4)
Organic matter (g/kg)	656.5 ± 16.92 (n = 4)
N (g/kg)	21.2 ± 2.05 (n = 4)
Ammonium-N (g/kg)	0.08125 ± 0.1125 (n = 4)
Organic-N	21.2 ± 2.05 (n = 4)
NO3--N	0.5 ± 0 (n = 4)
P total P2O5	14.05 ± 1.59 (n = 4)
Ktotal K2O (g/kg)	40.325 ± 8.24 (n = 4)
S (g/kg)	4.35 ± 1.06 (n = 4)
Ca total (CaO)	17.5 ± 3.20 (n = 4)
Mg total (MgO)	7.605 ± 0.52 (n = 4)
Na	2.355 ± 0.62 (n = 4)
Cu	0.02495 ± 0.005 (n = 4)
Zn	0.1525 ± 0.005 (n = 4)
Ni	0.0031375 ± 0.002 (n = 4)
Pb	0.0025 ±0.002 (n = 4)
Cr	0.004842± 0.002 (n = 4)
Cr VI	0
Hg	0.00009075 ± 8.64 E-05 (n = 4)
As	0.00128 ± 0.0015 (n = 4)
Cd	0.0001 ± 0.0001 (n = 4)
Salmonella	0
Enterococcaceae /g	12.25 ± 15.20 (n = 2)

Biochar, due to its stability and low degradation rate, has minimal storage requirements. It is not prone to spoilage, making it easy to store for extended periods without losing its beneficial properties. Biochar, while stable in normal conditions, is a combustible material and can pose a fire risk if not stored properly. It is important to store biochar in a well-ventilated area and away from sources of ignition, as fine biochar dust can be particularly flammable when dispersed in the air. Therefore, the moisture content of the product should be adjusted to <20%.

Mono ammonium phosphate (MAP), being a dry granular fertilizer, also requires dry conditions to prevent clumping and maintain its nutrient availability. Like biochar, MAP is easy to store long-term in standard agricultural storage facilities, but it should be protected from moisture and high humidity to ensure the product remains in good condition for future application. Both products offer significant flexibility in terms of storage, reducing logistical concerns for farmers.

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A notable co-benefit of the pyrolysis process is the recovery of nitrogen, which can be used to produce mono ammonium phosphate (MAP). This MAP can be stored or sold, offering farmers an additional revenue stream or a strategic resource to balance soil nutrient levels according to seasonal or regional needs.

In terms of soil health, biochar offers several agronomic benefits. When added to soils, biochar improves water retention, enhances soil structure, and increases microbial activity. Importantly, biochar retains essential nutrients such as phosphorus and potassium, which are made available to plants over time, enhancing soil fertility. These nutrients, unlike nitrogen, are stable in biochar and do not leach easily, making them a long-lasting source of nutrition for crops.

In summary, converting cattle manure into biochar not only reduces environmental risks but also enhances soil health, making essential nutrients such as phosphorus and potassium available to plants. The process provides a stable, storable, and environmentally beneficial product that supports sustainable agricultural practices while recovering valuable nitrogen as MAP for future use or sale.

3.4 BBFs from the Belgian pilot plant

3.4.1 The pilot plant in Belgium

The Belgian stripping/scrubbing pilot is installed at the Bio Sterco farm, located in Hooglede, Belgium, and has the capacity to house 454 sows, 5 boars, and 5524. The farm operates its own manure treatment facility with a maximum capacity of 52000 t y^{-1}. The manure treatment system comprises a conventional processing setup, featuring a centrifuge for mechanical separation, an activated sludge tank primarily focused on nitrification-denitrification (NDN) removal, and a settling tank to eliminate activated sludge from the effluent. The stripping/scrubbing pilot valorises part of the manure generated in the farm by including an additional step that allows the partial recovery of nitrogen before introducing the manure in the conventional treatment train. The pilot tested includes an ammonia (NH_3) stripping -scrubbing unit and this unit is one of the several manure treatments steps that were implemented at Bio Sterco (Figure 3.4). Firstly, animal manure (95% pig manure and 5% cattle manure) is separated into a liquid (LF) and solid (SF) fraction by centrifugation. The SF of manure is subsequently composted while a part of the nitrogen (N)-rich LF of manure is treated in the stripping-scrubbing unit to recover NH_3 as ammonium nitrate (**BE-AN**) or ammonium sulphate (**BE-AS**). The scrubbed LF of manure is mixed with the non-scrubbed LF and biologically treated via nitrification/denitrification (NDN) system. The effluent of NDN system is polished in a constructed wetland.

In the NH_3 stripping-scrubbing unit, the NH_3 is stripped by an air ventilation flow. NH_3 volatilisation is induced by increasing pH (adding NaOH) and temperature of the LF from manure. The stripping pH and temperature range are between 7.5-9 and 42–55 °C, respectively. The pilot has a capacity 15,000–20,000 tonne of LF of manure per year with a 30-60% NH_3-reduction. The stripping column has a ventilation flow of 1,000–1,800 m^3 h^{-1} with an air speed of 0.2-0.8 m s^{-1}. The stripped NH_3 is sent to a scrubber column where nitric acid or sulfuric acid are added as a sorbent, resulting in BE-AN or BE-AS. The pathway of N recovery at DETRICON plant can be seen in Figure 3.4.

Next to the BE-AN and BE-AS, ammonium water (**BE-AW**) was characterised (WP2) and tested in WP4. The BE-AW was not produced at the DETRICON pilot plant but was collected from a Flemish anaerobic digestion facility that evaporates LF of digestate, and as such it produces ammonium water. This product is currently sold as reductant in the DeNOx system of a local incineration plant.

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Industrial-scale pig farms with a similar scale (15000-100000 tonne manure per year) and treatment facilities are common in Belgium, therefore the addition of a stripping-scrubbing unit at these farms with an already existing treatment line would be optimal to upgrade them partially recovering nitrogen while increasing the treatment capacity of the conventional treatment line and potentially reducing their operating costs.
The Belgian pilot is classified as TRL7 as it is demonstrated in the relevant environment (pig farm with its own manure treatment installation) and relevant scale (+15000 t of manure treated per year).

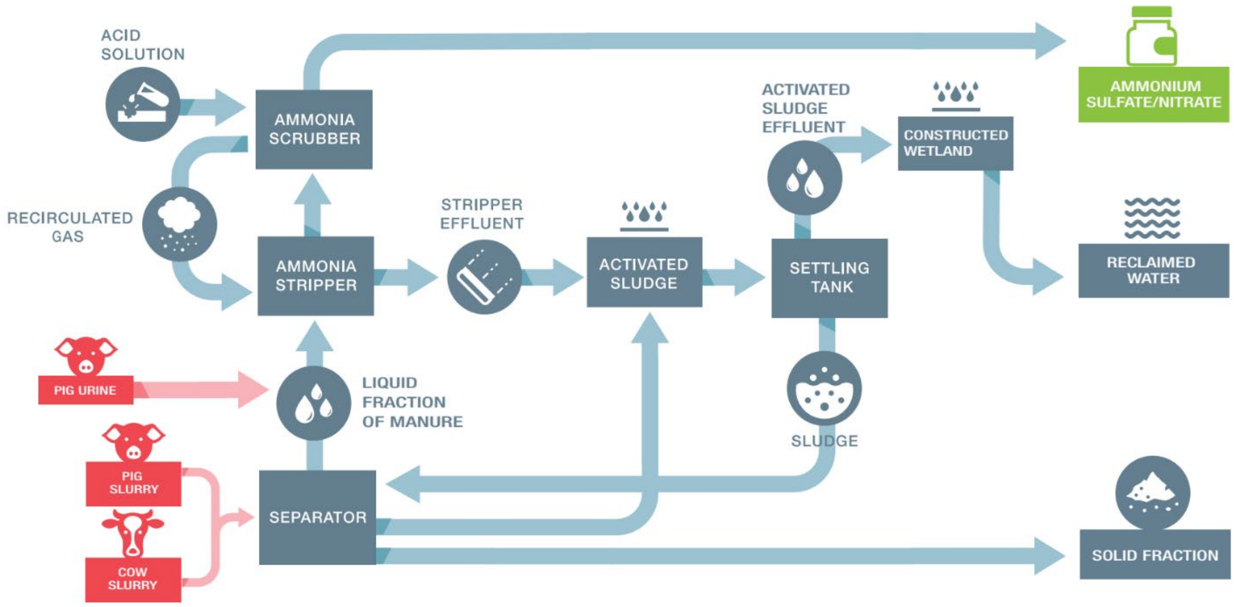

Figure 3.4. Belgian pilot infographic, including feedstock and train of technologies

3.4.2 BBFs characterisation

The Belgium pilot produced two of the BBFs during its operational stage: BE-AN and BE-AS (Table 3-17), whereas BE-AW was obtained from two other pilots in the Flemish region (Waterleau and Op de Beeck). The BBFs produced by the Belgium pilot, including BE-AN (n=5) and BE-AS (n=13), were characterised from February 2022 till current date, while BE-AW was sampled 12 times between July 2021 and February 2022. The three BBFs are all N biobased fertilisers in liquid form (BE-AN, BE-AS and BE-AW).
It is important to note that the reported amounts are estimations regarding the whole Flemish.

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Table 3-17. Definitions of the BBFs from the Belgian pilot plant and their estimated production amounts

#BBF	Description	Type	Product form	Estimated production
BE-AN	Ammonium nitrate	N	Liquid	285 tonne/year
BE-AS	Ammonium sulphate	N	Liquid	285 - 85,300 tonne/year
BE-AW	Ammonium water	N	Liquid	724 tonne/year

The estimated production amount (Table 3-17) of ammonium nitrate is slightly less than indicated in PTR1 (500 tonnes/y) as the recovery efficiency and treatment capacity indicated at the start of the project by the installer were an overestimation. While the estimated production amount of ammonium sulphate is estimated between 285 – 85,300 tonne/year. When only the Belgium pilot is considered, a production capacity of 285 tonne/year can be expected, whereas the production capacity of the whole Flemish market equals 85,300 tonne AS/y. The different sources across the Flemish market were monitored and characterised from July 2021 till February 2022 which was reported in D2.2. Therefore, production capacity of the whole market can be considered for ammonium sulphate. Ammonia water is produced at two different plants with a combined production capacity of 724 tonne/year.

The main chemical-physical characteristics of Belgium BBFs are reported in Table 3-18, while the nutrient concentrations can be found in Table 3-19 and main pollutants and pathogens content is reported in Table 3-20.

Similarly to synthetic N fertilisers, the recovered ammonium nitrate, ammonium sulphate and ammonium water contain total N entirely in mineral form. Ammonium nitrate contains N in the form of Ammonium-N and Nitrate-N, whereas the total N in ammonium sulphate and ammonium water is present in the form of Ammonium-N. Ammonium nitrate is characterised by higher N concentration (2x) as compared to ammonium sulphate.

The increased N concentration of ammonium nitrate is due to the use of nitric acid instead of sulfuric acid in the scrubbing process. Since ammonium sulphate is obtained by scrubbing with sulfuric acid, ammonium sulphate contains a considerable amount of sulphur. The amount of added acid determines the pH and conductivity of the BBF.

Usually, higher pH values are measured for ammonium nitrate compared to ammonium sulphate, which reduces the risk of machinery corrosion, but also increases the rate of ammonia volatilization. Instead, the application of ammonium sulphate leads to significantly higher soil EC values as compared to ammonium nitrate, which could be of concern while utilizing ammonium sulphate as fertiliser for salt sensitive crops. This was also highlighted in the work done by Sigurnjak et al. (2019).

Table 3-18. Chemical-physical characterisation of the BBFs produced by the Belgian pilot plant

Parameter	Unit	BE-AN	BE-AS	BE-AW
pH	-	6.0 ± 0.6 (n=5)	5.6 ± 1.0 (n=15)	10.3 ± 0.8 (n=12)
Density	kg/L	1.3 ± 0.1 (n=5)	1.2 ± 0.1 (n=15)	1.1 ± 0.0 (n=12)
Dry matter	g/kg	390.8 ± 63.2(n=5)	308.5 ±47,5 (n=15)	- [a]
Organic Matter	g/kg	<1 (n=5)	<1 (n=15)	-
Organic C	g/kg	0.12 ± 0.03 (n=4)	0.82 ± 0.11 (n=4)	0.56 ± 0.16 (n=4)

- not applicable or unknown
[a]Dry matter was not determined for BE-AW as all compounds are volatilised during the drying process

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Table 3-19. Macro- and micro- nutrients content of the BBFs produced by the Belgian pilot plant

Parameter	Unit	BE-AN	BE-AS	BE-AW
Total N	g/kg	153.1 ± 26.4 (n=5)	74.2 ± 7.8 (n=15)	158.2 ± 27.9 (n=12)
Ammonium-N	g/kg	76.2 ± 18.1 (n=5)	74.1 ± 7.5 (n=15)	154.9 ± 31.8 (n=12)
Nitrate-N	g/kg	77.4 ± 15.0 (n=5)	-	-
Total P	g/kg	0.06 ± 0.01 (n=5)	0.05 ± 0.01 (n=13)	0.03 ± 0.01 (n=4)
Total K	g/kg	0.55 ± 0.16 (n=5)	0.68 ± 0.20 (n=13)	0.89 ± 0.23 (n=4)
S	g/kg	0.37 ± 0.06 (n=5)	81.3 ± 11.8 (n=13)	0.55 ± 0.09(n=4)
Ca	g/kg	0.37 ± 0.12 (n=5)	0.56 ± 0.16 (n=13)	0.30 ± 0.06 (n=4)
Mg	g/kg	0.08 ± 0.01 (n=5)	0.04 ± 0.02 (n=13)	0.07 ± 0.01 (n=4)
Na	g/kg	0.58 ± 0.08 (n=5)	0.78 ± 0.13 (n=13)	0.41 ± 0.23 (n=4)
Cu	mg/kg DM	1.2 ± 0.7 (n=5)	2.2 ± 0.9 (n=8)	3.4 ± 0.9 (n=4)
Zn	mg/kg DM	3.4 ± 1.3 (n=5)	5.5 ± 2.4 (n=8)	8.6 ± 3.1 (n=4)
Fe	mg/kg DM	12.3 ± 4.1 (n=5)	18.2 ± 5.8 (n=8)	22.8 ± 4.3 (n=4)
Mn	mg/kg DM	0.4 ± 0.1 (n=5)	1.3 ± 0.6 (n=8)	1.2 ± 0.3 (n=4)

- not applicable or unknown

Table 3-20. Heavy metals and pathogens content of the BBFs produced by the Belgian pilot plant

Parameter	Unit	BE-AN	BE-AS	BE-AW
Cd	mg/kg DM	<0.028 (n=5)	<0.028 (n=8)	<0.028 (n=4)
Ni	mg/kg DM	0.23 ± 0.06 (n=5)	15.2 ± 8.6 (n=8)	0.32 ± 0.09 (n=4)
Pb	mg/kg DM	0.1 ± 0.1 (n=5)	0.1 ± 0.1 (n=8)	0.9 ± 0.6 (n=4)
Cr	mg/kg DM	0.13 ± 0.02 (n=5)	3.52 ± 0.38 (n=8)	0.26 ± 0.03 (n=4)
Hg	mg/kg DM	<0.003 (n=3)	<0.003 (n=3)	-
As	mg/kg DM	<0.1 (n=3)	<0.1 (n=3)	-

- not applicable or unknown

3.4.3 Resulting upgrading from the original manure and specific storage needs for the recovered BBFs

Ammonium sulphate and ammonium nitrate produced by stripping/scrubbing process is similar to a synthetic ammonium sulphate or ammonium nitrate as it only consists of a solution of $(NH_4)_2SO_3$ or NH_4NO_3. Due to its

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high purity and its purely mineral composition, it can be used as a mineral fertilizer, with the same technical and regulatory constraints.

