WaysTUP!

VALUE CHAINS FOR DISRUPTIVE TRANSFORMATION OF URBAN BIOWASTE INTO BIOBASED PRODUCTS IN THE CITY CONTEXT

D1.1: Laboratory analysis protocol and methodology for executing WAC

WP1 – Identification of opportunities and barriers to utilisation of urban biowaste sources

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Executive summary

This is Deliverable D1.1 of the WaysTUP! "Value chains for disruptive transformation of urban biowaste into biobased products in the city context" project which documents the progress of Task 1.1 "Systematic characterization of urban biowaste". The objective of this deliverable is to report on the development of a representative methodology for the conduction of Waste Analysis Campaigns (WACs) of all different types of urban biowaste that will be used as feedstocks for the biobased products within WaysTUP!. These include: meat waste, fish waste, coffee waste, source separated biowaste, used cooking oils, coffee oil from spent coffee grounds, cellulosic rejections of MSWTP, nappies, carton and paper rejections of MSW, sewage sludge, olive oil mill waste and sawdust. In order to set up this methodology, a questionnaire was constructed and all relative partners were contacted. Valid responses were received by the submission deadline.

Based on the questionnaires' responses and the state-of-the-art standards for the sampling and characterization of waste, the methodology for setting up a WAC is proposed suggesting strict sampling plans, sampling procedures and preservation times in order to ensure that the outcomes of the samplings are representative. This report also includes a common protocol for laboratory analysis for the detailed physicochemical characterization of urban biowaste according to the needs and specifications of each pilot plant and feedstock. The laboratory protocol includes the methods of typical analyses that must be performed according to the type (solid, oil etc.) of samples. Guidelines for the uniform representation of results are also included indicating that the sampling records must also accompany the characterization reports.

1. Introduction

The main scope of this report is to develop a methodology for conducting the Waste Analysis Campaigns (WACs) as well as a common protocol for laboratory physicochemical characteristics that will be applied in order to effectively map the composition of urban biowaste that will be valorized within WaysTUP!.

Since one of the main objectives of WaysTUP! is to demonstrate a diverse range of technologies for urban biowaste utilization (7 Pilot plants), resulting in new end products and value chains, the knowledge of their intrinsic characteristics, especially with respect to evaluating their response to various treatments, and their potential impacts on the environment is essential.

Urban biowaste to be valorized arise in a wide variety of types and sampling situations (e.g. during a waste production process, from stockpiles, tanks, drums). More specifically, feedstocks will include:

- Meat and fish by-products and spent coffee grounds (SCG) from Valencia Region, Spain (SAV, VAL);
- SCG from London, UK (BIO-BEAN);
- Source separated biowaste from households located in Attica Region, Greece (SUST, NTUA) and from Valencia Region, Spain (SAV, VAL);
- Used cooking oils from restaurants and canteens (NFG),
- Cellulosic rejection material from MSW and WWTP from Metropolitan Area of Barcelona (AMB), and
- Sewage sludge from WWTP at Crete, Greece (TUC).

Because of their variability, instability, and widely contrasting compositions, it is of prime importance to specify the procedural steps to be taken in the preparation and application of appropriate sampling plans, taking into account:

■ Strong heterogeneities;

■ Occasional complex behaviors during the sampling and preparation stages, due to instability or other physicochemical characteristics;

■ The result of a specific production process, which may be necessary to consider for a proper sampling strategy.

Thus, this deliverable provides a framework that can be used to produce standardized WACs for use under routine circumstances, with a view also to be able to incorporate specific sampling requirements demanded by European or national legislations, and finally to design and develop sampling plans for use on a case-by-case basis.

Furthermore, the present deliverable develops a common laboratory protocol including the methods of typical analyses that must be performed according to the type of WaysTUP! feedstocks.

2. Methodology and overall concept

In order to obtain accurate and representative results, a methodology for WACs needs to be set up before the first sample is taken. The potential scope of an overall WAC may be complex, and for that it would be useful to identify its essential elements. Figure 1 presents the overall concept of D1.1 and defines 7 discrete key steps.

Step 1. Sampling plan: Initially appropriate sampling methods need to be selected or designed, that will ensure representative samples.

Step 2. Field sample extraction: According to the sampling plan, field samples are taken and are converted to laboratory samples after pretreatment, if necessary. The whole procedure is recorded.

Step 3. Delivery to laboratory: The laboratory samples are transferred to the assigned laboratory taking all necessary precautions. Procedures predefined for sample packaging, storage, preservation, transport and delivery should be followed.

Step 4. Test sample preparation: The samples are prepared for analysis. The preparation procedures are defined by the parameters to be measured.

Step 5. Extraction: For certain analysis, an additional extraction procedure should be followed as defined by standard methods.

Step 6. Analysis: According to standard methods, analysis of feedstocks takes place.

Step 7. Measurement report: Data analysis is evaluated and an overall measurement report is produced.