Regarding storage strategies of the obtained products, the produced ammonia salts can be stored/transported in plastic tanks or inox tanks. Due to corrosive and reactive properties of ammonium nitrate, and to avoid contamination, other type of materials should be avoided such as galvanised iron, copper, lead, and zinc in bins, or other handling devices for storage.

3.5 BBFs from the French pilot plant

3.5.1 The pilot plant in France

The French pilot focuses on producing ammonia and carbon concentrated products from pig/cattle/poultry manure. The French pilot was also designed to be mobile. The objective is to treat manure sources on-farms, with the opportunity of using bio-based fertilisers (BBFs) directly on field. Main goals were: (i) to treat liquid manures with the stripping tower for ammonia recover therefore producing two products: ammonium sulphate and liquid K fertiliser, and (ii) to treat solid manures through the pyrolysis process for carbon stabilization and biochar production.

The pilot plant was used on various farms located in Grand Est and Brittany regions and to carry the pilot on-site, the treatment plants were built and installed on two trucks (Figure 3.5).

Within the FERTIMANURE project, the substrates tested with priority were both the liquid pig slurry for nitrogen stripping and poultry manure for carbon stabilization into biochar. During 2022, the stripping process was also tested on liquid phase of digestate and pyrolysis process was carried out on solid phase of digestate.

The nitrogen stripping process has been improved by insulating the stripping column and by the addition of a heater to heat up liquid manure (60°C) during M18-M22. The first step of the stripping process was done in a column with an air/liquid ratio of 200 (ventilation flow of 1 m^3/h and liquid 5 L/h). NH_3 volatilization was further induced by increasing pH to 10 (adding NaOH) and temperature of the manure liquid phase. During the second step, the stripped NH_3 was then sent to a second column where sulfuric acid was added as a sorbent, resulting in ammonium sulphate production: **FR-AS**. In order to double the exchanging time between effluent and airflow the second tower was used as an extension of the first one for effluent circulation and NH_3 is recovered by bubbling in containers placed in series and containing sulfuric acid (50%). The liquid manure from which the mineral nitrogen has been extracted was recovered providing the BBF called **FR-LK**.

Before the pyrolysis process, manures were dried to reach a DM content at least of 70% fresh weight. The pyrolysis pilot used an endless screw to move the substrate forward. The process was carried out as “slow pyrolysis process” with a temperature of 700°C for poultry manure and 550°C for solid phase of digestate. Residence time was between 25 to 30 min for both tested manures. Anaerobic conditions were ensured by continue injection of N_2 (between 15 to 20 NL/min) to reach an oxygen content below 2%. Pyrolysis by-products were biochar (**FR-BC**) and pyrolysis gases. The gases were evacuated to a flare where they were burned.

The French pilots reached a TRL6 level. We were able to extensively demonstrate their effectiveness on several demonstration sites and we were even able to produce significant quantities of BBF for field trials. The design as a mobile pilot that could be shared by various farmers was an innovation demonstrated to be technically feasible.

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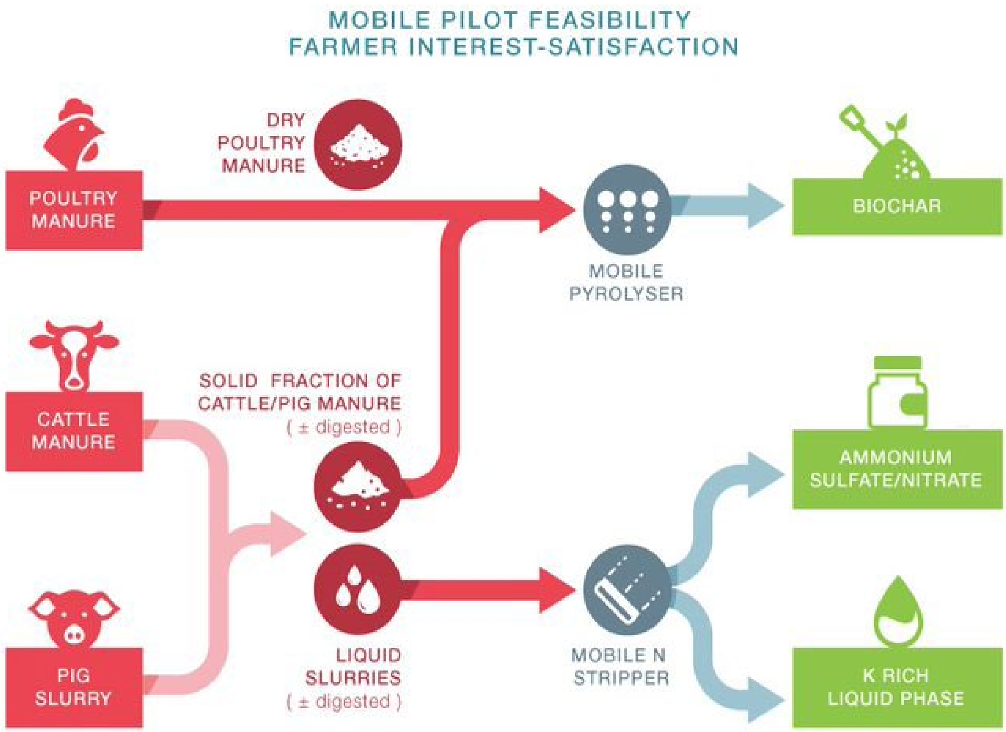

Figure 3.5. French pilot infographic, including feedstocks and train of technologies

3.5.2 BBFs characterisation

Table 3-21 reports main information concerning the BBFs estimated from the France mobile pilots. The pyrolytic unit produces biochar (FR-BC, solid soil amendment), whereas the stripping mobile unit produces the other two BBFs (FR-AS, N; FR-LK, K).

Table 3-21. Definitions of the BBFs from the French pilot plant and their estimated production amounts

#BBF	Description	Type	Product form	Estimated production
FR-BC	Biochar from poultry manure (700 °C)	Soil amendment	Solid	4.2 tons/year*
FR-BC	Biochar from digestate (550°C)	Soil amendment	Solid	7.2 tons / year*
FR-AS	Ammonium sulphate	N	Liquid	00.188 ton/year**
FR-LK	K-fertiliser	K	Liquid	11.7 tons/year**

** considering following parameters: Working days = 221 days by year; 8 hours / day; Treatment of 1 T poultry manure (10kg/h) = 40 kg of biochar and treatment of 1 T dried solid digestate (max 10kg/h)= 410 kg of biochar.*

***With following parameters: Working days = 221 days, treatment capacity=53 L slurry/day and final production rate = 0,85 L FR-AS/day at 48,8 g N-NH4/L.*

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The main chemical-physical characteristics of France BBFs are reported in Table 3-22 while macro- and micro-nutrients contents of France BBFs are reported in Table 3-23. Data on heavy metals and pathogens from the BFFs produced by the French pilot plant are shown in Table 3-24.

Table 3-22. Chemical-physical characterisation of the BBFs produced by the French pilot plant

Parameter	Unit	FR-BC1	FR-BC2	FR-AS	FR-LK
pH	-	11.8 ± 1 (n=5)	10.3 ± 0.4 (n=5)	4.75 ± 2.1 (n=12)	10.9 ± 2.21 (n=5)
CE	mS/cm	14.9 ± 0.9 (n=3)	1.66 ± 0.6 (n=5)	199.1 (n=1)	36.66 ± 8.83 (n=2)
Density	kg/L	0.21 ± 0.02 (n=4)	0.13 ± 0.017 (n=5)	1.12 ± 0.01 (n=2)	1.01 ± 0.01 (n=3)
DM	g/kg	979.00 ± 19.82 (n=4)	955.48 ± 1.55 (n=5)	302.9 ± 148.5 (n=2)	25.2 ± 8.9 (n=4)
Ash	g/kg	416.6 ± 21.9 (n=2)	308.2 (n=1)	-	-
Organic Matter	g/kg	700.00 ± 200 (n=5)	621.60 ± 46.37 (n=5)	<1 (n=3)	8.5 ± 6.2 (n=4)
Organic C	g/kg	350.00 ± 109 (n=5)	359.84 ± 117.92 (n=5)	<1 (n=3)	4.25 ± 3.16 (n=4)

-: not applicable or unknown

Produced biochars are all characterised by large amounts of organic matter, organic carbon, potassium and to a lesser extent phosphorus. They have also low pathogenic potential, little quantity of volatile elements (ammonia, sulphur). Biochars from digestate are slightly different from biochar from poultry manure, i.e. lower density, higher copper and zinc for poultry manure biochar (due to veterinary products for animals), less potassium in digestate biochar, and a higher organic matter in biochar from digestate.

On the other hand, parameters did not show any significant differences for pH, nitrogen and ammonia lost during the process.

Table 3-23. Macro- and micro- nutrients content of the BBFs produced by the French pilot plant

Parameter	Unit	FR-BC1	FR-BC2	FR-AS	FR-LK
Total N	g/kg	25.3 ± 4.2 (n=5)	15.9 ± 2.36 (n=5)	48.76 ± 5.47 (n=4)	1.92 ± 1.05 (n=5)
Ammonium -N	g/kg	<1 (n=5)	<0.1 (n=2)	48.8 ± 0.56 (n=14)	<1.1 (n=5)
Total P	g/kg	24.8 ± 2.31 (n=5)	17.71 ± 2.55 (n=5)	<1 (n=2)	0.26 ± 0.28 (n=5)
Total K	g/kg	76.7 ± 10.04 (n=5)	38.92 ± 10.98 (n=5)	<1 (n=2)	2.23 ± 0.67 (n=5)

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S	g/kg	7.1 ± 0.84 (n=4)	9.91 ± 5.98 (n=4)	130.6 ± 15.6 (n=14)	0.22 ± 0.07 (n=3)
Ca	g/kg	35.7 ± 2.71 (n=4)	35.83 ± 11.59 (n=4)	13.65 (n=1)	0.43 ± 0.27 (n=3)
Mg	g/kg	16.7 ± 7.75 (n=4)	17.60 ± 5.09 (n=4)	<0.002 (n=1)	0.05 (n=1)
Na	g/kg	13.35 ± 1.48 (n=3)	-	-	6.75 ± 3.10 (n=3)
Cu	mg/kg DM	157.4 ± 9.25 (n=4)	38.70 ± 10.89 (n=2)	<1 (n=2)	85.09 ± 59.55 (n=4)
Zn	mg/kg DM	897.6 ± 149.5 (n=4)	164.74 ± 47.71 (n=2)	<1 (n=2)	172.1 ± 81.44 (n=4)
Fe	g/kg DM	2.02 (n=1)	5.05 ± 21.12 (n=2)	0.02 (n=1)	<0.0001
Mn	mg/kg DM	-	430 (n=1)	<2 (n=2)	-

-: not applicable or unknown

Table 3-24. Heavy metals and pathogens content of the BBFs produced by the French pilot plant

Parameter	Unit	FR-BC1	FR-BC2	FR-AS	FR-LK
As	mg/kgDM	<1.3 (n=3)	0.94 ± 0.66 (n=2)	<0.245 (n=2)	2.47 ± 1.57 (n=3)
Cd	mg/kgDM	<0.19 (n=3)	0.15 ± 0.06(n=2)	<0.245 (n=2)	< 0.43 (n=3)
Cr	mg/kgDM	20.22 ± 15.49 (n=3)	2.65 ± 3.61(n=2)	<0.29 (n=2)	3.34 ± 1.59 (n=2)
Cr VI	mg/kgDM	<0.32 (n=3)	<0.50 (n=2)	<0.10 (n=2)	<2.03 ± 0.10 (n=3)
Hg	mg/kgDM	<0.13 (n=3)	<0.14 (n=2)	<0.0245 (n=2)	<0.43 (n=3)
Ni	mg/kgDM	67.54 ± 90.27 (n=3)	12.69 ± 12.42 (n=2)	<0.29 (n=2)	4.26 ± 2.5 (n=3)
Pb	mg/kgDM	<3.2 (n=3)	2.39 ± 1.57 (n=2)	<0.76 (n=2)	<10.52 (n=3)
Tl	mg/kgDM	<0.0105 (n=1)	-	-	-
Salmonella spp.	unit/25g	0 (n=1)	0 (n=1)	0 (n=1)	<100 (n=3)
Escherichia coli	CFU/g	<10 (n=1)	<10 (n=1)	<10 (n=1)	1,344 ± 2,288 (n=3)
Enterococcaceae	CFU/g	<23 (n=1)	<23 (n=1)	<10 (n=1)	0 (n=1)
PAH (16)	mg/kgDM	2.25 ± 1.46 (n=2)	1.272 (n=1)	<0.05 (n=1)	<1.26 (n=1)

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WHO-TEQ (2005) dl-PCB	ng/kg MS	0.40 (n=1)	-	-	-
WHO-TEQ (2005) PCDD/F	ng/kg MS	1.26 (n=1)	-	-	-
Cl	g/kg	22.06 ± 0.008 (n=3	10.30 ± 2.82 (n=2)	<0.01 (n=1)	<23.72 ± 5.46 (n=2)

-: not applicable or unknown

3.5.3 Resulting upgrading from the original manure and specific storage needs for the recovered BBFs

Biochar is a material reworked and concentrated from the original material. The volatile elements (nitrogen, sulphur and part of the carbon) are partly lost by the pyrolysis process, but the other elements (phosphorus, potassium) are preserved and concentrated in a material that is very stable over time. Pyrolysis process also have an obvious effect on pathogen elimination, however attention must be paid to the concentration of non-volatile pollutants such as certain heavy metals.

Ammonium sulphate produced by stripping is in every way similar to a synthetic ammonium sulphate: it only consists of a solution of $(NH_4)_2SO_3$. Due to its high purity and its purely mineral composition, it can be used as a mineral fertilizer, with the same technical and regulatory constraints.

The remaining liquid fraction FR-LK keeps all the other nutrients of the original slurry. In comparison with the original slurry, the potassium concentration remains identical, however, thanks to the nitrogen stripping process, the spreading of this FR-LK should potentially be opened to areas where the Nitrogen intake is regulated. The concentrations remain quite low because they are linked to the concentrations in the original material, which was not already very concentrated, but can provide on the one hand additional potassium already present on the farm and therefore avoid purchasing fertilizer, or even a contribution of organic potassium which will have the advantage of longer-term availability than a mineral fertilizer.

The other advantage that stripping offers is the reduction of olfactory nuisances linked to the use of FR-LK compared to the original effluent: in the tested effluents, odours were essentially due to the presence of ammonia. Thanks to the ammonia extraction, the odour of FR-LK is significantly lower than the row material odour.

Regarding storage specifications FR-LK can be stored under the same storage conditions as the treated effluent without any restriction (same volume, same composition except for the elimination of ammonia).

FR-AS is acid and liquid, so it must be stored/transported in plastic tanks. All farmers equipments intended for synthetic ammonium sulphate use of synthetic ammonium sulphate (tanks, spray booths) is perfectly suited to the FR-LK given that the products are identical

Biochar FR-BC is solid, so it can be stored in piles. On the other hand, to preserve its properties and avoid losses due to wind, it is strongly recommended to cover it (tarpaulin or roof). Some processes moisten the biochar to prevent self-heating and the start of a fire. For dry biochars, a fire detector and a safety sprinkler system are strongly recommended.

3.6 BBFs antibiotic resistance genes (ARGs) characterisation and monitoring in agriculture soils

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Presence of ARG (Antibiotic resistance genes) in BBFs fertilisers was studied to evaluate potential transference of these genes to the soil after the fertilization process. Firstly, products obtained from the pilot plant of Spain, including poultry manure, biodried solid fraction and biostimulants were analysed for ARGs identification.

The selection of the BBFs to be assessed was done based on the potential risk of presence of ARGs. Low risk of presence of ARGs was considered for mineral products coming from off-gases or that had beared strong acid/alkaline conditions. Same consideration was taken for those products obtained from thermal processes such as combustion or pyrolysis. Some of the BBFs obtained were considered not attractive enough for the market due mainly to their low concentration of nutrients (Liquid K fertilisers and nutrient rich concentrate) and thus, they were not considered relevant for the analysis of ARGs. Initially, the project plan stated that some samples would be analysed using metagenomics to obtain a complete profile of antibiotic resistance genes, and then the most relevant ones would be further assessed by qPCR. However, we decided to perform all the analyses using shotgun metagenomics, as it would provide more comprehensive information.

In order to perform metagenomic sequencing, DNA from the extracted samples is required. In this case, the BBFs identified as interesting for this part of the assessment were the biodried solid fraction and biostimulants from the Spanish pilot. Initially soil conditioner from the Dutch pilot was also included in the study, however, the samples that arrived from the Netherlands did not contain sufficient DNA after extraction, so they could not be analyzed as they did not meet the minimum required concentration.

All in all, to evaluate potential introduction of these ARGs in soil systems, LEITAT collaborated with UVIC in their wheat cropping trial of 2021-2022. The trial consisted of four treatments:

- Control: negative control, no fertilization during the whole experiment
- Mineral: chemical commercial fertiliser
- Raw manure
- Tailor made fertiliser (TMF):
  Ammonium sulphate: Dutch pilot plant
  Biodried solid fraction: Spain pilot plant
  Biostimulant: Spain pilot plant

The three fertilizations were applied with two nitrate percentage (50 and 100%) based on the maximum concentration allowed in agricultural soils (170kg/a). For each treatment, three parcels were sampled and at three timepoints (T1: Initial timepoint, previous to the crop; T2: 4 months after the beginning, biostimulant and biodried solid fraction was applied only in TMF; T3: final timepoint of crop, 4 months after T2, all treatments were applied).

In total, 68 samples were used for this study. Total DNA was extracted from all samples with the Soil DNA Isolation Plus Kit (NORGEN, BIOTEK CORP) and sequenced on the Illumina NextSeq platform (PE150) by Genome Québec Inc. (Centre d'expertise et de services Génome Québec, Montréal (Québec), Canada). The metagenomic raw sequencing data quality was analysed with FASTQC (V0.12.1), and high quality paired-end sequences (q >30) were filtered with Trimmomatic (V0.39). ARG identification was performed blasting the processed reads against the comprehensive antibiotic resistance database ResFinder 4.0, with the cutoff of e-value (10^15) and an alignment length of over 50, with >1 counts per sample. The ARGs data was analysed using phyloseq and ggplot packages from R software (Version 1.4.1717).

Overall, analysed metagenomic data across 68 samples identified 115 ARGs. Top 10 most abundant ARGs were plotted in a stacked bar plot with log10 scale. The most abundant genes include tetracycline (tet), erythromycin (erm), chloramphenicol–florfenicol (cfr), and macrolide (msr), oleosin (ole) and efflux pump genes (OqxB). When studying the ARGs identified, we found the most counts were detected in pig slurry products, having an overall average count of 1e+30 (Figure 3.6). This is dominated by tetracycline (tet()) (9e+08). This was found present in all condition's treatments and timepoints. This was closely followed by erm() gene. Following the step of Biodried solid fraction, ARGs count was found to be 1e+22, having predominantly ole() which was not found in pig slurry samples. Finally, biostimulants, showed significant lower counts of ARGs,

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being dominated by OqxB gene. This ARG profile was closely related to the soil profile characterisation. Overall, fertilisers were found to have a higher count of ARG, and their composition to be distinct from the fertilised soil.
It can be observed how the presence of ARGs in pig slurry, biodried solid fraction and bioestimulants did not influence the ARGs in soil after the fertilisation treatments. Soil profiles were found to have a lower ARG count, as well as conserving their initial ARG profile independently from the fertilisation treatments. Unique genes can be found in the fertilisers and in soil. Cfr() can only be seen in pig slurry fertilisers and biostimulants. Overall, the soil ARG characterisation does not change with the effects of BBF, nor their concentration. ole() can be found in the soil profile through time, with no variation on the treatments. This is also the case for OqwxB().

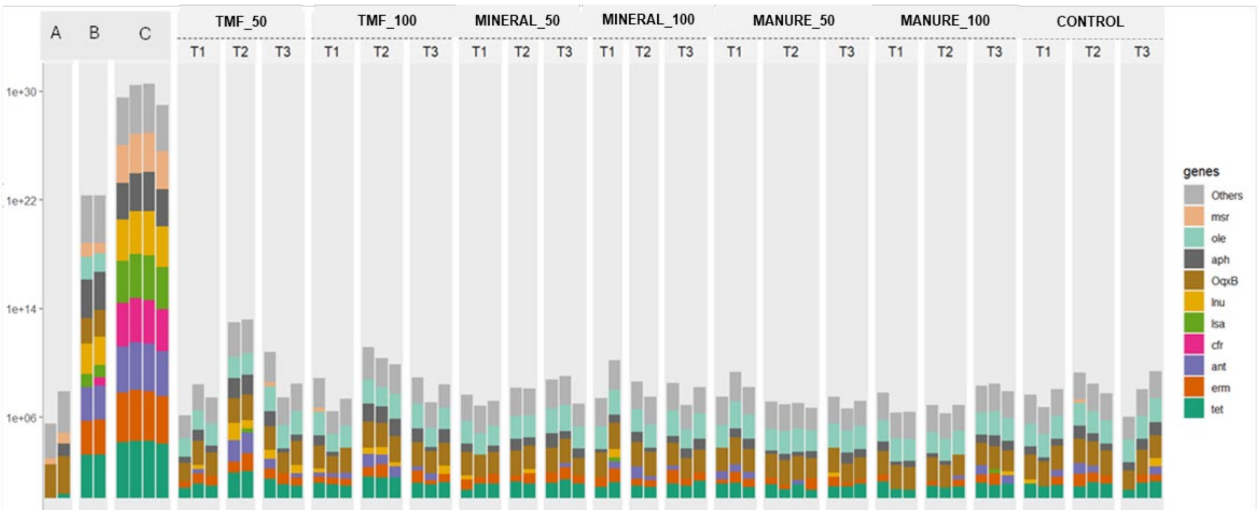

Figure 3.6. Composition plot of ARGs genes at log10 scale of pig slurry products and fertilization treatments are different concentrations through time

Furthermore, we investigated how the different fertilisers ARGs change through time, analysis the composition of soil ARGs. The different treatments were plotted as well as the different times (T1- previous to treatment, T2- implementation of treatment and T3- final implementation of treatments simultaneously). It can be observed how on the tailor-made fertiliser, abundance of ARGs increases at both concentrations once the treatment has been used. This pattern cannot be observed in the mineral treatment (positive control, chemical fertilisation), in the manure (poultry manure directly) nor in the negative control (no fertilisation). The soil profile of the samples does not change in terms of treatment nor though time. This indicates the soil ARGs to be stable, independently from fertilisations. Therefore, at the different timepoints ARGs characterisation is stable, and although it appears that some treatments fertilisations may influence their abundance, they do not change their composition.

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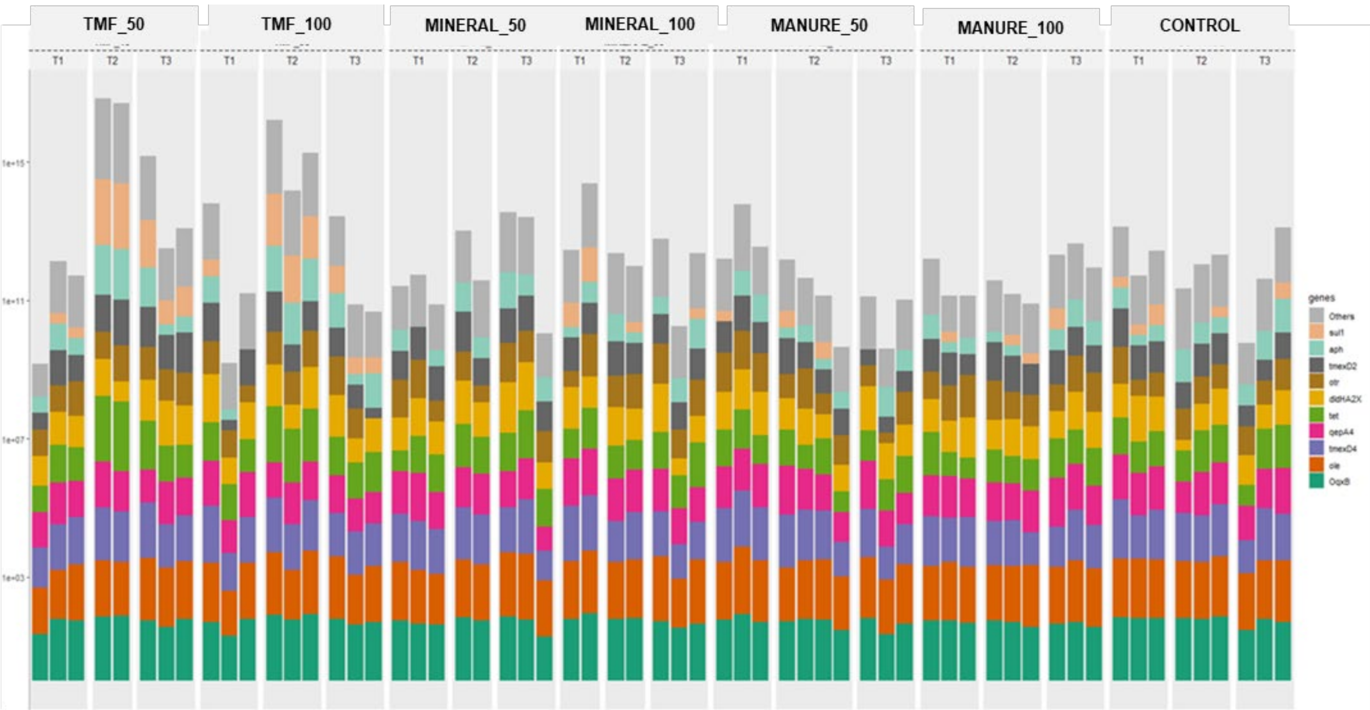

Figure 3.7. Composition plot of ARGs genes at log10 scale of fertilization treatments at different concentrations through time

4. Pilot plant optimisation process

During the operation time of the pilot plants, some bottlenecks have been identified and pilot plant equipment and/or operation conditions were slightly modified in order to solve these bottlenecks. These modifications resulted in the production of BFFs with different qualities and composition than reported in previous tables. The modifications carried out as well as the screening of solutions are reported in the following sections.

It is important to notice that not all FERTIMANURE pilot plants needed to be optimised as some of them were already well stablished such as Dutch and Belgium pilot plants.

4.1. Spain Pilot Plant

Modification of separation unit

During 2022, several modifications were made in the units of the Spanish pilot plant aimed to improve the efficiency of the whole biorefinery.

The first improvement is related to the main bottleneck found when the operation of the biorefinery started. The characteristics of separation unit were designed according to what conventionally is found in the market, thus, separation mesh size installed was of 450 µm. However, this mesh size was found to be inefficient to remove some particles that were leading to the malfunctioning of the microfiltration unit, generating a clogging problem. In April 2022, the mesh size of the separation unit was modified to 280 µm. In addition, microfiltration

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membrane cut off was increased from 130 nm to 400 nm to further reduce membrane clogging while reducing the specific energy consumption of the train of technologies. It was observed that the obtained MF permeate is suitable to be used in the subsequent steps without negative effects and the MF step's specific energy consumption was reduced by up to 10%.

Figure 4.1 and Figure 4.2 show the evolution of the characteristics of slurry compared to the main characteristics of the liquid fraction obtained after separation. The summertime and wintertime periods are marked in slightly orange and blue background colours, respectively.

In summary, an improvement of 20% less dry matter and organic carbon was achieved after the modification. However, this improvement was found only in the period between May and October (marked in slightly orange background), while during wintertime (between November and April) there was no relevant difference between the two periods, before and after mesh size modification. It seems that the feeding and mainly drinking routines of the animals change throughout the year modifying therefore the characteristics of the slurry generated. It is important to remark that the optimal performance of the separation unit was found during summertime, when the slurry was more diluted. During that period microfiltration membrane clogging was successfully avoided.

However, in wintertime, reducing the slope of the separation unit was needed to retain the slurry a longer time in the separation drum, allowing in turn the satisfactory operation of the separation unit. However, still, there was a difference in the characteristics of the liquid fraction produced during wintertime, which are not the most appropriate for the optimal performance of the microfiltration unit. Overall, the operation time needed to generate the same volume of liquid fraction was significantly longer during wintertime, leading to an increased energy consumption of the unit.

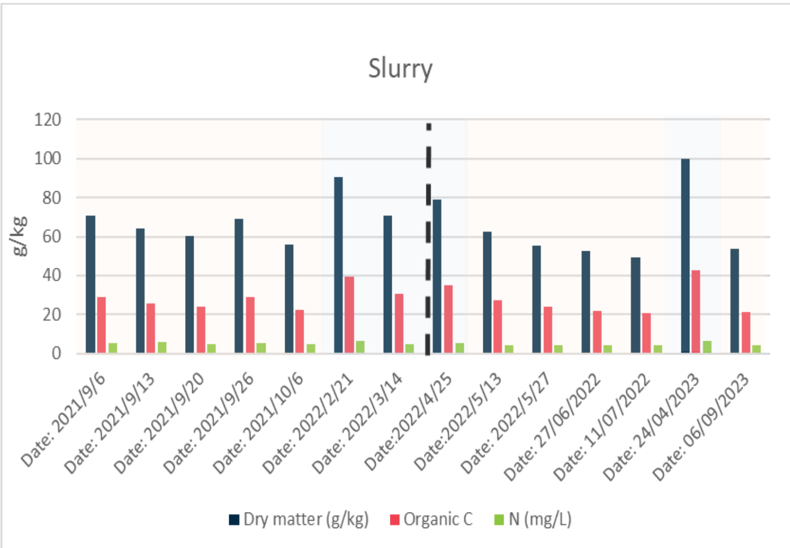

Figure 4.1. Variability of the dry matter, organic carbon and nitrogen contents in slurry along the operational period of the separation unit. The discontinuous line identifies the modification of the mesh size in the unit. Orange background marks the hot season is marked in blue background.