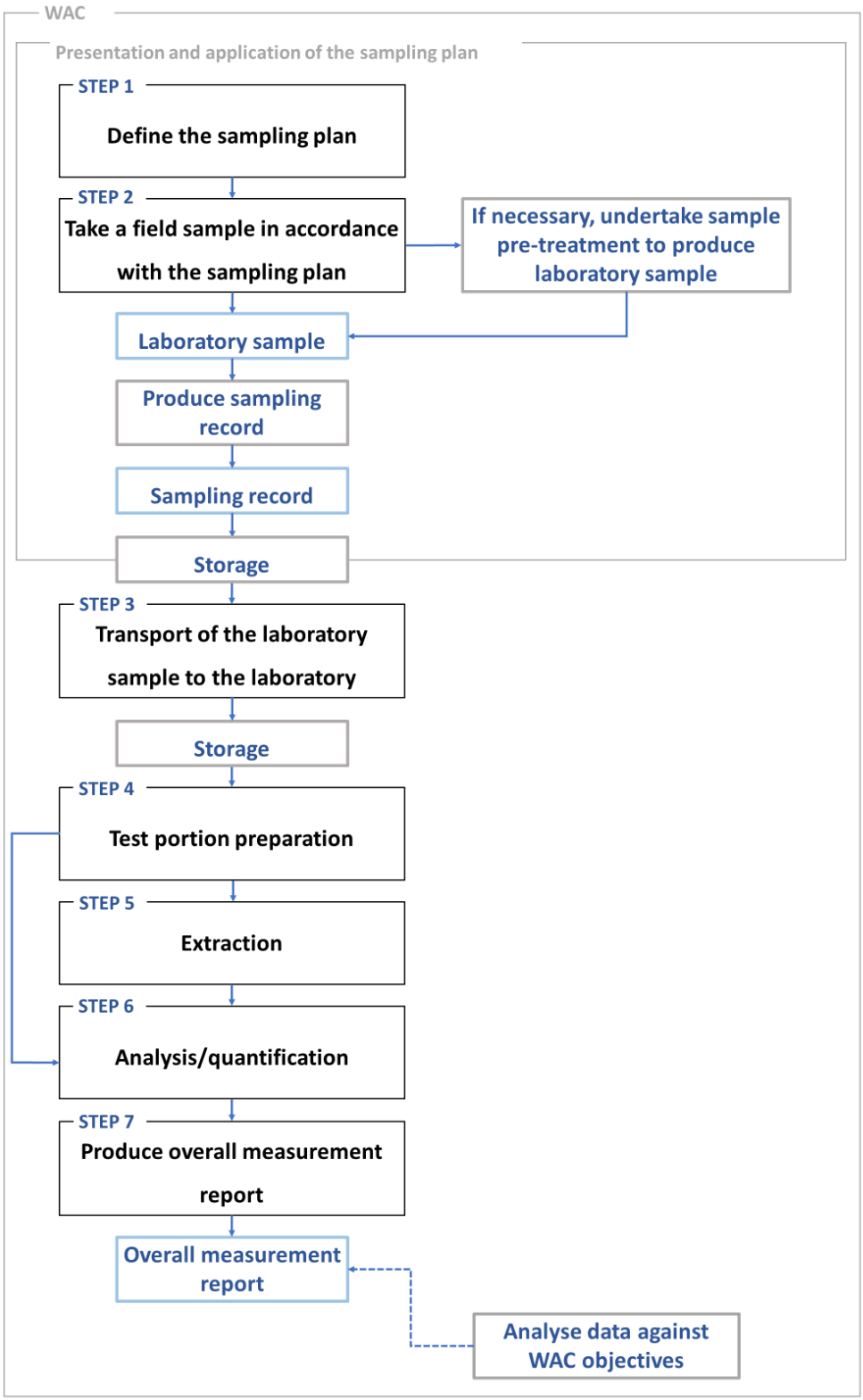

Figure 1. Overall concept of the methodology for executing WACs

The methodology for executing WACs that will be described in detail in the following chapters will also be in line with all relevant standard documents on "characterization of waste – Sampling of waste materials" such as:

EN 14899: Framework for the preparation and application of a sampling plan;

CEN/TR 15310-1:2006: Guidance on selection and application of criteria for sampling under various conditions;

CEN/TR 15310-2:2006: Guidance on sampling techniques;

CEN/TR 15310-3:2006: Guidance on procedures for sub-sampling in the field;

CEN/TR 15310-4:2006: Guidance on procedures for sample packaging, storage, preservation, transport and delivery;

CEN/TR 15310-5:2006: Guidance on the process of defining the sampling plan.

In order to tailor the developed methodology to the needs of WaysTUP!, the following crucial points were adressed by the involved partners via a questionnaire (Annex 1):

- Source of feedstock (Industries, WWTP, Established Collection Network, New Developed Collection Network)
- Protocols and methodologies already adopted
- Quality characteristics necessary for each process
- Laboratory analysis methods already used.

3. Waste Analysis Campaigns

A Waste Analysis Campaign (WAC) includes all the procedures that need to be followed in order to end up to a successful feedstock characterization. The characterization must be representative of the wastes physical and chemical properties and consider any variability in the WaysTUP! feedstock. According to the objectives of a WAC, a sampling plan should be designed and developed for use on a case-by-case basis prior by providing specific and practical instructions to the sampler.

3.1. Sampling plan

A sampling plan is defined as "all the information pertinent to a particular sampling activity". The sampling plan includes the taking of the sample, the production of a laboratory sample, and the transport (to the laboratory), and may include the storage of the laboratory sample. In other words, a sampling plan shall be completed prior to undertaking any sampling and shall provide the sampler with detailed instructions on how sampling should be carried out. In the process of defining a sampling plan the key elements of the WAC, shown in Figure 2, shall be addressed.