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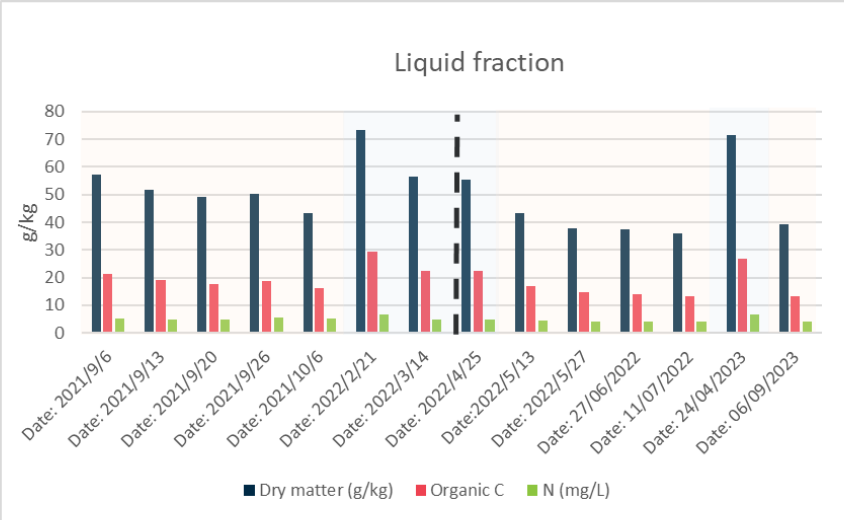

Figure 4.2. Variability of the dry matter, organic carbon and nitrogen contents in the recovered liquid fraction along the operational period of the separation unit. The discontinuous line identifies the modification of the mesh size in the unit. Orange background marks the hot season is marked in blue background.

Optimisation of membrane-assisted stripping

Membrane-assisted stripping process has been continuously optimised for increasing nitrogen content in the final product. Stripping acid volume used per batch was reduced from 15 – 20 L/batch at initial configuration to 2.5 L/batch in the most optimised conditions. This modification led to an increased nitrogen concentration in the acid side from 8 g/L obtained in the first batch to 44 g/L in the most optimised configuration.

Screening on the best configuration in membrane systems coupled with freeze concentration unit

Different configurations of membrane systems and freeze concentration were assessed to identify the most promising configuration. Figure 4.3 shows schematically the different configurations assessed where a) freeze concentration of the retentates of MF and RO mixed according to their production ratio to obtain the product codified as ES-NC-MFRO; b) freeze concentration of the retentate of MF to obtain the product codified as ES-NC-MF; c) freeze concentration or the retentate of RO when skipping MC in the membrane treatment train to obtain the product codified as ES-NC-RO skipping MC; d) freeze concentration of the retentate of RO to obtain the product codified as ES-NC-RO.

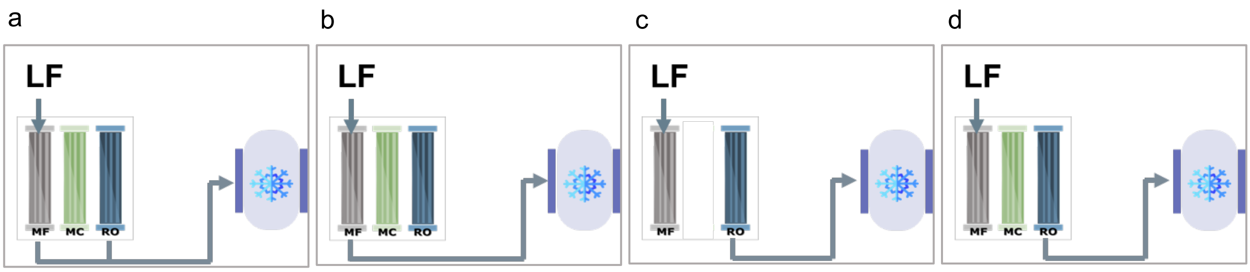

Figure 4.3. Schematic description of the configurations assessed in membrane systems coupled with freeze concentration.

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In terms of freeze-concentration process efficiencies, the volume reduction was lower than expected (around 30%) in the configurations freezing retentates from reverse osmosis and unaffected by the skipping or not of the MC step. In terms of products obtained, in both cases organic nutrient-rich concentrate is far below the targeted values and they must be concentrated by 14 and 16-fold, respectively. Considering the current treatment train design the concentration of the nutrients to such extent does not seem feasible. Freezing the retentate from MF was relatively more efficient (almost 60% of volume reduction) however, in that configuration, the distribution of nutrients in the concentrate and in the melted ice fractions was roughly equal. Melted ice retained an important part of the particulate material (2.6% DM) and since concentration was not achieved, this configuration does not meet the expected performance. Finally, the most promising configuration was found to be the one freeze-concentrating the mixture of both retentates (MF and RO) in the same mixing ratio as they are produced. In this case, the volume reduction was in average of 48% although the longer contact time of refrigerant per volume of retentates treated allowed a volume reduction over 65%. In average, 60.5% of nitrogen, 55% of phosphorus and 64% of potassium were concentrated in 51.6% of the initial liquid fraction volume. Still, and as it was mentioned before, the products obtained did not gather the appropriate nutrient levels to be regarded as organic- or organo-mineral fertiliser. Moreover, considering Zn content, this product is above the limits established in the European Fertilizing Products Regulation (with a limit established of 800 mg/kg DM). Table 4-1 shows the most relevant quality parameters analysed in the products obtained during screening of configurations.

Table 4-1. Characterisation of main parameters during optimisation process in Spain pilot plant

Parameter	Unit	ES-NC-MFRO	ES-NC-MF	ES-NC-RO skipping MC	ES-NC-RO
pH	-	8.03±0.7 (n=3)	7.4 (n=1)	7.6 (n=1)	9.0 (n=1)
CE	mS/cm	26.9±6.8 (n=3)	27.4 (n=1)	17.2 (n=1)	15.1 (n=1)
Density	kg/L	1.0	-	1.0	1.0
DM	g/kg	41.0±13.5 (n=3)	50.0 (n=1)	10.2 (n=1)	13.1 (n=1)
VS	g/kg	24.7±3.9 (n=3)	33.9 (n=1)	2.6 (n=1)	6.5 (n=1)
Organic C	g/kg	13.8±2.2 (n=3)	19.0 (n=1)	4.6 (n=1)	3.6 (n=1)
Total N (NTK)	g/kg	3.8±0.1 (n=3)	5.3 (n=1)	4.6 (n=1)	1.4 (n=1)
Ammonium-N	g/kg	2.8±0.1 (n=3)	3.7 (n=1)	1.6 (n=1)	1.3 (n=1)
P	g/kg	0.58±0.14 (n=3)	1.1 (n=1)	0.04 (n=1)	0.004 (n=1)
K	g/kg	2.21±0.66 (n=3)	2.4 (n=1)	1.2 (n=1)	1.0 (n=1)
Cu	mg/kgDM	195±74 (n=3)	250 (n=1)	-	-
Zn	mg/kgDM	838±272 (n=3)	1119 (n=1)	-	-

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A last strategy was assessed at lab scale, i.e. the solubilization of phosphorus was tested at lab scale tests by reducing pH of the liquid fraction with the addition of a strong acid solution. Solubilization was successful at pH 5 but this strategy was not tested in the on-farm pilot, thus technical feasibility at pilot scale was not assessed. This modification was expected to solubilize the nutrients that are normally retained in the MF retentate, therefore reaching to the subsequent units. In this scenario, membrane contactor would be skipped to avoid likeable phosphorus precipitations (as calcium phosphates, etc.) due to pH adjustments. Nutrients are expected then to be concentrated in the retentate of RO that could afterwards be further concentrated via freeze concentration. However, this scenario also has some challenges to be faced as a higher ammonium concentration in RO permeate that could report inhibitory effects for microalgae growth and could result in a setback in the potential use of RO permeate as reclaimed water.

regarding the amino acid-based biostimulant production (ES-AA), several optimisations at pilot scale (10-L batch) have been performed. In the previous trials, endoproteases and exopetidases were evaluated to produce protein rich hydrolysate from lyophilised *Scenedesmus sp.* biomass. The selected conditions for enzymatic hydrolysis that achieved higher protein solubilization values were: 2% DM endoprotease, 1% DM exoprotease, at 65ºC for 6h, pH 7 and a solid:liquid ratio 1:12. However, it needs to be highlighted that lyophilised biomass has undergone a mechanical process which may help to disrupt microalgae cell walls. Typical Scenedesmus sp., biomass, provided by ALGE to LEITAT in this project, is in the form of frozen biomass paste. Without lyophilization, pilot process needed to be upgraded to improve process yield while enhancing cell wall breakdown and protein solubilization. For this reason, the following approaches were considered:

- Optimisation of solid: liquid ratio to maximize, as possible, protein and AA content in final product.
- Optimisation of enzymatic treatment to ensure cell wall breakdown and improve solubilization yield.

Typical Scenedesmus sp. biomass in the form of frozen paste had a dry matter content ranged between 10 and 20% which represents a solid: liquid ratio of 1:9 to 1:4. First, from a known enzymatic process (2% cellulases, 2% of proteases at 65ºC), different solid-liquid ratios have been tested to optimize process yield (minimize biomass dilution but ensuring good agitation). Trials were performed with fresh biomass (frozen paste) having a 20% of dry-matter content (solid-liquid ratios1:4) and diluted biomass (solid: liquid ratio 1:8) with RO permeate was obtained in the Spanish Pilot Plant.

Results in terms of total (N), protein and amino acid content in the obtained product are shown in Table 4-2.

Table 4-2. Characterisation of produced products (solid-liquid ratio optimisation)

Batch	Solid: liquid ratio	Total N (NTK, g/100g fw) (n=2)	Protein content (g/100g fw) (n=2)	AA content (g/100g fw) (n=1)
1	1:4	0.88 ± 0.001	5.51 ± 0.08	1.33
2	1:8	0.57 ± 0.001	3.57 ± 0.04	1.02

**The protein content was estimated from the nitrogen content of the samples by using the conversion factor 6.25*
Initial microalgal biomass dry weight 19.6%

As it can be observed that enzymatic hydrolysis worked well on initial biomass, further studies were performed using fresh biomass (frozen paste) without dilution. Note that the dry weight of initial biomass varies in a range and, consequently, the minimum solid: liquid and protein and free amino acid content in the produced biostimulant also change. To optimize the enzymatic treatment, screening trials, detailed in the following table (Table 4-3), were performed.

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Table 4-3. Optimisation of enzymatic hydrolysis

	Cellulase			Proteases				
Batch	**Temperature (ºC)**	**pH adjustment**	**Time (h)**	**Temperature (ºC)**	**Concentration endopeptidase (% DM)**	**Concentration exopeptidase (%, DM)**	**pH adjustment**	**Time (h)**
1	65	-	2	65	2	1	8	6
2	65	-	2	65	2	1	-	6
3	50	-	2	50	2	1	8	6
4	50	-	2	50	2	1	-	6
5	-	-	-	65	2	1	8	6
6	-	-	-	65	2	1	-	6
7	-	-	-	50	2	1	8	6
8	-	-	-	50	2	1	-	6
9	-	-	-	50	2	2	8	7
10	-	-	-	50	2	2	-	7

It is important to note that in batches 9 and 10, 1% of exopeptidase was added in combination with the endopeptidase, and the other 1% was added to the liquid fraction. Results in terms of total (N), protein and amino acid content in the obtained product are shown in Table 4-4.

Table 4-4. Characterisation of optimisation of batches

Batch	**Total N (NTK, g/100g fw) (n=2)**	**Protein content (g/100g fw) (n=2)**	**AA content (g/100g fw) (n=1)**
1	0.510 ± 0.018	3.19 ± 0.11	0.37
2	0.354 ± 0.001	2.21 ± 0.01	0.79
3	0.516 ± 0.005	3.22 ± 0.03	1.21
4	0.406 ± 0.003	2.54 ± 0.02	0.93
5	0.469 ± 0.008	2.93 ± 0.05	1.05
6	0.388 ± 0.001	2.42 ± 0.01	0.79
7	0.512 ± 0.020	3.20 ± 0.12	1.35
8	0.397 ± 0.016	2.48 ± 0.10	1.12
9	0.453 ± 0.028	2.83 ± 0.18	1.47
10	0.383 ± 0.001	2.41 ± 0.01	1.10

**The protein content was estimated from the nitrogen content of the samples by using the conversion factor 6.25*
Initial microalgal biomass dry weight 10.3%

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From the results obtained (Table 4-5), it can be concluded that the addition of cellulase (batches 1-4) did not result in an increase of the free amino acid content. Moreover, the addition of 1% of exopeptidase to the liquid fraction (batch 9) help to increase the free amino acid content in the final AA-based biostimulant.

Finally, the selected conditions for the enzymatic hydrolysis were pH 8, 2% DM. exopeptidase and 1% DM. endopeptidase at 50ºC for 6h, and then 1% endopeptidase in the aqueous phase at 50ºC for 1h.

Considering selected optimal operational conditions, different batches (pilot scale 10L-batch) were developed to analyse the replicability of biostimulant (protein hydrolysate) composition. It is important to be highlighted that the previous processes showed difficulties in hydrolysate filtration (high viscosity). In this sense, the effect on viscosity as well as on final biostimulant composition of adjusting pH at 8 or not was also evaluated. The Table 4.5 show the composition, the protein extraction yield and the hydrolysis grade found for each of the four hydrolysates produced. Batch 1 (B1) and 2(B2) are performed at pH 8, batch 3 (B3) and 4 (B4) are performed without pH controlling (process pH:6).

Table 4-5. Characterisation of liquid hydrolysates B1, B2, B3 and B4.

	Biostimulant composition (proces: 2%endopetidase + 1% exopetidase pH 8)		**Biostimulant composition (proces: 2%endopetidase + 1% exopetidase without pH control)**	
	B1	**B2**	**B3**	**B4**
pH	7.55	7.9	5.94	5.94
DM g/Kg	64.60	66.30	60.20	54.10
N g/Kg	4.7328	4.3312	4.33	3.85
Protein g/Kg	**29.60**	**27.07**	**24.04**	**24.09**
TOTAL Free AA g/Kg	**14.69**	**12.47**	**11.02**	**10.30**
Protein solubilisation yield %	28.44	28.55	19.83	18.52
Hydrolysis grade %	49.62	46.06	45,84	41.63

As observed from the table 4.5, B1 and B2 showed higher protein content (29.6-31.9 g/Kg) and free-AA content (12.5-14.7gKg) in final hydrolysate in comparison with B3 and B4 with 24.1-27.0g/Kg and 10.3-11.0 g/Kg of protein and Free-AA, respectively. In addition, the protein solubilisation yield, as well as the hydrolysis grade (calculated as percentage of total protein that it is hydrolysed into free-AA), were also higher in batch 1 and 2 than batch 3 and 4. It is important to highlight that the process at pH 8 achieved a hydrolysis grade above 50%. That means that almost half of the protein of final biostimulant is hydrolysed into free-AA.

Analysing the free- AA profile of the produced hydrolysates, some difference between process at pH 8 and process without controlled pH can be also observed. In the figure below, the free-AA profile of four batches is detailed. Data is expressed as average of B1 and B2 and average of B3 and B4

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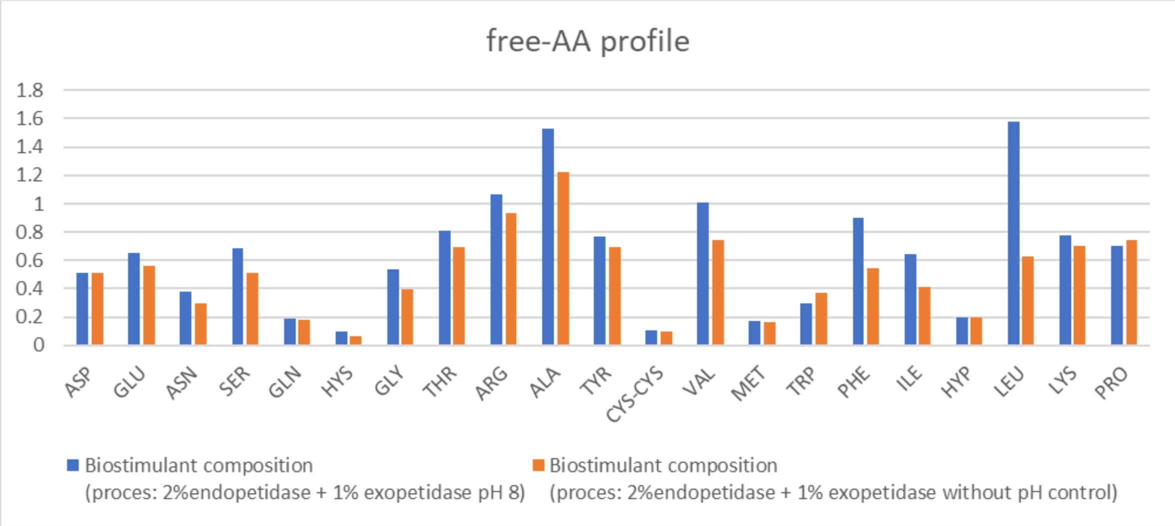

Figure 4.4. Free-AA profile. Comparison of enzymatic process at pH 8 and enzymatic process without pH controlling. ASP:Aspartic acid, GLU: Glutamic acid, ASN: Arparagine, SER:Serine, GLN:glutamine, HYS:Histidine, GLY:Glycine, THR: Threonine, ARG:Arginine, ALA:Alanine, TYR:Tyrosine, CYS-CYS: Cystein,

As observed from Figure 4.4, most of the free-AA were higher in B1+B2 than B3+B4. Leucine content, however, stands out above the rest, being significatively higher in B1+B2 than B3+B4. Free-AA showing higher content were Alanine (ALA), Leucine (LEU), Arginine (ARG), and Valine (VAL).

As observed from the Table 4.6, produced biostimulant showed and average nitrogen content of 4.5g N/Kg of liquid biostimulant (69.2g N/Kg DM), protein content of 28.3g/Kg liquid biostimulant (432.7g/Kg DM) and Free-AA content of 13.6 g/Kg liquid biostimulant (215g/Kg DM). Besides, biostimulant showed a high content in C and K, with 25.2 g C/Kg liquid biostimulant (377g C/Kg DM) and 1.2g K /Kg of liquid biostimulant (18g K/Kg DM), respectively. No presence of heavy metals was observed.

Considering selected operational conditions, hydrolytic unit has been operated. Process developed in the hydrolytic unit is the process selected according to the results commented before, pH 8, 2% DM. exopeptidase and 1% DM. endopeptidase at 50ºC for 6h, and, then 1% endopeptidase in the aqueous phase at 50ºC for 1h. The process operates at 50ºC during 7h. Besides, the process includes a final step (enzyme inactivation) at 85-95ºC for 15 min. To ensure temperature maintenance during the 7h of operating as well as to improve temperature curve and achieve the required temperature as fast as possible, the hydrolytic unit have been updated incorporating a broiler and an external thermal jacked.

The hydrolytic unit has a total capacity of about 100L, but the used capacity was about 65L. Hydrolytic unit operation has been performed using commercial lyophilised *Scenedesmus sp.* biomass as raw material. *Scendesmus* paste produced within the project could not be used due to the difficulties to get the enough amount of the inlet biomass (65L). Lyophilised biomass has been diluted to 10-11g/Kg w/w solution to simulate *Scenedesmus sp.* paste. Some differences were observed when lyophilised microalgae were used in comparison with process with microalgae paste. During hydrolytic unit operation, although enzymatic process were developed at pH:8, no viscosity problems were observed in the final hydrolysate. Besides, the filtration process were developed without further limitations. In this case, conditioning characteristic (lyophilised vs. fresh paste) was observed as a key aspect affecting process operationality. Besides, final produced biostimulant from commercial biomass showed significance difference in its composition compared with biostimulant produced from *Scenedesmus sp.* paste. In the following figure comparison is performed evaluating difference between produced biostimulants composition.

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Table 4-6. Individual and average composition of produced biostimulants (ES-AA) under selected conditions

	Biostimulant composition (process: 2%endopetidase + 1% exopetidase pH 8)		
	B1	**B2**	**Average B1+B2**
pH	7.55	7.9	7.72±0.24
DM g/Kg	64.60	66.30	65.40±0.12
Organic matter g/Kg	59.60	61.30	60.40±1.20
Density	0.98	0.99	0.99±0.01
N g/Kg	4.7328	4.3312	4.53±0.28
Protein g/Kg	**29.60**	**27.07**	28.32±1.77
TOTAL Free AA g/Kg	**14.69**	**12.47**	13.58±1.56
C g/Kg	25.32	25.00	25.16±0.23
K g/kg	1.26	1.20	1.23±0.04
P g/kg	0.92	0.80	0.86±0.08
Na g/Kg	0.32	0.35	0.33±0.02
Mg g/Kg	0.20	0.10	0.15±0.07
S g/Kg	0.35	0.40	0.37±0.03
Ca g/Kg	<0.10	<0.10	<0.10
Cr g/Kg	<0.01	<0.01	<0.01
Mn g/Kg	0.001	<0.01	0.001
Fe g/Kg	0.007	0.005	0.006 ±0.001
Ni g/Kg	<0.001	<0.001	<0.001
Cu g/Kg	<0.001	<0.001	<0.001
Zn g/Kg	0.002	0.004	0.003±0.01
As g/Kg	<0.001	<0.001	<0.001
Cd g/Kg	<0.001	<0.001	<0.001
Hg g/Kg	<0.001	<0.001	<0.001
Pb g/Kg	<0.001	<0.001	<0.001

As observed from the Figure 4.5, produced biostimulant in hydrolytic unit showed and average nitrogen content of 5.1g N/Kg liquid product (91.1g N/Kg DM), a protein content of 31.9 g/Kg liquid product (569,6,7g/Kg DM) and a Free-AA content of 15.3 g/Kg liquid product (273.2g/Kg DM). In comparison with biostimulant from *Scenedesmus sp* paste, nitrogen, protein and free-AA are slightly higher. It must be highlighted that Initial biomass shows different composition. Nitrogen and protein content of Scenedesmus paste was higher than those in lyophilised biomass. Even so, protein content in biostimulant from lyophilised biomass is higher than biostimulant from paste, showing higher nutrient recovery when commercial lyophilised biomass is used (70% in commercial biomass versus 30% achieved with paste). These results are in line with those reported in the bibliography (see section 5) where difference between using fresh or paste biomass or lyophilised biomass were evaluated, reporting higher recovery nutrients from lyophilised biomass.

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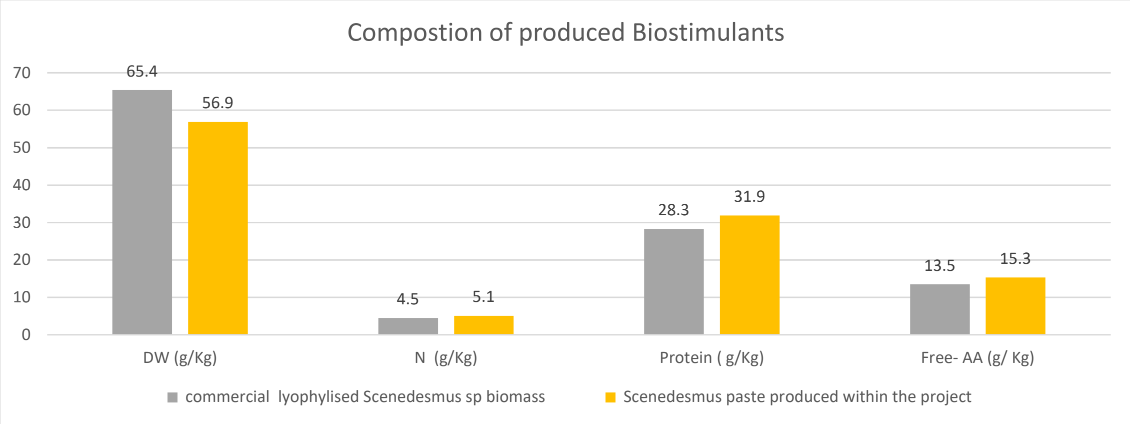

Figure 4.5. Comparison of biostimulants produced using commercial lyophilised *Scenedesmus sp.* biomass and biostimulant produced using *Scenedesmus sp* paste produced in the project. Results are expresses as g/Kg of liquid biostimulant.

Finally, it is important to highlight that during the hydrolytic process a solid residual fraction is produced. Residual fraction from process using *Scenedemus sp.* paste was characterised. This residual fraction is still rich in nutrients, and it could be once more an agriculture input, ensuring the zero-waste strategy of the biorefinery process. In particular, the nutrient content of this solid fraction is as follows: DM: 58.73g/Kg, Nitrogen: 30.5 gN/Kg, Carbon: 306.1 gC/Kg and potassium: 1.2gK/Kg.

4.2. German Pilot Plant

By using TCR gas for MAP production, there is no selective conversion of phosphoric acid with ammonia to mono ammonium phosphate in the cases investigated. This is confirmed by the analysis of the ammonia content of the perlite samples from the different trials. Differences in MAP reactor temperature and reaction time had no influence on the outcome of the experiments.

A broad preliminary analysis of all products showed that both the pH and the amount of ammonia are quite low. The pH of app. 1.6 indicates that only small amounts of the ammonia present in the gaseous phase have reacted with the phosphoric acid on the perlite. This is supported by the vanishingly small amounts of ammonia detectable on the perlite. Furthermore, analytical results show that the theoretical phosphoric acid content of the perlite differs from the experimentally determined content. This can be explained by non-uniform distribution on the perlite.

In addition, it is noticeable that the usually colourless product has brown to red discolorations and a strong aromatic smell mostly attributable to various organic compounds. As can be seen from elemental analysis (Table 4-7) almost no nitrogen is present after processing the perlite within the packed be reactor. The presence of carbon indicates the presence of hydrocarbons or other organic compounds, and it is in agreement with the detectable smell of the products.

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Table 4-7. Elemental analysis of the perlite from the different pilot trials

Sample name	N [%]	C [%]	H [%]	S [%]
Pilot trial 1	0.01	0.042	3.21	0
Pilot trial 2	0.02	0.035	2.87	0
Pilot trial 3	0.02	0.031	2.9	0
Pilot trial 4	0.01	0.030	3.14	0
Pilot trial 5	0.43	0.058	3.02	0
Pilot trial 6	0.03	0.075	2.67	0

Due to the described problems with MAP production at the TCR30 plant with the MAP reactor, a change to a TCR2 laboratory plant, which is equipped with two modular bypass reactors, has been made. The bypass can be filled with both solids and liquids.

Different approaches to optimize the reaction were persecuted: (i) drying of the TCR gas by equipping the bypass with CaO to rule out that water is the interfering factor in this reaction; (ii) abandon perlite during the reaction in order to obtain pure monoammonium phosphate from liquid medium and (iii) substitute active charcoal by 100 mL of phosphoric acid.

Despite the efforts, there was not obtained promising results in any of the configurations as few amounts of MAP was obtained due to both low concentration of ammonia in TCR and the presence of side-reactions within phosphoric acid which inhibits the formation of MAP. Therefore, more trials will be performed by using diluted phosphoric acid.

Finally, it was detected that the condensate from pyrolysis contains around 50 g/L of ammonia. Therefore, partners from other pilot plants where stripping processes are used, will try to recover the ammonia contained in condensates.

4.3. French Pilot Plant

The presented results are the combined results obtained since 2021.

Concerning pyrolysis, lower temperature was used during 2022 (550°C instead of 700°C used in 2021) in order to assess the impact of this parameter on the quality of biochars (and also to reduce the energy consumption of the pyrolysis process). Trials on these biochars are still running, and other manures are currently being tested. Obtained results will be present in the next deliverable.

Ammonia stripping:

The first conception of the stripping tower is described in Figure 4.6.

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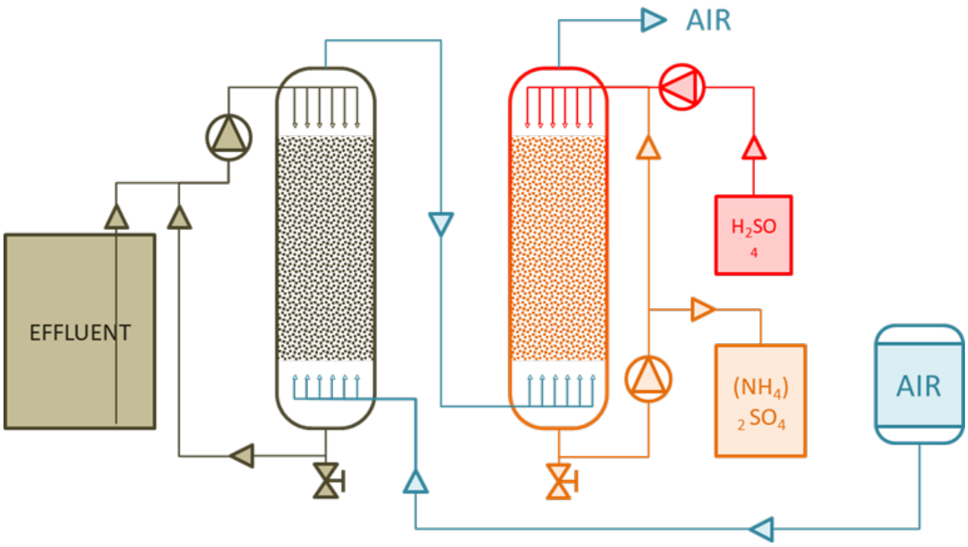

Figure 4.6. First conception of ammonia stripping tower

The two columns were functioning simultaneously, one for liquid effluent circulation, and the second for sulfuric acid circulation.

With this configuration several problems appear: without optimisation of physicochemical properties of effluent, ammonia volatilization is extremely poor, and on the other hand acid circulation highly reduce its trapping capacity (the volume capacity of the circulation is very low compared to the volume of the column). Furthermore, circulation of sulfuric acid in the system severely damaged all the system (stainless steel tower, fittings, pumps…). That is why we made some modifications to the system and tested step by step every change in order to get closer to industrial yields. The Figure 4.7 shown the final configuration which gave us the bests yields.

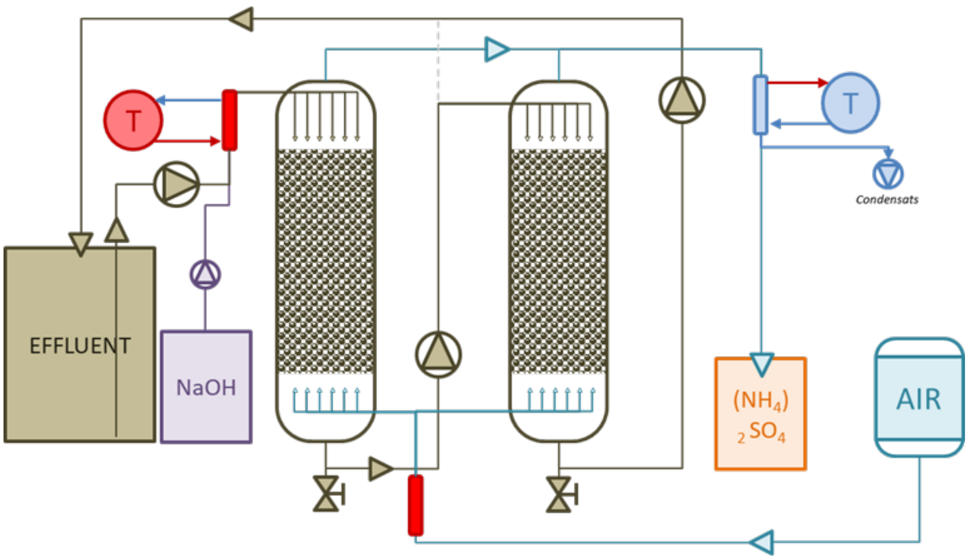

Figure 4.7. Final conception of ammonia stripping tower

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Details of the whole modifications brought to the stripping tower are presented in deliverable D2.1. Shortly, in the final system, the ammonia volatilization is optimised thanks to:

- the addition of caustic soda (NaOH) into the effluent in order to reach a pH above 10.
- The heating of effluent and airflow at 60°C thanks to 2 heating exchangers
- The usage of the second tower as an extension of the first one for effluent circulation, in order to double the exchanging time between effluent and airflow.