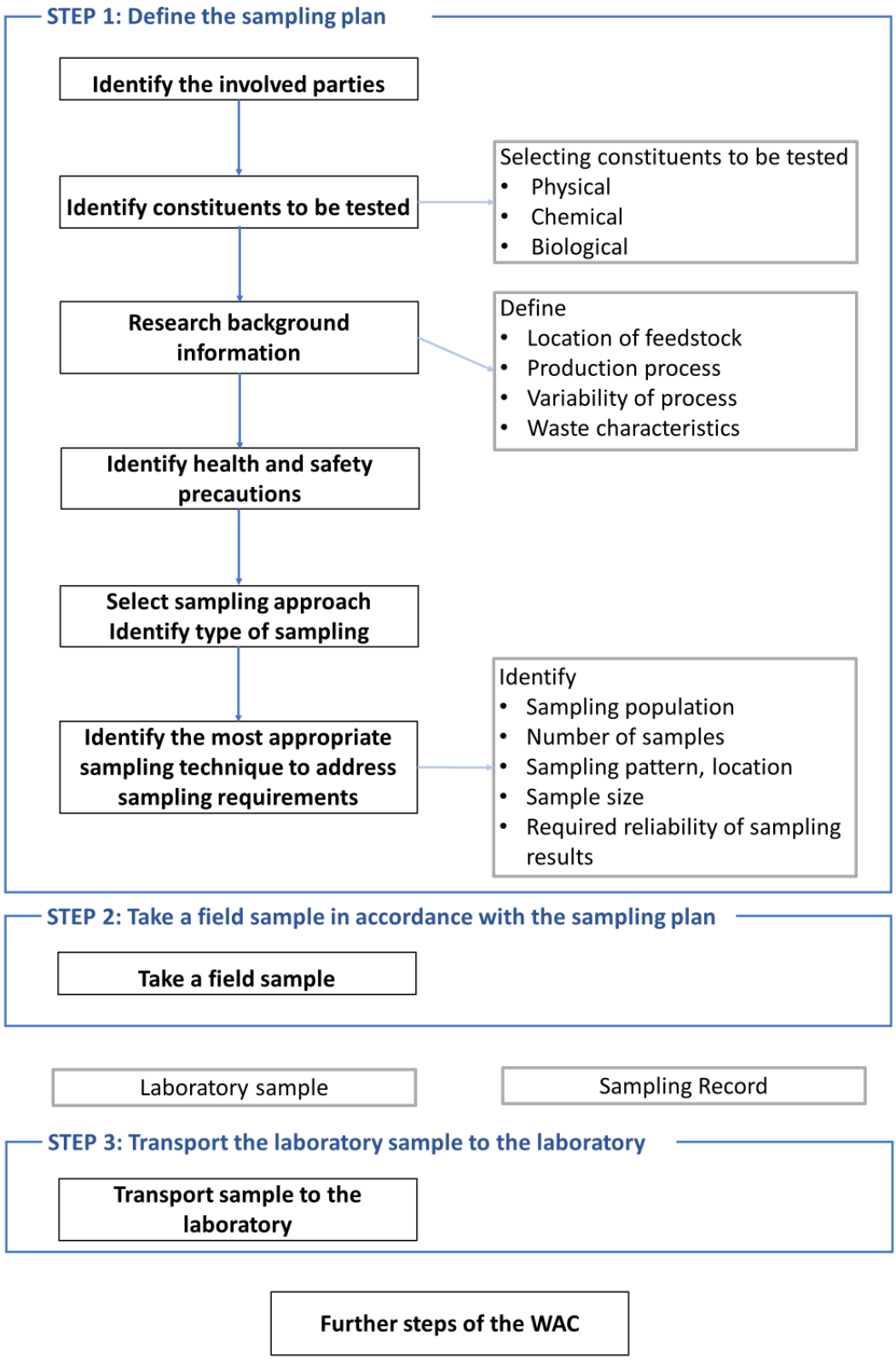

Figure 2. Key elements of sampling methodology of WAC

Within WaysTUP!, when defining a sampling plan, the following points must be systematically addressed.

Identification of involved parties

The parties involved may as a minimum be the Feedstock provider and Pilot owner, but usually also the waste producer and manager, the Process partner, the sampler, the laboratory analyst, etc. Table 1 indicates the main involved parties for each feedstock. In view of improving the quality of the WAC, all involved parties should contribute a fair share of their expertise and background.

Table 1. Main involved parties for each WaysTUP! feedstock

Feedstock	Pilot Plant	Feedstock provider	Pilot Owner	Process partner
Meat waste	1 – Food & Feed	SAV	SAV	BIOPOLIS
Fish waste	1 – Food & Feed	SAV	SAV	BIOPOLIS
Coffee waste	1 – Food & Feed	SAV	SAV	BIOPOLIS
Coffee waste	2 – Coffee Oil	BIO-BEAN	BIO-BEAN	BIO-BEAN
Source Separated biowaste	3 – Insect protein	SAV	UA	UA
Meat & Fish waste	3 – Insect protein	SAV	UA	UA
Used Cooking Oils	4 – Bioplastics	NFG, Fabio Produkt	NFG	NFG, NVMT
Coffee oil from Spent coffee grounds	4 – Bioplastics	BIO-BEAN	NFG	NFG
Source Separated biowaste	5 – Biosolvents	SUST	NTUA	NTUA
Cellulosic Rejections of MSWTP	6 – Perseo	WWTP-Besos	IMECAL	IMECAL, Ciemat
Nappies	6 – Perseo	AMB	IMECAL	IMECAL, Ciemat

Carton and paper rejections of MSW	6 – Perseo	AMB	IMECAL	IMECAL, Ciemat
Sewage sludge	7 – Sludge Biochar	Municipal wastewater treatment plant of Chania, Greece	TUC	TUC
Olive Oil mill waste	7 – Sludge Biochar	Olive mill owners, Chania, Greece	TUC	TUC
Sawdust	7 – Sludge Biochar	Carpenters, Chania, Greece	TUC	TUC

Identification of constituents to be tested

The sampling plan shall identify the characteristics or components to investigate, taking into account all known information, such as origin of the feedstock; intended valorisation scheme of the feedstock (Pilot plant); total volume of feedstock to be assessed and processed. It is also important to collect background information on the feedstock, in order to identify details of the site location or to define the production process, the variability of the process and the feedstock properties (e.g. type and flow rate). Special attention should be paid on assessing the human health and / or environmental risks posed by each test material.

Selection of the most suitable sampling approach

The sampling plan shall take into account the variability within the lot, or the population and/or sub-population. In addition, the sampling plan shall identify when, where, by whom and how samples shall be taken and collected to ensure that the sample is appropriate to meet the sampling objectives of WAC.

At this stage, it is important:

- To define the lot/population to be sampled. The lot/population is defined as the total amount (volume or mass) of feedstock about which information is required through sampling.
- To identify the scale that defines the volume or the mass of waste material that a sample shall represent taking into account its heterogeneity.
- To choose the desired reliability of the sampling approach, mainly in terms of "confidence interval" and precision.

In general, the sampling plan shall specify either 'probabilistic' sampling, which ensures that each unit within the population being sampled has an equal chance of being sampled, or

'judgmental' sampling when representative sampling is not possible due to the heterogenic character. Within WaysTUP!, a probabilistic sampling approach is recommended.

The sampling approach should at least include:

- The increment size, representing the amount of feedstock (mass or volume) that is obtained through one single sampling action. An increment is not analysed as an individual unit, but is combined with other increments to form a composite sample.
- The sample size.
- The use of composite or individual samples.
- The required number of samples.
- The sampling location.
- The sampling frequency.

Identification of sampling technique

The sampling plan shall identify the technique(s) selected to collect the samples of feedstock, and shall identify the consequences of deviation from the designated sampling technique or equipment. Any requirements for the production of composite samples from incremental samples and for sub-sampling in the field shall also be identified, along with the procedure(s) selected for packaging, preservation, storage, and transport of the laboratory samples.

3.1.1. Sample size

The appropriate number of samples to collect is the least number required to generate a sufficiently precise estimate of the true mean concentration of a chemical component of a feedstock. It is always prudent to collect a greater number of samples than indicated by preliminary estimates.

As heterogeneity increases, the number of sub-samples should be increased. If insufficient numbers of samples are collected, analytical results will not represent the feedstock in question.

The number of samples to be collected is calculated based on statistical criteria defined by the WACs.

The number of samples (n) required to achieve a desired level of measurement precision is a function of the component(s) under consideration and the confidence level (ASTM, D 5231 – 92). The governing equation for n is as follows:

$$n = \left(\frac{t^* \cdot s}{e \cdot \bar{x}}\right)^2$$ Equation (1)

where:

t* = student t statistic corresponding to the desired level of confidence,

s = estimated standard deviation,

e = desired level of precision, and

$\bar{x}$= estimated mean.