The ammonia trapping is also optimised with bubbling charged airflow into an acid trap instead of making an acid circulation through the stripping tower (this also helps protect the process from acid corrosion)

Given that production was very variable and unstable before reaching this configuration, previous productions carried out with previous stripping systems were not considered in the final mass and element balance presented below. Ammonia stripping was carried on pig slurry and liquid digestate. Unfortunately, the second effluent was more difficult to use with our configuration. The liquid digestate is more concentrated with suspended matter, the pumps and columns saturate more quickly, requiring recurring cleaning of the whole system.

5. Comparison of BBFs with literature

The resulting BBFs within FERTIMANURE can be classified within 5 categories, namely organic amendments, organo-mineral BBFs, mineral BBFs, and biostimulants. The composition and characteristics of FERTIMANURE BBFs are compared with commercial equivalent products and those obtained at laboratory and bench scale by using organic wastes as feedstock (i.e., sewage sludge, manure, digestates, etc.)

5.1. Organic amendments

Biodried organic amendment from Spanish pilot

There are not many biodrying studies assessing the biodried product as an organic fertilizing product rather than a biomass fuel. Only 3 works were found reporting agronomic quality parameters of biodried manure (Table 5-1). Phosphorus contents in both organic amendments obtained from the solid fraction of pig slurry and poultry manure are generally low (0.3-0.4% phosphorus) whereas potassium content is three times higher in biodried poultry manure (1.6% of potassium) compared to the product obtained from the solid fraction of pig slurry (0.5% of potassium). Considering their main nutrients content, in both cases, the product must be regarded more as an organic amendment rather than an organic fertiliser. When comparing nutrient contents to the literature reported data phosphorus and potassium contents reported are generally higher than in our products (Sadaka and Ahn, 2012). In the mentioned work, the products obtained from poultry manure contain 4 times more phosphorus while only 50% higher content of potassium (calculated in dry basis). In the case of the biodried product obtained from pig manure, phosphorus content per dry product was equivalent to the product obtained in the Spanish pilot whereas the potassium was 2.5 times less concentrated than in the product reported by Sadaka and Ahn (2012). Regarding other nutrients, Spanish product obtained from pig slurry is slightly richer in calcium, magnesium, and copper than the one obtained by Sadaka and Ahn (2012), while the difference is more relevant for zinc. The differences found for the product obtained from poultry manure are not as different except for Calcium which was four times higher in the work mentioned. As mentioned, there is very scarce information regarding agronomic quality of biodried products (even produced from other feedstocks such as sewage sludges). Therefore, it is also relevant to benchmark the organic amendments obtained from pig and poultry manure to composted manure, or even composted sludge or organic fraction from municipal solid wastes (OFMSW). In this regard, it is relevant comparing the safety aspects of the obtained products. In terms of heavy metal contents, biodried manure contain, in general, equivalent metal concentrations to composted manures (Ravindran et al., 2019) but lower than the compost obtained from OFMSW, except for copper and zinc (Vázquez and Soto, 2017) (Table 5-2). Analogous to the

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content of phosphorus and potassium, the heavy metal contents are directly dependent on their content in feedstock materials. In this specific case, zinc and copper are usually used as feeding additives in pig farms (as zinc and copper oxides), and although their addition has been recently limited in Europe (EU Regulation 1095/2016 in force since 2022), their content in the slurry still affects the final quality of the product. Pathogen presence in the biodried products was also assessed with positive results in general. Biodrying seems promising as it was demonstrated to be able to reach a good sanitization of manure, which would be probably intensified in plant scale. Therefore, in terms of safety, it could be said that biodried product could be an attractive fertilizing product. However, biodrying has not been typified as a conventional process to obtain fertilizing products. The biological stability of reached at the end of the process (DRI >2 gO2/h/kg VS) indicates a moderate biological activity in the range of what was found for biodried municipal solid waste (Adani et al., 2002). This was in fact expected as stabilization of material is not the aim of biodrying process but the maximum conservation of carbon.

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Table 5-1. Comparison of the main characteristics of biodried or composted organic amendments reported in literature

Reference	Spanish pilot		Sadaka and Ahn, 2012			Rékási et al., 2019		Vázquez and Soto, 2017	Ravindran et al., 2019
Feedstock/processing	Biodried solid fraction of pig manure	Biodried poultry manure	Biodried beef manure with corn stover	Biodried swine manure with corn stover	Biodried poultry manure with corn stover	Composted sewage sludge digestate with green waste	Vermicomposted sewage sludge digestate with green waste	Home-composted OFMSW (n>70)	Composted swine manure
pH	7.2±0.2 (n=5)	8.6±0.6 (n=2)				6.86±0.04	6.80±0.05		7.4±0.44
EC (mS/cm)	2.8±0.8 (n=5)	5.6± 4.1 (n=2)				4.0±0.4	4.8±1.1		0.84±0.03
density (kg/L)	0.38 (n=1)							0.68±0.21 (n=90)	
DM (g/kg)	487.4±175.2(n=5)	665.3±59.9 (n=2)	768.4	773.9	760.9			319±96 (n=87)	
OM (g/kg)	433.9±159.3 (n=5)	556.1± 36.9 (n=2)	622	717	696			481±190 (N n=90)	
Org C (g/kg)	243.0±89.2 (n=5)	311.4±20.6 (n=2)				206±12	197±7	215±97 (n=57)	
N (g/kg)	11.54±3.8 (n=5)	22.0±3.5 (n=2)				24.3±1.3	24.1±0.6	21±11 (n=90)	
TP (g/kg)	2.7±0.8 (n=5)	3.6±1.4 (n=2)	4.53	5.42	18.28	0.022±0.00	0.022±0.00	6.1 (n=36)	
TK (g/kg)	5.0±1.6 (n=5)	16.1±0.1 (n=2)	11.21	20.35	27.54	7.56±0.14	7.95±0.09	25.3 (n=36)	
S (g/kg)	5.2±2.4(n=4)	4.1 (n=1)							
Ca (g/kg)	10.6±2.3 (n=4)	13.7 (n=1)	31.46	14.51	67.51			37.0 (n=36)	
Mg (g/kg)	2.7±0.7 (n=4)	4.3 (n=1)	2.84	2.46	5.68			7.3 (n=36)	
Zn (mg/kg DM)	719.9±489.4 (n=4)	455.3±182.0 (n=2)	222.5	193.8	391.6			148.8±90.9 (n=87)	496±17.16
Cu (mg/kg DM)	62.1±3.4 (n=4)	78.7±57.0 (n=2)	37.7	42.6	72.3			39.7±23.2 (n=89)	206.3±8.49

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Table 5-2. Comparison of the heavy metal contents of biodried or composted organic amendments reported in literature

Reference	Spanish pilot		Vázquez and Soto, 2017	Ravindran et al., 2019
	Biodried solid fraction of pig manure	Biodried poultry manure	Home-composted OFMSW (n>70)	Composted swine manure
Cd (mg/kg DM)	<0.5 (n=3)	25 (n=1)	0.38±0.32 (n=88)	1.1±0.03
Hg (mg/kg DM)	<0.4 (n=3)	< 0.4 (n=1)	0.09±0.07 (n=88)	
Pb (mg/kg DM)	3.8±2.2 (n=3)	21.5 (n=1)	21.3±18.0 (n=87)	55.2±1.79
Cr (mg/kg DM)	<10 (n=3)	< 10 (n=1)	20.7±24.8 (n=89)	
Cr VI (mg/kg DM)	<0.5 (n=3)	< 0.5 (n=1)		
Ni (mg/kg DM)	4.84±2.0(n=3)	5.4 (n=1)	15.2±13.8 (n=90)	7.23±0.42
As (mg/kg DM)	<2 (n=3)	< 2 (n=1)	13.6±14.6 (n=89)	

Soil conditioner from cattle manure digestate from Dutch pilot:

The soil conditioner produced in the Dutch pilot is a carbon rich and fibrous material obtained from the separation of the digestate produced in the anaerobic digestion of cattle manure with feed residues. The soil conditioner is slightly basic (average pH of 8.4) although higher pHs are reported in literature (Table 5-3). The product is similar, in terms of organic carbon and phosphorus content, to other reported solid fractions of digested manures. However, nitrogen content is, in this case, in the low range of what was reported for the solid fraction of digested cattle manure and digested pig manure. It is noticeable the difference in the nitrogen species in the digestates obtained from different feedstocks. In the Dutch pilot almost 37% of the total nitrogen in the soil conditioner appears in ammonium form, similar to other reported solid fractions of cattle manure digestate (Cavalli et al., 2018). The percentage in which nitrogen appears as ammonium nitrogen reported for the solid fractions of pig manure digestate are slightly higher (Morey et al.,2023) probably due to the inherent differences in the manure used to feed the digestor.

Heavy metal contents are only reported for pelletised cattle manure digestate in the table below. In general terms, heavy metals content is very low in this kind of soil amendment finding higher presence of zinc and copper. Compared to the organic amendments obtained in the Spanish pilot, cattle manure seems to contain markedly lower content of those elements compared to pig manure or poultry manure. This fact must be carefully considered when designing and implementing certain technologies to obtain targeted BBFs from certain manures as it might become the limitation to put some products in the fertilisers' market.

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Table 5-3. Comparison of the main characteristics of Dutch soil conditioner from cattle manure digestate with digestates and solid fractions of digested manure reported in literature

Reference	Dutch pilot	Morey et al., 2023	Risberg et al., 2017				Valentinuzzi et al., 2020	Cavalli et al., 2016
Feedstock/ processing	SF of Cattle manure co-digested with feed residues and others (mesophilic)	SF of Pig manure and agro-industrial wastes (mesophilic)	Digestate 96% pig slurry, 4% cabbage (mesophilic)	Digestate 95% manure, 5% organic waste (mesophilic)	Digestate 100% cow manure (mesophilic)	Digestate 75% manure (cow, pig, chicken), 25% wastes from food industries	Pelleted cow manure-based digestate	Solid fraction of cattle manure digestate (range of 3 years value)
pH	8.4 ± 0.5 (n=14)						9.75	9.0-9.8
DM (g/kg)	260 (± 84) (n=14)	230	60	31	74	39	890	256.5-276.0
OM (g/kg)	205 ± 61 (n=14)	142.6					729.8	
Org C (g/kg)			27.0	11.0	34.0	15.0	373.8	431.6-439.8
N (g/kg)	6.5 ± 1.4 (n=14)	12.65	4.3	4.5	3.4	4.8	17.5	20.9-22.9
Ammonium N (g/kg)	2.4 ± 0.7 (n=14)	5.29	3.1	3.7	2.2	3.6	0.36	5.1-7.8
TP (g/kg)	2.4 ± 0.5 (n=13)	2.71					7.78	
TK (g/kg)	5 ± 0.5 (n=14)	0.57					13.2	
TS (g/kg)	1.4 ± 0.4 (n=14)						4.2	
Ca (g/kg)	4.4 ± 2(n=14)							
Mg (g/kg)	2.1 ± 0.7 (n=14)						7.5	
Zn (g/kg DM)	277 ± 204(n=12)						242	
Cu (g/kg DM)	154 ± 362 (n=12)						59	

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Cd (g/kg DM)	<0.4 ± 0 (n=7)						0.4	
Hg (g/kg DM)	<0.05 ± 0 (n=7)						0.2	
Pb (g/kg DM)	<5 ± 0 (n=7)						6	
Cr (g/kg DM)	6.6 ± 1.6 (n=7)	<0.5					16	
Ni (g/kg DM)	5.2 ± 0.2 (n=7)						11	
As (g/kg DM)	<1 ± 0.1 (n=7)							

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Phosphorus and potassium rich biochar from German and French pilots:

The biochar DE-BC from the German pilot plant is produced by TCR-technology with a two-step process. In the first step the cattle manure is processed at 450°C in a continuously working auger reactor. In a second step the biomass is promoted to the post reforming unit where a reforming of the gases and the char at 650°C takes place. Compared to other pyrolysis processes this unique process management minimizes the formation of polyaromatic carbo hydrogens (PAH) and therefore ensures a high quality of the resulting product. Compared to the literature, all biochars obtained from German and French pilots are similar, in terms of nutrient contents, to other reported biochars in literature (Table 5-4).

In general terms, the biochar from the German TCR process and the one from poultry manure from Frech pilot show a higher pH than the other biochars reported in literature, even for the equivalent feedstock. Biochar produced from poultry litter show relevantly higher content in N, while P and K content are also below the values found in literature, but that is mainly linked to the raw material content. Carbon content seems to be higher in biochars obtained from poultry and cattle manure rather than the ones obtained from pig slurry. However, there is not a clear trend for the rest of the nutrients (P, K, S, Ca, Mg) as they report high variability among different works.

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Table 5-4. Comparison of the main characteristics of German and French biochars obtained from cattle manure and poultry litter with manure derived biochars reported in literature

Parameter	**DE-BC**	**FR-BC**	**FR-BC**	**Uzoma et al., 2011**	**Sikder and Joardar 2018**	**Tsai, C.-C.; Chang, Y.-F. 2021**					**Chaves et al. 2020**	**Sarfaraz et al. 2020**	**Gunamantha et Widana 2018**		**Stylianou et al. 2020**	**Sarfaraz et al. 2020**	
Feedstock	**Cattle manure**	**Poultry litter**	**Digestate**	**Cattle manure**	**Poultry litter**	**Poultry litter**					**Poultry litter**	**Poultry litter**	**Cattle manure**	**Pig slurry**	**Cattle manure**	**Cattle manure**	**Pig slurry**
Temperature (ºC)	650	700	550	500	300	200	300	400	500	600							
pH	12.3	12.1	10.1	9.2	9.1	6.9	6.8	7.1	9.7	9.8	9.5	10.0			10.4	10.2	9.6
Total N (g/kg)	10.06 ± 1.49 (n=5)	27.4	17.2	1.51	20.4	31.7	31.9	35.3	35.5	33.9		18.2	8.0	7.0	15.8	30	9.5
Total C (g/kg)		324.2	388.3	33.61		364.0	391	410	464	463	397.7	221.1		188.0	284.6	382.7	164.2
Ammonium-N (g/kg)	0.14 ±0.09 (n=7)																
Total P (g/kg)	30.4 ± 2.58 (n=8)	6.4	7.8	8.14	46.8	9.3	11.6	13.4	20.9	19.8		33.3	34.0	33.0		48.8	9.4
Total K (g/kg)	95 ± 23.4 (n=8)	20.4	15.8		43.0	24.0	28.8	33.4	49.4	47.9		56.0	46.0	46.0		36.7	26.6

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S (g/kg)	2.5 ± 0.14 (n=5)	1.9	4.0		6.3						7.4		4.0	4.0	4.8		
Ca (g/kg)	22.9 ± 1.5 (n=2)	33.6	30.7		21.0	17.8	22.8	26.2	40.3	38.3	68.3	238.9	98.0	52.0		70.2	13.6
Mg (g/kg)	6.7 ± 0.3 (n=2)	15.7	11.5		10.7	6.1	7.5	8.7	13.8	13	13.4	27.9	25.0	25.0		58.4	0.7
Cu (mg/kg DM)	51.30	151.4	32.4								40.0	7.7				20.7	31.2
Zn(mg/kg DM)	358.50	767.3	198.5								80.0	35.9				508.6	28.8
Fe (mg/kg DM)	3,402	2,020.0	3,550.0		3.0						4,600.0	75.3	3,2943.0	6,6726.0		476.6	855.4
Mn (mg/kg DM)	403																

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5.2. Organo-mineral BBFs

Nutrient-rich concentrate from Spanish pilot:

As mentioned before, nutrient-rich concentrate is too diluted in nutrients and carbon to be regarded as an organic fertiliser. Assuming an enhanced concentration efficiency, the most appropriate type of fertilizing product for the nutrient-rich concentrate would be probably an organo-mineral liquid amendment. The information of freeze concentration used to obtain fertilizing products from secondary streams is very scarce and the data deported in terms of nutrient content in the concentrates obtained from RO retentates (Uald-Lamkaddam et al., 2021) and urine (Noe-Hays et al., 2021; Moharramzadeh et al., 2022) are very promising (Table 5-5).