All numerical values for the symbols are in decimal notation. For example, a precision value (e) of 20% is represented as 0.2.

Suggested values of s and $\bar{x}$ for food waste are 0.03 and 0.10 respectively. For 95% level of confidence, from equation (1), it may be estimated that a minimum of 15 samples should be collected for each sampling.

3.1.2. Sampling pattern

A representative sample defines a material's average characteristic, typical for the entire material being sampled. A representative sample of raw feedstock is not easily obtained; and sampling must be repeated over time to compensate for naturally high variations.

The main types of sample collection that may be used within WaysTUP! are the following (Figure 3):

Point sampling: site-specific sample collection from within the general mass is used to identify and quantify points of extreme variability, hot spots or problem zones. Point-sampling alone should not be used unless special conditions exist.

Composite-sampling: a single sample for laboratory analysis composed of multiple, well-blended point- or sub-samples uniformly distributed throughout the entire volume that, after mixing, accurately represents an average or median value of the property or trait of interest for a batch or general mass. Properly implemented composite sampling is preferable for most sampling plans. In most cases, composite sampling is satisfactory when the amount of variability within the mass is known to be insignificant.

Stratified sampling: A modified composite sampling scheme is used to document gradients and define heterogeneity as a function of position within the bulk or general mass of sampled material, where the general mass is subdivided into separate zones and a series of point-samples are collected and composited within each zone. Stratified sampling should be used

when heterogeneity of raw material is unknown and when regulatory constraints require knowledge of the relative spatial and temporal variability. This is most often based upon the standard deviation and mean.

Interval sampling: Sampling from moving conveyor belts.

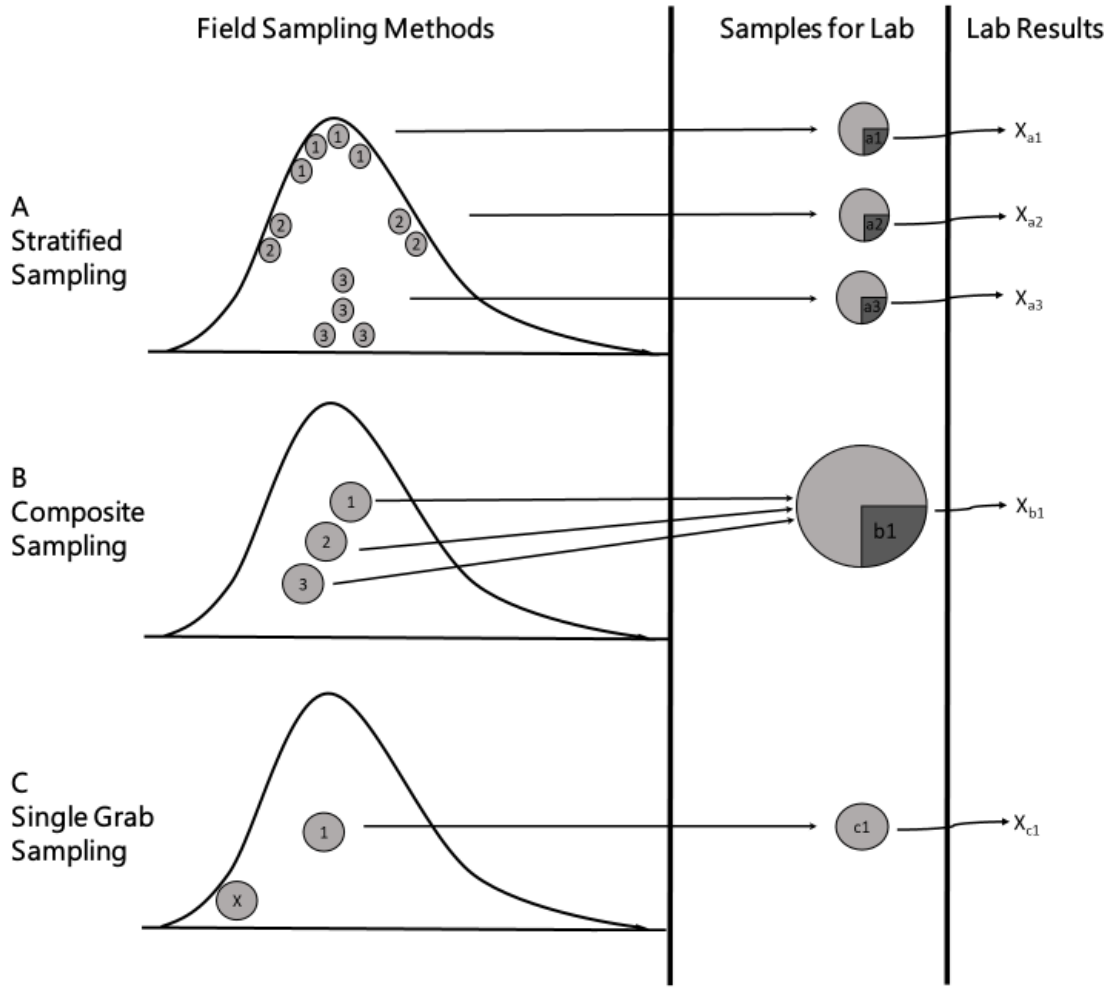

Figure 3. Schematic representation of sampling

For WaysTUP! feedstocks, stratified random sampling is recommended, unless otherwise dictated. In all cases, the recommended minimum quantity for each incremental sample is 8-12% of the total amount of sampled material. The incremental samples must be then mixed thoroughly to create the composite sample. Given the large size of the composite sample it may be necessary to reduce it in order to obtain the final samples using the Sample Division System. To subsample, first divide a sample unit into small portions. Then choose a second sample from these portions in order to achieve a small representative sample. Special attention

should be drawn to the selected sub-sampling technique, especially for the case of large, inhomogeneous samples.

Specific parameters such as seasonality, collection system, socio-economic influences etc. might have an influence on the feedstocks' composition. A case-by-case evaluation should be performed and respective WACs should be planned and executed accordingly. A minimum of 3 WAC per WaysTUP! feedstock (Table 1) should be performed by the associated partners in order to ensure the accuracy of the feedstocks characterization and to meet the performance indicators of WaysTUP!

3.2. Sampling

3.2.1. Sampling procedure

The steps presented below should be followed during the sampling procedure:

1. Sample Collection—Collect an appropriate number of subsamples needed to ensure a reliable analytical result.
2. Place each subsample into a sampling (subsample) container.
3. Transfer the contents of the subsample container onto (into) mixing surface (container) and proceed to the next randomly selected sample point.
4. Repeat steps 2 and 3 until the predetermined number of subsamples is obtained.
5. Sample Mixing—Place subsamples on clean tarp or other similar mixing platform, mix sub-samples thoroughly using a wooden spatula or comparable sampling tool.
6. Sample Splitting—Subdivide sample into quarters, thoroughly mixed composite sample into fourths.
7. Repeat steps 5 and 6 until sample size is appropriate for intended analysis.
8. Sample Storage and Shipping—Place sample in clean container, preferably a Teflon pail or similar inert material.
9. Transfer blended feedstock to fill plastic freezer-safe bags.
10. Line the shipment pail with aluminum foil to minimize heat exchange.
11. Place the plastic freezer-safe bags containing the feedstock samples in the plastic pail and interleave with cold packs for shipping if necessary.
12. Seal the square pail with its lid. Seal and secure lid with duct tape.
13. Send the square plastic pail containing samples to the selected laboratory for analysis as soon as possible but they should be received after no later than 2 days.

3.2.2. Variables that Compromise Quality of Sampling

Sample collection technique may affect the relative accuracy of sampling and the reliability of laboratory analytical determinations. Failure to adjust sampling protocols according to the nature and source of variations may invalidate test results.

3.2.2.1. Bias Introduced by the Sampler

Inaccurate sample collection is often due to systematic or intentionally selective sampling introduced by the sampler. Significant error will result from attempts by the sample collector to counteract perceived variability.

3.2.2.2. Sample Heterogeneity

The following are key sources of non-uniformity that can give rise to significant sampling errors.

- Sub-sample size affects sampling accuracy. In general, a representative composite sample contains large (> 1000 cm^3) and plentiful sub-samples (>12 samples).
- Complete and thorough mixing of raw material -waste
- Soil, stones and/or other impurities may be picked up during sampling. These pose problems for good sampling.
- Varying particle size is one of the most common sources of sample variability.

3.2.3. Sample Handling

Collect samples from areas of the feedstock pile that are representative of the general appearance.

For most feedstock, use containers made of stainless steel, plastic, glass or Teflon. These materials will not change the feedstock's chemical quality. Laboratories provide advice on appropriate sample containers, preservatives and shipping instructions when requested.

A representative sample must be collected from appropriate sampling locations and consists of no less than 15 point-samples.

Determine the number and types of sampling and shipping containers to be used. The composite sample is placed in a sanitized container and thoroughly mixed. Follow proper quality assurance/quality control procedures for sample preservation, storage, transportation and transfer.

Each sample collected should be tagged with a unique identification reference code, capable of use in wet conditions.

Usually, the laboratory test method and analytical parameter of interest dictate the method of sample collection, type of container for shipping and storage of samples and sample handling procedures required. Table 2 provides an indicative list of analytical traits that are affected by sample collection and handling. In general, volatile compounds and elements, physical bulk factors and microbiological samples require special considerations when developing the sampling plan.

Table 2. Indicative list of test parameters that require special sampling and handling considerations

Test Parameter	Principle Constraint	Associated Error	Alteration of Sampling for Collective Action
Total-N	Volatilization loss of NH_3 during sample handling	Underestimation of total N and volatile N	Place in container quickly with minimal stirring
Volatile fatty acids (VFA)	Volatilization loss of VFA during sample handling	Underestimation of VFA content	Place in container quickly with minimal stirring
Microbiology (pathogens)	Contamination from tools, buckets, air	Over or under estimation of pathogens	Use only clean, sterile containers and implements
Bulk Density	Excess sample moisture	Overestimation of volume/weight	Take large, oversized samples

In each case the determination for a trait of interest can be changed adversely by improper sample collection and handling, and consequently lead to erroneous conclusions. Analytical precision or relative variability may not be affected by inappropriate sampling, but accuracy of the expected determination may be biased and incorrect.

For each type of parameter measured after sampling, specific containers and holding times should be observed prior to and during transport to a laboratory (Tables 3- 6). Use as many containers to preserve sample integrity as necessary.

When plastic containers are acceptable, use double freezer-safe plastic bags marked on the exterior with a marking pen with insoluble ink, and placed with several cool-packs in a large polystyrene cooler or similar insulated container.

Ship the samples to the laboratory for delivery within 24 h or less. Request that the laboratory staff store samples at 4°C when delays in lab preparation are anticipated.

Collection and storage of samples for organic compound analysis e.g. volatile fatty acids (VFAs) - require glass containers with Teflon lids, or exclusively Teflon containers. Sample containers should be filled to overflowing with material to minimize airspace in the container and reduce volatilization of organic compounds during storage.

Table 3. Physical Parameters: Sampling containers and conditions for compost and source ingredient testing

Test Parameter of Interest	Container	Conditions	Maximum Holding Time Allowed in Lab
Bulk Density, Hydraulic Conductivity, Porosity, Water Holding Capacity, Total-N	P.G	4°C	7 d
Temperature	NA	NA	Immediate, no delay
Total Solids	P.G	4°C	24 h

P=Plastic, G=Glass

Table 4. Organic and Biological Properties: Sampling containers and conditions for compost and source ingredient testing

Test Parameter of Interest	Container	Conditions	Maximum Holding Time Allowed in Lab
Respirometry	P.G	4°C	24 h
Organic Carbon	P.G	4°C	14 d
Volatile Fatty Acids	G (2L CWM)	4°C	14 d
Volatile Solids	P.G	4°C	14 d

P=Plastic, G=Glass

Table 5. Organic and Biological Properties: Sampling containers and conditions for compost and source ingredient testing

Test Parameter of Interest	Container	Conditions	Maximum Holding Time Allowed in Lab
Acidity/Alkalinity (pH),	P.G	4°C	48 h

Electrical Conductivity, Kjeldahl Nitrogen, Nitrate Nitrogen (NO_3-N), Nitrite Nitrogen (NO_2-N), Ammonia Nitrogen and Ammonium Nitrogen (NH_3-N, NH_4-N). Sulfide			
All other Metals	P.G	4°C	6 months
Chloride, Sulfate	P.G	4°C	28 d
Chromium VI	P.G	4°C	24 h
Mercury	P.G	4°C	28 d

P=Plastic, G=Glass

Table 6. Pathogens: Sampling containers and conditions for compost and source ingredient testing

Test Parameter of Interest	Container	Conditions	Maximum Holding Time Allowed in Lab
Enteric Virus	G	-70°C	> 8 h
Enteric Virus	SP, G	4°C	8 h
Coliforms and other bacteria	SP, G	4°C	48 h
Helminth Ova	SP, G	4°C	1 month

SP = Sterilized Polypropylene, G = Sterilized Glass

3.2.4. Personal protection

The sampling should be performed by persons specially trained to do so. A wide range of personal protection equipment may be needed including specialist equipment requiring training in its use, e.g.:

- Protective/disposable gloves
- Protective clothes
- Respiratory protection: with appropriate filter for organic vapors if necessary

Always wash your hands before and after handling any products!