Table 5-5 Comparison of the main characteristics of the nutrient-rich concentrates obtained via freeze concentration in the Spanish pilot with the ones reported in literature

Reference	Spanish pilot	Uald-Lamkaddam et al., 2021		Noe-Hays et al., 2021	Moharramzadeh et al., 2022	Samanta et al., 2022	Chiumenti et al., 2013
Feedstock/ processing	Retentates from microfiltration and reverse osmosis (1.5:1, v:v) from the treatment of the liquid fraction of pig slurry	Reverse osmosis retentate from the treatment of the liquid fraction of digestate of agro-industrial waste		Urine (2 stage FC)	Stabilised urine (with peracetic acid)	Microfiltration-vacuum evaporation of pig manure, N recovery in condensate	Vacuum evaporation of digestate
	Suspension FC	Progressive FC	Suspension FC		Progressive FC (at different cooling temperatures)		
pH					6-6.5		8.6
EC (mS/cm)					16.3-39.6		
DM (g/kg)	41.0±13.5 (n=3)	4.02±0.01	3.49±0.01				15.1
OM (g/kg)	24.7±3.9 (n=3)						11.4
Org C (g/kg)	13.8±2.2 (n=3)						
N (g/kg)	3.8±0.1 (n=3)	22.66	20.86	29.7	21	up to 10-30 (depending on feedstock)	5.35 and 55 with acidification
TP (g/kg)	0.58±0.14 (n=3)	0.05	0.04	1.68			
TK (g/kg)	2.21±0.66 (n=3)	2.9	2.49	9.37			
Zn (g/kg DM)	838±272 (n=3)	117.9	46.39				
Cu (g/kg DM)	195±74 (n=3)	150.2	41.8				
Cd (g/kg DM)	< 0.5 (n=1)	<QL	<QL				
Hg (g/kg DM)	< 0.4 (n=1)	<QL	<QL				
Pb (g/kg DM)	< 5 (n=1)	<QL	<QL				
Cr (g/kg DM)	< 10 (n=1)	<QL	<QL				
Cr VI (g/kg DM)	< 0.5 (n=1)						
Ni (g/kg DM)	10 (n=1)	4.138	8.972				
As (g/kg DM)	2.5 (n=1)						

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Freeze concentration applied to membrane retentates (RO retentate) at large scale was promising in terms of nitrogen content in the liquid product (Uald-Lamkaddam et al., 2021) while the phosphorus, potassium and heavy metal contents were in general equivalent to the one obtained in the Spanish biorefinery, except for the zinc which was significantly higher in our study. Considering the similar volume reduction and slightly lower concentration yield obtained in our pilot, the high concentration of nutrients already in the feedstock obtained at the industrial plant seems to be the key to obtain an attractive fertilizing product. Moreover, we used MF retentate which retains a major part of particulate material and the metals associated with them (being the zinc and in a less extent the copper the most problematic ones due to their use as veterinary additives). When our liquid product is compared to other concentration technologies such as vacuum evaporation of manures or digestates, the nitrogen concentrations are again significantly higher (up to 30g/kg in concentrated manure in Samanta et al., 2022 and up to 55 in concentrated digestate with acidification in the evaporation process in Chiumenti et al., 2013). As mentioned, the characteristics and mainly the initial concentration of nutrients in the feedstock are critical as even though the concentration ability of the technologies compared are quite equivalent, the final product quality of the compared products differ significantly.

5.3. Mineral BBFs

Within FERTIMANURE, several mineral BBFs were produced, that can be classified in major groups such as N-rich, P-rich, K-rich, and mixed mineral fertilisers.

5.3.1. Ammonium-based BBFs from Spanish, Dutch, French, and Belgium pilot

Within FERTIMANURE, ammonium sulphate and ammonium nitrate solutions are produced by using stripping processes. Spanish pilot plant used membrane assisted stripping by using membrane contactors while the rest of the FERTIMANURE pilots used conventional stripping-scrubbing processes. In Table 5-6-6 summarises the characterisation of the BBFs obtained within the project as well as other BBFs reported in the literature.

Table 5-6. Comparison between FERTIMANURE ammonium-based BBFs and previous studies Comparison of the main characteristics of the ammonium sulphate solutions obtained in the different FERTIMANURE pilots with the ones reported in literature

Reference	Spanish pilot	Dutch pilot	French pilot	Belgium pilot (BE-AS)	Belgium pilot (BE-AN)	Oudad et al. (2022)	Vecino et al. (2020)
Feedstock/ processing	Pig slurry microfiltration permeate	Digested cattle manure	Mixed pig slurry and cattle manure	Mixed pig slurry and cattle slurry		Compost leachate	Wastewater
	Membrane-assisted stripping	Stripping/scrubbing					Membrane-assisted stripping + Electrodialysis
pH	5.5	5.3	4.75	5.6	6.0	-	-
EC (mS/cm)	56.6	66.6	199.1			-	-
DM (g/kg)	235	323	302.9	308.5	390.8	-	-
OM (g/kg)	-	334	<1	<1	<1	-	-
Org C (g/kg)	-	0.89	<1	0.82	0.12	-	-
TN (g/kg)	44	65.3	47.92	74.2	153.1	1.5	51 - 101
TP (g/kg)	<1	<0.03	<1	0.05	0.06	-	
TK (g/kg)	<1	<0.4	<1	0.68	0.55	-	

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Zn (g/kg DM)	<0.1	<250	<2	5.5	3.4	-	
Cu (g/kg DM)	<0.1	<50	<2	2.2	1.2	-	

It is important to note that most of the previous studies are focused on the removal of nitrogen from wastewaters and other waste streams but not in the recovery and use of recovered products as BBFs. Therefore, it was noticed a lack of agronomic and full characterisation of the recovered products.

As can be observed in the previous table, membrane-assisted stripping resulted in an ammonium-based BBF with lower TN content due to the osmotic pressure in the stripping acid side that could cause reverse permeation of salts to the feed side. This limitation could be solved by adding a further concentration step such as electrodialysis as reported by Vecino et al. (2020), but techno-economic feasibility of the solution should be deeply analysed in an upscaled scenario. In addition, it is important to note that the product obtained with membrane-assisted stripping reported the highest purity as it contains pure ammonium salt with no detectable presence of pollutants.

In the case of BBFs obtained with conventional stripping-scrubbing processes, FERTIMANURE pilot plants reported ammonium-based BBFs that accomplish with European regulation on fertilizing products, so have the potential to be commercialised. However, one of the main challenges to be faces is logistics as the product still contains huge amount of water (>90%) that could make transportation expensive and inefficient.

Finally, as reported by all authors, and also noted by FERTIMANURE partners, the quality and nitrogen content of the final product is highly dependent on feedstock initial nitrogen content, obtaining higher mass transfer coefficient and higher nitrogen content in the final product as higher is the feedstock nitrogen content.

5.3.2. Phosphorus-based BBFs

Phosphorus rich ashes from Spanish pilot

The combustion process of biodried solid fraction of pig manure concentrate most of the nutrients (macro-, meso-, and micronutrients) present in the organic amendment. However, heavy metals are also highly concentrated. The ashes obtained in the Spanish pilot are very rich in phosphorus (6.8%) and potassium (6.1%). However, the phosphorus values previously reported for combusted solid fraction of pig manures (Thygesen and Johnsen, 2012; Christel et al., 2014) and digestates (Thygesen and Johnsen, 2012) are normally at a higher range (10-12%) (Table 5-7). In the case of sewage sludge, usually the phosphorus content is not reported to be as rich (Li et al., 2017; Fang et al., 2018), although several technological advancements have been able to obtain phosphorus enriched (up to 10-15% of phosphorus) ashes from sewage sludge (Egle et al., 2015; Herzel et al., 2016).

As it was clarified before, the extensive use of zinc and copper as veterinary additives in animal feed are responsible for the presence of such metals in the products. Although the values are highly variable among different batches, the strong concentration effect of combustion makes the ashes obtained reach in average very high zinc (>3600 mg/kg DM) and copper (>600 mg/kg DM) concentrations, in the range of the values reported before (Thygesen and Johnsen, 2012; Christel et al., 2014). Heavy metal concentrations are only reported for sewage sludge ashes. They are significantly lower in the combusted biodried solid fraction of pig slurry (Li et al., 3017; Fang et al., 2018) than in sewage sludge ashes. However again, there were several technological industrial approaches (Ashdec ®, Reco-Phos ®, etc.) developed to significantly reduce the content of heavy metals in the ashes (Egle et al., 2015).

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Table 5-7. Comparison between phosphorus rich ash obtained in the Spanish pilot with previous studies

Reference	Spanish pilot	Christel et al., 2014	Thygesen and Johnsen, 2012			Li et al., 2017	Fang et al., 2018	Egle et al., 2015	Herzel et al., 2016
Feedstock/processing	Combustion of biodried solid fraction of pig slurry	Solid fraction of pig slurry, combustion at different temperatures (400.600ºC)	Combusted pig digestate fibers	Combusted pig manure	Combusted cattle manure	Sewage sludge incineration ash (industrial)	Sewage sludge incineration ash (industrial)	Sewage sludge ash obtained from different technologies	Sewage sludge ash
pH	11.9 (n=1)					8.45			
EC (mS/cm)	6.35 (n=1)								
density (kg/L)	-								
DM (g/kg)	997 ± 5.7 (n=3)								
OM (g/kg)	-								
Org C (g/kg)	27 (n=1)	47-128.9							
N (g/kg)	1.4 (n=1)	4.9-21.8							
TP (g/kg)	68.0±6.9 (n=3)	99.2-118.5	112±7.5	123±3.1	51±10	53.59	40.46	85-166	93.7±3.16
TK (g/kg)	61.6±21.3 (n=3)					23			15±1.28
S (g/kg)	14.2±5.3 (n=3)					13.4	16.3		8.9±0.44
Ca (g/kg)	170.9± 26.1(n=3)					45.3	69.5	30-170	114±7.52
Mg (g/kg)	36.4±6.8 (n=3)						10.01		16.4±0.93
Na (g/kg)	28.2±8.5 (n=3)						17.95		5.3±0.46
Zn (mg/kg DM)	3,632± 2,308(n=1)		2,632.5±254	4,293±497	959	5,703	6,186	21-1,950	2,330±29
Cu (mg/kg DM)	648.7± 171 (n=2)		785.5±179.3	1,269±190	362	1,118	1,917	29-664	767±43
Cd (mg/kg DM)	0.15±0.14 (n=2)					< QL		0.07-3.2	2.1±0.2
Hg (mg/kg DM)	0.10± 0.14 (n=2)							<0.1-0.7	1.1±0.1
Pb (mg/kg DM)	15.5±21.6 (n=2)							3.4-120	122.6±0.8
Cr (mg/kg DM)	107.5± 21.9(n=2)		360.3±15.6	519.6±66.9	19	753		22-118	159±11
Cr VI (mg/kg DM)	<0.1 (n=2)								
Ni (mg/kg DM)	81±20 (n=2)		40.35	65.14	21.2		314.3	0.2-75	73.3±3
As (mg/kg DM)	1.87± 1.23 (n=2)						151.5	9-28	11.1±0.4
Fe (mg/kg)	55.9						314.3		58,500±3390
Mn (mg/kg)	2.0					0.85			1,190±22
Al (mg/kg)	17.3		16.8±12.7	2.8±0.2	3.4±0.16	76422	62873		67,200±4680

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Phosphoric acid *from Spanish pilot*

The ashes obtained from the combustion of the biodried solid fraction of pig slurry were treated by acidic treatment to obtain a phosphorus rich leachate or extract that could be regarded as a mineral fertiliser analogous to phosphoric acid. From the upscaled trials assessed, a product with a 1.1% of phosphorus was obtained (2.5% reported as P2O5). It should be mentioned that after the extraction process, a formation of gel was identified, probably due to a very low ash to acid ratio used in the extraction. This phenomenon was already reported as an issue for the Eco-Phos® process, together with the high viscosity of the supernatant to be filtered (Prayon webpage, 2023).

Very few works on phosphorus extraction on ashes report a rather complete characterisation of ash extracts and they are only limited to the use of sewage sludge ashes (Donatello et al., 2010; Gorazda et al., 2012). Only Gorazda et al. (2012) reached a 3% of phosphorus in their extracted product (Table 5-8). In this case, the ash to acid ratio used was lower (1 to 2.5) than ours (1 to 5) and they achieved very good phosphorus recovery (up to 95%) conversely to us (around 22% of P recovery).

Table 5-8. Comparison between phosphoric acid obtained in the Spanish pilot with previous studies

Reference	Spanish pilot	Donatello et al., 2010	Gorazda et al., 2012	Weigland et al., 2013	
Feedstock/processing	Ash from the combustion of biodried solid fraction of pig slurry	Sewage sludge ash (extraction with H2SO4)	Sewage sludge ash (extraction with HNO3)	RecoPhos product	TSP
pH	2.61 (n=1)				
EC (mS/cm)	0				
density (kg/L)	-				
DM (g/kg)	10.4 (n=1)				
OM (g/kg)	-				
Org C (g/kg)	-				
N (g/kg)	-				
TP (g/kg)	11.337 (n=1)	3.1-3.4	31.8	176 ± 11	174
TK (g/kg)	7.465 (n=1)				
S (g/kg)	13.17 (n=1)	0.31			
Ca (g/kg)	0.68 (n=1)	0.434	43.5		
Mg (g/kg)	6.53 (n=1)	0.372	4.6		
Na (g/kg)	4.86 (n=1)				
Zn (g/kg DM)	1,956 (n=1)	21.4	820	1,580±278	439
Cu (g/kg DM)	171 (n=1)		113	663±31.5	36.5
Cd (g/kg DM)	< 0.5 (n=1)		2.2	2.16±0.25	20.0
Hg (g/kg DM)	< 0.4 (n=1)			0.7±0.15	<0.05
Pb (g/kg DM)	< 5 (n=1)		19	51.4±6.55	55.1
Cr (g/kg DM)	< 10 (n=1)		5.6	118±24.9	120
Cr VI (g/kg DM)	< 0.5 (n=1)			<0.01	<0.01
Ni (g/kg DM)	49.9 (n=1)		6.1		
As (g/kg DM)	3.6 (n=1)			9.1±1.82	8.30

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Therefore, if the optimisation of the phosphorus extraction process from manure-derived ashes is performed it seems feasible to obtain to an even more attractive mineral fertilizing product. Moreover, other post-treatment steps could be effective to obtain a marketable product. For instance, the Reco-Phos® process is able to obtain a recovered solid product (after precipitation) equivalent in terms of phosphorus content (around 17% of phosphorus) to the TSP fertiliser (Weighland et al., 2013).