During the sampling microbiological or other contamination of the sample must be avoided.

Certain waste may retain living microorganisms. Aerosols can be generated and inhaled when handling. Wear a mask if necessary.

3.2.5. Sampling Record

On completion of sampling, a Sampling Record should be completed by the Sampler. An example is provided in Annex 2. In the Sampling Record, the following information should be included:

b) all procedures and observations from the sampling exercise;

c) all variations from the intended Sampling Plan;

d) unique sampling number (e.g. reflect site location, material and date);

e) date and time of sampling;

f) place and point of sampling;

g) persons present (if witnesses are present, including name and address);

h) difficulty of access (obstacles), including information on those areas or volumes of the material that sampled or not sampled;

i) condition of material:

— color;

— consistency/homogeneity/grain size (uniform or diverse);

— observations during sampling (e.g. gassing out, reactions, development of heat, odor);

j) details of on-site determinations (e.g. pH and conductivity measurements);

k) identify sample amount (estimate volume and mass);

l) sub-sampling methodology (recording which samples are mixed, in what volumes, time and date) (if undertaken);

m) name of sampling personnel;

n) place, date and signature.

4. Analysis Protocol

Once a sample enters the laboratory, there are a number of steps needed prior to testing. These pre-examination steps include: • verifying the sample is properly labelled, adequate in quantity, in good condition, and appropriate for the test requested. The test request must be complete and include all necessary information; • recording sample information into a register or log; • enforcing procedures for handling sub-optimum samples, including sample rejection, when necessary.

The laboratory should establish rejection criteria and follow them closely. It is sometimes difficult to reject a sample, but remember that a poor sample will not allow for accurate results. It is the responsibility of the laboratory to enforce its policies on sample rejection so that results accuracy is not compromised. The following are examples of samples that should be rejected: • unlabeled sample; • broken or leaking tube/container; • insufficient sample information; • sample collected in wrong tube/container; • insufficient quantity; • prolonged transport time, or other poor handling during transport.

The laboratory should keep a register (log) of all incoming samples. A master register may be kept, or each specialty laboratory may keep its own sample register. Assign the sample a laboratory identification number – write the number on the sample and the requisition form. The register should include: • date and time of collection; • date and time the sample was received in laboratory; • sample type; • laboratory assigned identification (e.g., number 276_01_06_2009); • tests to be performed.

4.1. Testing and analytical methods

Testing and analytical methods for each parameter must be specified. Analytical methods should be chosen by considering the physical state of each feedstock, analyses of interest, and required detection limits. Sample preparation and clean-up methods should also be specified, if required.

Generally, all testing and analytical methods should be standard methods, such as ASTM, U.S. EPA SW-846 methods, European standards etc. When this is the case, it is sufficient to only reference the method by name, number, and source. However, any changes to the standard methods, or other methods used (e.g., facility specific methods), must be accompanied with a standard operating procedure for the method in the waste analysis plan or the laboratory quality document. Deviations from the methods presented in the WACs should be documented. As a general information basis, Table 7 contains a non-exhaustive list of standards for the characterization of feedstocks within WaysTUP!.

Table 7. List on standards for the characterization of WaysTUP! feedstocks in solid/slurry form

Parameter		Reference	Description
pH	pH	EPA Method 9045D ASTM D4972-19	Soil and Waste pH Standard Test Methods for pH of Soils
Dry Matter/ Moisture	TS	APHA-AWWA-WEF 2540 B. ASTM D2216 - 19	Total solids dried at 103–105°C
Volatile Solids/Ash	VS	APHA-AWWA-WEF 2540 G. ASTM E1755 - 01	Total, Fixed, and Volatile Solids in Solid and Semisolid Samples Standard Test Method for Ash in Biomass
Total Organic Carbon	TOC	APHA-AWWA-WEF 5310 ASTM D6316	Characterisation of waste. Determination of total organic carbon (TOC) in waste, sludges and sediments
Total Kjeldahl Nitrogen	TKN	APHA-AWWA-WEF 4500	The Kjeldahl methods (4500-N_{org}.B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. "Kjeldahl nitrogen" is the sum of organic nitrogen and ammonia nitrogen
Ammonium, Nitrates	NH_4^+, NO_3^-	ISO/TS 14256-1:2003 ISO 7890-1-2- 1986 EPA Method 350.2	The concentrations of the inorganic nitrogen compounds nitrate, nitrite and ammonium in the extracts are determined using spectrophotometric methods.
Olsen Phosphorus	P	ISO 11263, 1994 EPA Method 365.1	Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.
Chlorides	Cl^-,	EPA Method 1312, EPA Method 9038	Thiocyanate ion (SCN) is liberated from mercuric thiocyanate through sequestration of mercury by chloride ion to form un-ionized mercuric chloride. In the presence of ferric ion, the liberated SCN forms highly colored ferric thiocyanate in a concentration proportional to the original chloride concentration.
Sulfates	SO_4^{2-}	EPA Method 1312 EPA Method 9251	Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined by a nephelometer, filter photometer, or spectrophotometer and compared with a curve prepared from standard sulfate solution.
Phenols		EPA Method 1312 DIN 38409-16:1984-06	Distillation of samples

			containing phenol and subsequent measurement of the phenol content using the 4-Nitroaniline method.
Oil & Grease	Oil	APHA-AWWA-WEF 5520E	Method 5520E is a modification of the Soxhlet method and is suitable for sludges and similar materials. Oil and grease" is defined as any material recovered as a substance soluble in the solvent.
Protein	P	NREL/TP-510-42625	Determination of Protein Content in Biomass. The nitrogen content of the biomass sample is measured by combustion or Kjeldahl methods and the protein content is estimated using an appropriate Nitrogen Factor (NF).
Starch		AACC Method 76-13.01, AOAC Method 996.11, ICC Standard Method No. 168 and RACI Standard Method	The Total Starch (AA/AMG) test kit (e.g. MEGAZYME) is used for the measurement and analysis of total starch. This kit now contains an improved a-amylase that allows the amylase incubations to be performed at pH 5.0 (as well as pH 7.0).
Cellulose	Cel	NREL/TP-510-42618	Determination of Structural Carbohydrates and Lignin in Biomass. Carbohydrates can be structural or non-structural. Structural carbohydrates are bound in the matrix of the biomass, while non-structural carbohydrates can be removed using extraction or washing steps.
Hemicellulose	Hem	NREL/TP-510-42618	Determination of Structural Carbohydrates and Lignin in Biomass. Carbohydrates can be structural or non-structural. Structural carbohydrates are bound in the matrix of the biomass, while non-structural carbohydrates can be removed using extraction or washing steps.
Lignin	Lig	NREL/TP-510-42618	Determination of Structural Carbohydrates and Lignin in Biomass. Lignin is a complex phenolic polymer. It fractionates into acid insoluble material and acid soluble material.
Sugars		NREL/TP-510-42623	Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples
Pectin			Gravimetric determination of pectin after its extraction with a 3% EDTA solution at pH 4.
Elemental Analysis C, H, S and N		EN15104	Flash 2000 Elemental Analyzer Thermo Scientific calibrated using BBOT standards (2,5-Bis(5-tert-butyl-2-benzo-oxazol- 2-yl)thiophene) containing carbon will be used for the elemental C, H, S and N analysis.

Metal content		EPA Method 3051a EPA Method 6010b	Microwave assisted acid digestion of sediments followed by inductively coupled plasma-atomic emission spectrometry
Thermo-gravimetric analysis	TG		Differential thermogravimetric analysis: continuously record of the rate and % weight loss as a function of time or temperature, under dynamic conditions, in the range of 25–850 °C, at atmospheric pressure, under nitrogen/air atmosphere, with a flow rate of 45 mL min^{-1} and a linear heating rate of 10 °C min^{-1}.

Table 8. Methods of analysis for the characterization of WaysTUP! feedstocks in oily form

Parameter		Reference	Description
Free fatty acid	FFA	ISO 660:2009 ASTM D5555 - 95	These Standards specify three methods (two titrimetric and one potentiometric) for the determination of the acidity in animal and vegetable fats and oils. The acidity is expressed preferably as acid value, or alternatively as acidity calculated conventionally. Acid value is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fat, while acidity is the content of free fatty acids.
Moisture, impurities, unsaponifiable	MIU	ISO 18609:2000	Determination of unsaponifiable matter — Method using hexane extraction. All the substances present in the product which, after saponification of the latter by potassium hydroxide and extraction by hexane, are not volatile under the specified operating conditions.
Saponification value		ASTM D5558 - 95	This test method is intended for use in the determination of the saponification value of fats and oils used in the manufacture of fat liquors for the purpose of quality assurance.
Iodine value		ASTM D5554 - 15	The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of

			centigrams of iodine absorbed per gram of sample.
Peroxide value		ISO 3960:2017	The peroxide value is a measure of the amount of oxygen chemically bound to an oil or fat as peroxides, particularly hydroperoxides. ISO 3960:2017 specifies a method for the iodometric determination of the peroxide value of animal and vegetable fats and oils with a visual endpoint detection.
Anisidine value		ISO 6885:2016	ISO 6885:2016 specifies a method for the determination of the anisidine value in animal and vegetable fats and oils. This is a measure of the amount of aldehydes present (principally α, β-unsaturated aldehydes).
Sulphur content		ASTM D5453 - 19a	This test method covers the determination of total sulfur in liquid hydrocarbons, boiling in the range from approximately 25 °C to 400 °C, with viscosities between approximately 0.2 cSt and 20 cSt (mm2/s) at room temperature.
Insoluble substances		ASTM D5557 - 95	This test method covers the determination of the amount of impurities that are insoluble in kerosine and petroleum ether contained in fats and oils
Lipid profile	FA profile		The fatty acid profile of lipids is analysed by capillary gas chromatography method (FID detector) after forming methyl ester of fatty acids

More specifically, feedstocks such as oil from spent coffee grounds as well as used cooking oils will be characterized according to Table 8, representing the oily waste of the project.

As far as the solid/slurry feedstocks are concerned, Table 9 presents all the analysis that will be performed in order to ensure reliable physical and chemical characterization of the examined urban biowaste of WaysTUP!. In Table 9, the questionnaires' responses of all relevant

partners are integrated with the objective to map the composition of urban biowaste. The technological scheme (Pilot Plant) that each substrate will feed was also taken into consideration during this integration. It should be pointed out that these analyses are indicative and not exhaustive.

Table 9. Proposed parameters for WaysTUP! feedstocks characterization

Parameter	Meat waste PP.1	Fish waste PP.1	Coffee waste PP.1	Coffee waste PP.2	Source Separated biowaste PP.3	Meat & Fish waste PP.3	Source Separated biowaste PP.5	Cellulosic Rejections of MSWTP PP.6	Nappies PP.6	Carton and paper rejections of MSW PP.6	Sewage sludge PP.7	Olive Oil mill waste PP.7	Sawdust PP.7
pH	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Dry Matter/ Moisture	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Volatile Solids/Ash	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Total Organic Carbon	✔	✔	✔	✔	✔	✔	✔				✔	✔	✔
Total Kjeldahl Nitrogen	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
Ammonium, Nitrates											✔	✔	✔
Olsen Phosphorus											✔	✔	✔
Chlorides											✔	✔	✔
Sulfates											✔	✔	✔
Phenols											✔	✔	✔
Oil & Grease	✔	✔	✔				✔						
Protein	✔	✔					✔	✔	✔	✔			
Starch							✔						

Cellulose			✔				✔	✔	✔	✔			
Hemicellulose			✔				✔	✔	✔	✔			
Lignin/Acid Insoluble Residue			✔				✔	✔	✔	✔			
Sugars							✔	✔	✔	✔			
Pectin			✔										
Elemental Analysis C, H, S and N											✔	✔	✔
Metal content											✔	✔	✔
Thermogravimetric analysis											✔	✔	✔

PP.x: Pilot Plant x

4.2. Evaluation and Presentation of Results

The format for the presentation of results is an important aspect of the WACs and affects the comparability of waste analysis results between the different urban biowaste feedstocks to be valorized within WaysTUP!. The fundamental aim of this deliverable (D1.1) is to improve the accuracy and comparability of urban biowaste characteristics and the format of presentation can assist in optimizing this. Presenting the results in a clear and logical format is one of the most important tasks for the person managing the characterization of feedstock. When presenting results, the format of the presentation should be tailored to address the aims and objectives of the D1.1 and to satisfy the potential users/partners of the results. The presentation needs to be effective, easy to understand and convey the main features of the data.