In terms of heavy metals and impurities, they are relevant in our product (mainly zinc and copper and in a lower extent, nickel or sodium). Donatello et al., (2010) added a purification step in which they were able to obtain an extracted product with higher quality. That type of approach could be implemented also to obtain a more attractive product. It is worth mentioning that the heavy metals content, except for copper, reported for the solid product obtained in Reco-Phos® and TSP are markedly higher than in our phosphoric acid. Therefore, a post-treatment stage of phosphoric acid based on precipitation should lead to a product with very attractive characteristics even in terms of safety.

5.4. Biostimulants

Both protein hydrolysates and amino acids represent a major category within plant biostimulants and are extensively used in sustainable agricultural practices (Bulgari et al., 2019). Protein hydrolysates are 'mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis' (Schaafsma, 2009). Protein hydrolysates are mainly produced by chemical (acid and alkaline hydrolysis), thermal and enzymatic hydrolysis of a wide range of both animal and vegetal sources, The high protein content of microalgae makes them a potential candidate for protein extraction being a promising feedstock for biostimulant production. Since the conceptualization of microalgae biorefinery, various researchers have investigated its feasibility for extracting different metabolites from many algal species. For biostimuant production, proteins must be released as amino acids, either through chemical or enzymatic hydrolysis. Enzymatic hydrolysis, however, is preferred because according to Romero García et al. (2012), the process is more effective, and the L-amino acids are better preserved. Several authors have reported *Scenedesmus sp* biomass hydrolysis to produce protein hydrolysates. Most of them, however, only evaluate protein hydrolysis grade (percentage of total protein hydrolysed into free-AA), reporting values from 48 to 65% (Romero García et al. (2012), Akberi et al (2019)) (Table 5-9). Composition of final hydrolysate and in particular, the protein and free-AA content of final hydrolysates are not reported. These values are in range with the ones found within the project with *Scenedesmus obliquus*, where a hydrolysis degree of about 48% was achieved. However, it has to highlighted that the other authors applied enzyme concentrations (Alcalase and Flavourzyme) higher than those used in the project, so that can affect significantly in the final hydrolysis degree. Nevertheless, to make process more sustainable and economically feasible, using low enzymes concentration is desirable.

Table 5-9. Comparison between project and literature of hydrolysis grade of Scenedesmus sp achieved by similar enzymatic processes

Reference	Spanish pilot	García et al. (2012)	Elvira Navarro et al (2020)	Akaberi et al (2019)
Processing (hydrolytic process)	Alcalase 2%+Flavourzyme 1%	Alcalse 4%+ Flavourzyme 5% w/w	Alcalase 4% fFlavourzyme 5% w/w	Alcalase 3% w/w and flavourzym 3% w/w
Hydrolysis grade %	48%	60%	57-65%	48%

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In addition, some authors have evaluated the effect of microalgae biomass concentration on the e enzymatic hydrolysis yield. Romero Garcia et al, reported that when the biomass concentration increases over 200 g/l, the hydrolysis degree drops from 55% at 200 g/l to 20% at 350 g/l. Consequently, the free-amino-acids concentration obtained is lower when the biomass concentration is increased. These data indicates that, despite the highest protein concentration in the culture when the biomass concentration is higher, the reduction in the yield of the enzymatic hydrolysis predominates, resulting in a lower final concentration of free-amino-acids when biomass concentration increases. These results are in line with those obtained within the project where microalgae solution of 100 to 110g/l has been used to ensure the maximum process yield.

The most commercial biostimulants containing protein hydrolysates use animal wastes or plant biomass as raw material treated by chemical (acid/alkali), thermal or enzymatic hydrolysis. They are produced in Spain, Italy, USA, India and China (Calvo et al., 2014).) and they are available as liquid extracts or in soluble powder and granular form, and may be side-dressed near the root or applied as foliar sprays (Colla et al., 2015a). Regarding to algal-based biostimulant, *Ascophyllum nodosum* (macroalgae) and *Arthosphira platensis* (microalgae) are main algae species used in commercial biostimulants. Regarding to their nitrogen and free-AA content, their total concentration of nitrogen ranges from 8 to 12 g/L and the content pf free amino acids is around 2%(w/w) in all cases (Table 5-10). Comparing with the biostimulant produced within the project, lower nitrogen (4,5g/L) and free-AA (14g/L) content were achieved. Most of the commercial products are rich in Leucine and Alanine which is in line with the composition of produced biostimulants. However, it has to be headlined that every algae specie has its own behaviour in front of hydrolytic conditions and has different initial composition. Besides, biostimulant produced in the project has a dry matter of only 6,5%. No data is reported about dry matter content of commercial biostimulant, so that makes difficult the comparison. In addition, some commercial biostimulants are a mixture of algae with other raw materials, such as, vegetal hydrolysates and/or micronutrients which helps to increase the nitrogen and free-AA content in final product.

Table 5-10. Comparison of biostimulant produced in the Spanish pilot with commercial biostimulants:

Reference	Spanish pilot	AgriAlgae Estrés (Algaenergy).	AgriAlgae Foliar (Algaenergy).	Mucigel ® (Promisol)	Spiragro (Neoalgae)	Spiragro floración (Neoalgae)
Biostimulant description	Liquid Biostimulant produced by enzymatic hydrolysis of microalgae	Liquid biostimulant from microalage	Liquid biostimulant from microalage	Liquid biostimulant from microalgae. External free- AA addition from hydrolysis of vegetal proteins.	Product produced from enzymatic hydrolysis of Spirulina	Product produced from enzymatic hydrolysis of Spirulina
Microalgae source	*Scenedesmus obliquus*	*Ascophyllum nodosum*	*Ascophyllum nodosum*	*Ascophyllum nodosum.*	*Arthorspira platensis*	*Arthorspira platensis*
Total N content	4.5 g/L	12.0g/L	12.0g/L	10.0g/L	12,5 g/L	10,0g/L
Free-A content	14 g/L	20 g/L	20 g/L	20.0g/L	21.0g/L	21.0g/L
Main Free-AA type	Leucine, Alanine Valine	Glutamic acid, Leucine, Alanine	Glutamic acid, Leucine, Alanine	No data	Leucine, Alanine	Leucine, Alanine

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5.5. ARGs in poultry and effect on soil

Slurry is the main source of the introduction of ARGs in soil, groundwater and surface water (Chee-Sanford et al., 2009). Tetracyclines, sulfonamides, macrolides and fluoroquinolones as well as multiresistant E. coli and several ARGs (tet, erm and sul genes) have frequently been detected in manure of pigs (Huygens et al., 2021; Rasschaert et al., 2020; Filippitzi et al., 2019; Van den Meersche et al., 2020; Patterson et al., 2007).

The use of tetracycline in food animal production has increase in recent years and is widely used in therapeutic treatment against infections in pigs. Tetracyclines are excreted as active compounds via faeces and urine and can be detected in slurry (Nielsen and Gyrd-Hansen, 1996; Sengeløv et al., 2003). Therefore, tetracyclines enter the soil environment primarily via slurry used as fertiliser. Moreover, accumulation of tetracycline in soil may pose a risk to the environment (Hamscher et al., 2002).

Erm() gene, macrolide-resistance genes, are frequently observed in *Streptococcaceae* (Ellabaan et al., 2021). Macrolides are broad-spectrum antibiotic. In soil, the most abundant ARG was the oleandomycin resistance gene. Most genes identified in the soil environment encoded resistance against macrolide antibiotics. The most widespread macrolide-resistant gene within this environment was ole(), which can be found in soil types including agriculture, desert, forest, grasslands, garden, permafrost, and soils, both with and without crop. Macrolide resistance genes have also been found to be most prevalent in poultry. The most widespread gene within poultry metagenomes is the erm() gene. Moreover, from literature we find that another of the most identified class is tetracycline resistance genes. The ole() gene confers resistance against oleandomycin through the production of a ribosome protection protein (Kerr et al., 2005). Oleandomycin is an active ingredient of a last resort drug used to treat mastitis in livestock where other treatments have failed.

6. Conclusions

Deliverable 2.5 (BBFs production and characterisation vs. time – list, average composition and composition variability) reported the production amount and quality assessment of BBFs produced from the five FERTIMANURE pilot plants. It can be observed that the production is slightly lower in almost all cases than the reported in Deliverable 2.2 as the first data reported are based on literature or theoretical installed capacity. Data reported in this D2.5 are more accurate as they are based on the live pilot plant continuous operation conditions at optimised conditions, and results.

This current deliverable reports the characterisation and quantity of the BBFs that have been used to produce TMFs (WP3) and experimental assessment of BBFs and TMFs (WP4). A total of 18 BBFs have been produced from the pilot plants, as introduced previously and summarised in Table 6-1.

Considering all BBFs, the majority are minerals BBFs (13), followed by organic amendments (4) and biostimulants (1). The largest part of mineral BBFs are N-fertilisers (mainly ammonium sulphate or ammonium nitrate solutions), followed by P and K fertilisers.

The understanding of the general characteristics of the BBFs has been crucial for WP3 and WP4 activities planning:

- TMFs will be formulated considering the characteristics of the BBFs in order to comply with the requirements of farmers, crops and soils.
- Assessment of the quality of BBFs through incubation, field and pot trials will be planned based on the priority characterisation provided by work package 2.

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Table 6-1 Summary of BBFs from FERTIMANURE pilots

Type of BBF	Product form	Amount	List
N	Liquid	6	Ammonium sulphate; Ammonium nitrate; Ammonium salts; Ammonium water
P	Semi-solid	1	Wet organic P-rich fertiliser
	Solid	2	P ashes; Dried organic P-rich fertiliser
K	Liquid	2	K fertiliser
NP	Solid	1	Mono ammonium phosphate
NPK	Liquid	1	Nutrient-rich concentrate
Organic amendments	Solid	4	Biochar; Soil conditioner; Biodried solid fraction
Biostimulants	Liquid	1	AA-biostimulant

The content of this deliverable is complemented with the data provided in deliverable 2.6 regarding mass and energy balances. The main outcome of this deliverable is that most o the FERTIMANURE BBFs accomplishes what is stablished in the European Regulation of Fertilizing products, thus can be potentially commercialised. Deeper studies regarding the marketability and business cases of produced fertilisers are expected within WP6.

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Annexes

LIST OF UNITS

°C	Celsius degree
µm	micrometer
g	gram
h	hour
kg	kilogram
L	liter
MJ	Mega Joule
m^3	cubic meter
mg	milligram
s	second
tonne	tonne
year	year

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FERTIMANURE

INNOVATIVE NUTRIENT RECOVERY FROM SECONDARY SOURCES-PRODUCTION OF HIGH-ADDED VALUE FERTILISERS FROM ANIMAL MANURE

PROJECT COORDINATOR

Fundació Universitària Balmes (Spain)

CONSORTIUM

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Wageningen Environmental Research (The Netherlands)
University of Milan (Italy)
Leitat (Spain)
GreenWin (Belgium)
European Landowners Organisation (Belgium)
IPS Konzalting (Croatia)
Fraunhofer (Germany)
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French Chamber of Agriculture (France)
Cooperativa Plana de Vic (Spain)
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RITTMO Agroenvironnement (France)
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PROJECT WEBSITE:

https://www.fertimanure.eu

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7. Brief project summary

The mission of the FERTIMANURE project is to provide innovative solutions (technology, end-products, and business models) that solve real issues, i.e. the manure challenge, and help farmers with the challenges that they are currently facing. FERTIMANURE will develop, integrate, test and validate innovative nutrient management strategies so as to efficiently recover and reuse nutrients and other products with agronomic value from manure, to ultimately obtain reliable and safe fertilisers that can compete in the EU fertiliser market.

The FERTIMANURE project will cover both technological and nutrient management approaches. The technological side will be addressed with the implementation of 5 innovative and integrated on-farm experimental pilots for nutrient recovery in the most relevant European countries in terms of livestock production (Spain, France, Germany, Belgium, The Netherlands), whereas nutrient management will be addressed through 3 different strategies adapted to mixed and specialised farming systems:

Strategy #1 with on-farm production and use of bio-based fertilisers (BBF)(1) , **Strategy #2** with on-farm BBF production and centralised tailor-made fertilisers (TMF)(2) production, and **Strategy #3** with on-farm TMF production and use.

Definition of Bio-based fertilisers (BBFs): *Bio-based fertilisers (BBFs) are fertilising products or a component to be used in the production of (Tailor-Made) Fertilisers that are derived* ***from biomass-related resources.***

The BBFs of FERTIMANURE are "obtained through a ***physical, thermal/thermo-chemical, chemical, and/or biological processes for the treatment*** *of manure or digestate that result into a change in composition due to a change in concentration of nutrients and their ratios compared to the input material(s) in order to get better marketable products providing farmers with nutrients of sufficient quality".*

However, just separation of manure in a solid and liquid fraction (as first processing step) is excluded. These products are not conceived as a BBF, although they are valuable sources to supply nutrients on agricultural land.

LIST OF BBFs Produced in FERTIMANURE

Number	BBF-code	BBF product description
1	NL-AS	Ammonium sulphate solution
2	NL-LK	Liquid K-fertiliser
3	NL-SC	Soil conditioner
4	NL-WP	Wet organic P-rich fertiliser
5	NL-DP	*90% dried organic P rich fertiliser (calc)*
6	ES-NC	Nutrient-rich concentrate
7	ES-DSC	Bio-dried solid fraction
8	ES-PA	Phosphorous (ashes)
9	ES-AM	Ammonium salts
10	ES-AA	AA-based biostimulants
11	DE-BC	Biochar (solid)
12	DE-AP	Ammonium phosphate on perlite (solid)
13	BE-AN	Ammonium nitrate
14	BE-AS	Ammonium sulphate
15	BE-AW	Ammonium water
16	FR-BC	Biochar
17	FR-AS	Ammonium sulphate
18	FR-LK	Liquid K-fertiliser

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Definition of Tailor-Made Fertilisers (TMFs): *A tailor-made fertiliser (TMF) is a customized fertiliser that meets with the nutrient requirements of a specific crop by taking into account the soil type, soil fertility status, and growing conditions and fertilisation practises.*

The TMFs obtained in FERTIMANURE are produced from BBFs (produced from manure or digestate and/or other recovered fertilising products that are available) and/or mineral fertilisers (MF) (and/or biostimulants).

Fully crop specific TMFs can be defined and centrally produced assuming e.g. a sufficient nutrient status of a soil type and no additional fertilisation practice.

However, on farm level the soil-crop requirements will be different due to another nutrient status of the soil and the fact that often manure/digestate will be applied on the fields which has to be taken into account as nutrient supplier. Consequently, the composition of the TMF (combination of BBF and MF) that will be used by the farmer can differ from the one produced in a centralised way.

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