When presenting the data, some form of a written report is essential. The report should convey the main features clearly and follow a logical progression, provide insight into the data and make the results as interesting as possible. As the author of the report, specific messages rather than generalised information should be conveyed. If appropriate, recommendations may need to included.

The contents of the report and its balance of words, tables and graphs will naturally depend on the feedstock characterised. Graphs and tables convey complex information clearly and can be used to add variety. At all times it should be remembered that the report is written to be read, and so it needs to make sense and be understandable. Such a report generally covers:

Introduction

The introduction states the purpose and aims of the report; gives the background to the research and defines terms and concepts.

Methodology

The methodology describes the WAC followed, the sampling plan applied, and information on the feedstock provider, as well as how the data was analysed and the statistical procedures which were used.

Results and Discussion

The findings and analysis section is the main part of the report which deals with details of the sample numbers, results and interpretation of tabulations etc. It is recommended to report and present the following data in this part:

1. Raw Data

Raw data along with the adopted methods of analysis should be presented and reported. The relevant WAC, Sampling Record and sample codes should be cited at this point.

2. Statistical Calculations

Statistical calculations should also be presented and reported. Excel (or any other statistical software) may be used for the calculation of the statistical parameters. The relative standard deviation confidence interval has to be calculated and represented as result.

3. Evaluation of single results of strata

The evaluation of single results of each stratum should also be presented and reported as a table.

4. Graphical presentation of results

Conclusions and Recommendations

The conclusions part summarises the major findings of the report and answers questions posed in the introduction. The recommendations outline what actions are indicated on the basis of the conclusions.

Appendices and References

Sampling records **MUST** be part of the Appendices. Any other items which may be useful but not essential to the report could be incorporated in the Appendices. References list the books, journals and papers referred to in the study.

Remembering that the report is likely to serve as a basis for the development of D1.2 (Report on urban biowaste composition & physicochemical characteristics), some other important considerations are the title, use of headings and sub-headings, the colour and design of the cover and the overall appearance of the report.

The ultimate aim of any report presenting the WaysTUP! feedstocks is to broaden the knowledge base on urban biowaste in a way that can be used to promote further decision making.

Conclusions

In summary, the purpose of this report was to provide WaysTUP! partners with guidance on acceptable and uniform protocols for the physical and chemical analysis of urban biowaste.

This report sets forth all the necessary program requirements for conducting an effective Waste Analysis Campaign. The minimum quality requirements presented in this document are designed to ensure that sample collection and laboratory analysis activities generate data which meet the pilot plants and project's requirements, and are technically valid, useable and legally defensible relative to the use for which the data are obtained.

Included in this document are the acceptable analytical method performance elements, a summary of minimum sample collection volumes, sample preservation requirements and maximum holding times, detailed requirements for analytical quality assurance and quality control and the necessary format for report.

Implementation of the developed methodology shall ensure the high data quality collection for the successful progress of D1.2 "Report on urban biowaste composition & physicochemical characteristics" and shall set the stepping stone for the successful initiation of WaysTUP! experimental part.

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Annex 1

WaysTUP! QUESTIONNAIRE

LABORATORY ANALYSIS PROTOCOL AND METHODOLOGY FOR EXECUTING WAC (WP1)

Questionnaire link:
https://docs.google.com/forms/d/e/1FAIpQLSdWcTxTKuYn8DbHPRo4HD6VpTD0-X5XjXQ3A4yxwOfDaUiBwQ/viewform?usp=sf_link

Email address *

Please designate a contact person (Name, email, Organisation) *

Pilot Plant:

Choose your feedstock:

Meat by-products

Fish by-products

Spent Coffee Grounds (SCG)

Source separated biowaste from households

Used cooking oils

Cellulosic rejection material

Sewage sludge

For each feedstock answer the following questions:

- Describe the feedstock that you are going to use for the Pilot plant operation.

- Is there a feedstock provider partner? If yes, name the feedstock provider.

- Is there an established collection network? If yes, please describe it by adding information about collection strategy, collection points, frequency, quantities etc.

- Is there a need for a collection network? If yes, please describe what you are planning to do to set up the network.

- Please describe the collection procedure.

- Describe the waste transportation procedure
- Describe the preservation procedure of samples prior to analysis

- Which parameters are you going to use to characterize your feedstock?

Please **attach in a file the methods** used for the determination of the parameters as well as any other relevant file regarding the characterization methods.

Additional comments and information.

Annex 2

Example information Sampling Record

SAMPLING RECORD	
Sample code: (Reflect site location, feedstock and date of collection)	
Date of sampling:	
Signature of sampler:	
GENERAL INFORMATION	
Waste producer: Contact:	Client (Company): Contact:
Location of sampling:	Carried out by (Company): Sampler:
SAMPLING OBJECTIVE	
SAMPLING APPROACH/PATTERN (with justification):	
MATERIAL	
Type of Feedstock:	Estimated moisture content:
Source and origin of the feedstock (e.g. form and nature of arising):	
Process/activity producing the feedstock:	
Description: (colour, odour. consistency/homogeneity/grain size - uniform or diverse)	
SAMPLING METHODOLOGY	
Describe/define sub-population or consignment sampled:	
Place and point of sampling:	

Access problems that affected areas or volumes of feedstock sampled:	
Date and time of sampling:	
Persons present (record name and address of witnesses present where appropriate):	
Procedure (describe procedure adopted):	
Equipment used:	
Number of increments/samples collected:	
Increment size/Sample size:	
Observations diving sampling (e g. gassing out, reactions, development of heat):	
Details of on-site determinations:	
Safety measures taken:	
SUB-SAMPLING AND PRE-TREATMENT	
Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)	
Procedure:	
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS	
Packaging:	
Preservation:	
Storage:	
Transport:	
DEVIATIONS FROM SAMPLING PLAN	
Detail:	
DELIVERY TO ANALYTICAL LABORATORY	
Company: Received by:	Delivery date: Signature: