Description of the SpiralG biorefinery model

Content: This document describes the methods used to collect and aggregate the pilot scale data of the SpiralG biorefinery which serve as a basis for the environmental life cycle assessment (LCA) studies. A general overview of the biorefinery is given before detailing the approaches used to convert the raw data into aggregated data. Then, the model constructed from the resulting datasets is presented with a focus on two influencing parameters: Spirulina biomass productivity and phycocyanin extraction efficiency. This model is used to analyse the aggregated data and perform a mass balance of the biorefinery. Finally, the framework coded in the `Python` programming language using `Brightway`, utilised to perform the LCA calculations and visualise the results, is described.

Contents

1 General description of the Spirulina biorefinery

1.1 Development of three marketable products

The SpiralG project gathers three industrial partners located in France and Italy which are each responsible for a portion of the Spirulina value chain. Livegreen[1] is a company established in Arborea, a city of approximately 4,000 inhabitants located in the province of Oristano in Sardinia (Italy). The economy of Arborea is based on the local agricultural activities revolving around the *Cooperativa Assegnatari Associati Arborea*[2] founded in 1956. Livegreen cultivates Spirulina in open raceway ponds (ORPs) and commercialises "pure" biomass in the form of tablets and spaghettini as well as transformed products inspired by traditional Sardinian cuisine (e.g. Fregula Sarda, Carasau Guttiau bread).

This work was limited to the assessment of the environmental impacts associated with the production of spaghettini. Indeed, only this form of biomass was used in the phycocyanin extraction process. The meteorological conditions in Arborea (e.g. light incidence, temperature) are favourable for the cultivation of Spirulina 90% of the year. The biomass is harvested daily, dewatered, shaped into spaghettini, and dried. These pre-processing steps allow the reduction of the water content of Spirulina biomass from 99% to 5% and facilitate its transportation for further processing. The dry spaghettini are shipped to Greensea[3], a company located in the city of Mèze, in the South of France. Phycocyanin is extracted using water as a solvent and approximately 50% of pure pigment is recovered in the blue extract after several separation (e.g. centrifugation) and purification (e.g. ultrafiltration) steps. This process co-produces two main biomass fractions with added value.

The co-product obtained from the first separation process, called co-product A (CPA), is transported to Algaia[4], a company located in Saint-Lô (France). After an acid hydrolysis to break down the proteins and two purification steps, the product enriched in amino acids is concentrated. Due to its high content in nitrogen, the CPA concentrate (CPAc) has promising applications in the agricultural industry. The second co-product, called co-product B (CPB), is obtained from the ultrafiltration of the blue extract and is treated at Greensea directly. CPB is concentrated and the product obtained, which contains small colourless proteins, can be used in the cosmetic industry. The three extracts produced in the Spirulina biorefinery, i.e. the blue extract, CPAc, and CPB concentrate (CPBc), are sold as ingredients to formulators and incorporated into food, cosmetics, and agricultural products (see Table 1). Due to confidentiality, the exact biochemical composition of each fraction is not detailed.

[1] https://livegreen.bio/
[2] https://www.arborea1956.com.
[3] https://en.greensea-all.com/
[4] https://www.algaia.com/

Table 1: Description of the Spirulina biorefinery products and their potential applications.

Biomass fraction	General composition	Potential application(s)
Blue extract	50% of pure phycocyanin	Natural food colouring & ingredient for cosmetics products
CPAc	Enriched in amino acids	Ingredient for agricultural & feed products
CPBc	Enriched in colourless proteins	Ingredient for food & cosmetics products

1.2 Construction of the biorefinery system diagram

In order to facilitate the assessment of the environmental impacts of the Spirulina biorefinery, three subsystems were defined (i.e. each corresponding to one industrial partners of the SpiralG project): Spirulina cultivation and biomass pre-processing in Arborea (Italy) (subsystem 1 or S1), phycocyanin extraction in Mèze (France) (subsystem 2 or S2), and co-product A treatment in Saint-Lô (France) (subsystem 3 or S3) (see Fig. 1). In the articles, the name of the companies are not mentioned and each section of the biorefinery is referred to as S1, S2, and S3. Each of these subsystems is divided into processes, also called "activities" representing the smallest unit for which data were collected on-site. The activities "S1.A0.Building" and "S1.A0.Operation", initially included in S1, were transferred to the subsystems "infrastructures" and "operation", respectively. Similarly. "S1.A8.Transport" and "S2.A8.Transport" were both transferred to the subsystem "transport".

2 Data collection and aggregation

2.1 Collection of the raw data

Six data collection campaigns were conducted at the three pilot scale facilities from 2019 to 2022 (see Table 2). The raw data collected on-site (i.e. primary data) correspond to the amounts of electricity, water, nutrients, chemicals, and materials used in the three subsystems. Data were obtained from direct measurements since no data logger or sensors allowed the retrieval of data for most of the equipment. Only the centrifuge and ultrafiltration machines in S2 had data loggers to measure the volumes of water and chemicals used during the cleaning programs. In S1, Spirulina biomass was harvested daily. The processes from "S1.A2.Filtration" to "S1.6.Packaging" were repeated, allowing the collection of daily datasets. The average values obtained over the week were used as inventory data in the LCA studies. Regarding S2 and S3, the processes were sequentially performed over the week. No data duplicates were measured and the datasets are based on unique values.

First written by hand in a notebook, the data were then digitalised and presented in the form of Excel files containing the original data, calculations, and text (e.g. description of the data collection procedure for each item, description of the data obtained). The data for S1, S2, and S3 were collected independently since the respective facilities are located in different regions of Italy and France. Regarding S1, the data were collected in July 2019 and July 2022, during the week 30 and 28, respectively. The climatic conditions were similar during the two data collection periods. This is of particular importance since the temperature of the air, light incidence, and cloudiness affect Spirulina growth in ORPs. In contrast, the data for S2 and S3 were collected in 2021 and 2022 at different periods. Since the phycocyanin extraction and co-product treatment are performed indoor, the meteorological conditions do not significantly affect the processes.

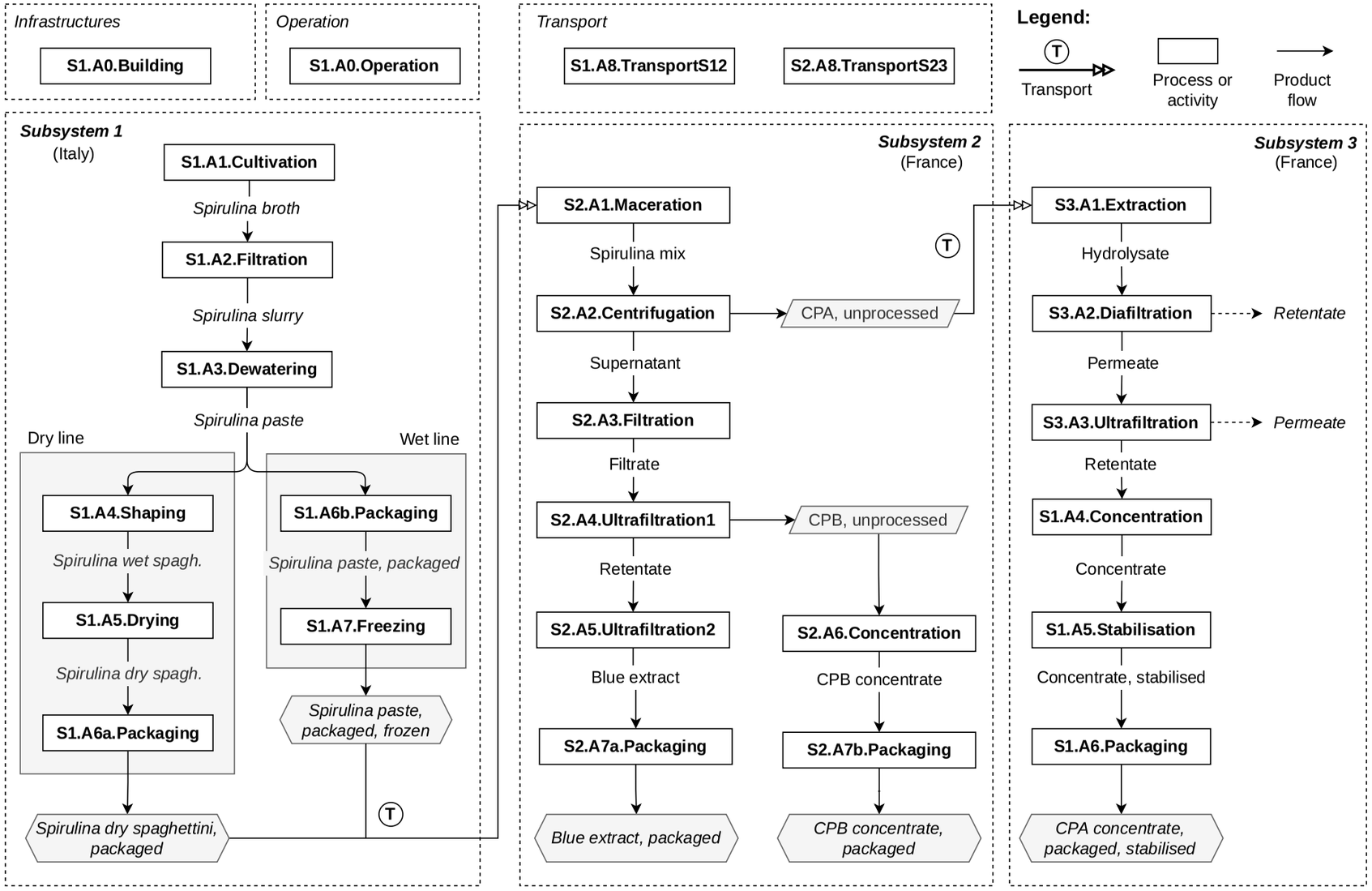


Figure 1: Simplified system diagram of the Spirulina biorefinery. The abbreviation “spagh.” refers to “spaghettini”.

In the case of S2, the temperature in the pilot hall influences the need to cool down the blue extract after extraction. In fact, the high temperature in the pilot hall in summer results in a larger consumption of energy to cool down the blue extract.

Table 2: Description of the data collection campaigns conducted in the frame of the SpiralG project.

No.	Period	Description
1	July 2019	Data collection at the Spirulina cultivation and biomass pre-processing facility in Arborea, Italy. The plant was inaugurated in April 2019 and it was therefore its first year of operation.
2	January 2021	Data collection at the phycocyanin extraction facility in Mèze, France. The pilot scale trial was performed using 50 kg of dry Spirulina spaghettini. The second ultrafiltration step was interrupted and the concentration of the CPB not conducted.
3	February 2021	Data collection at the CPA treatment facility in Saint-Lô, France. The pilot scale trial corresponded to the first based on acid hydrolysis to extract the proteins from CPA. The concentration of the permeate from ultrafiltration was interrupted.
4	April 2022	Data collection at the CPA treatment facility in Saint-Lô, France. Not all the permeate from diafiltration was ultrafiltered. The evaporation of the retentate from ultrafiltration was interrupted.
5	July 2022	Data collection at the Spirulina cultivation and biomass pre-processing facility in Arborea, Italy. No measurements of electricity consumption were performed.
6	August 2022	Data collection at the phycocyanin extraction facility in Mèze, France. The pilot scale trial was performed using 12.5 kg of dry Spirulina spaghettini. The CPB was concentrated using an evaporator. The two successive ultrafiltration steps worked.

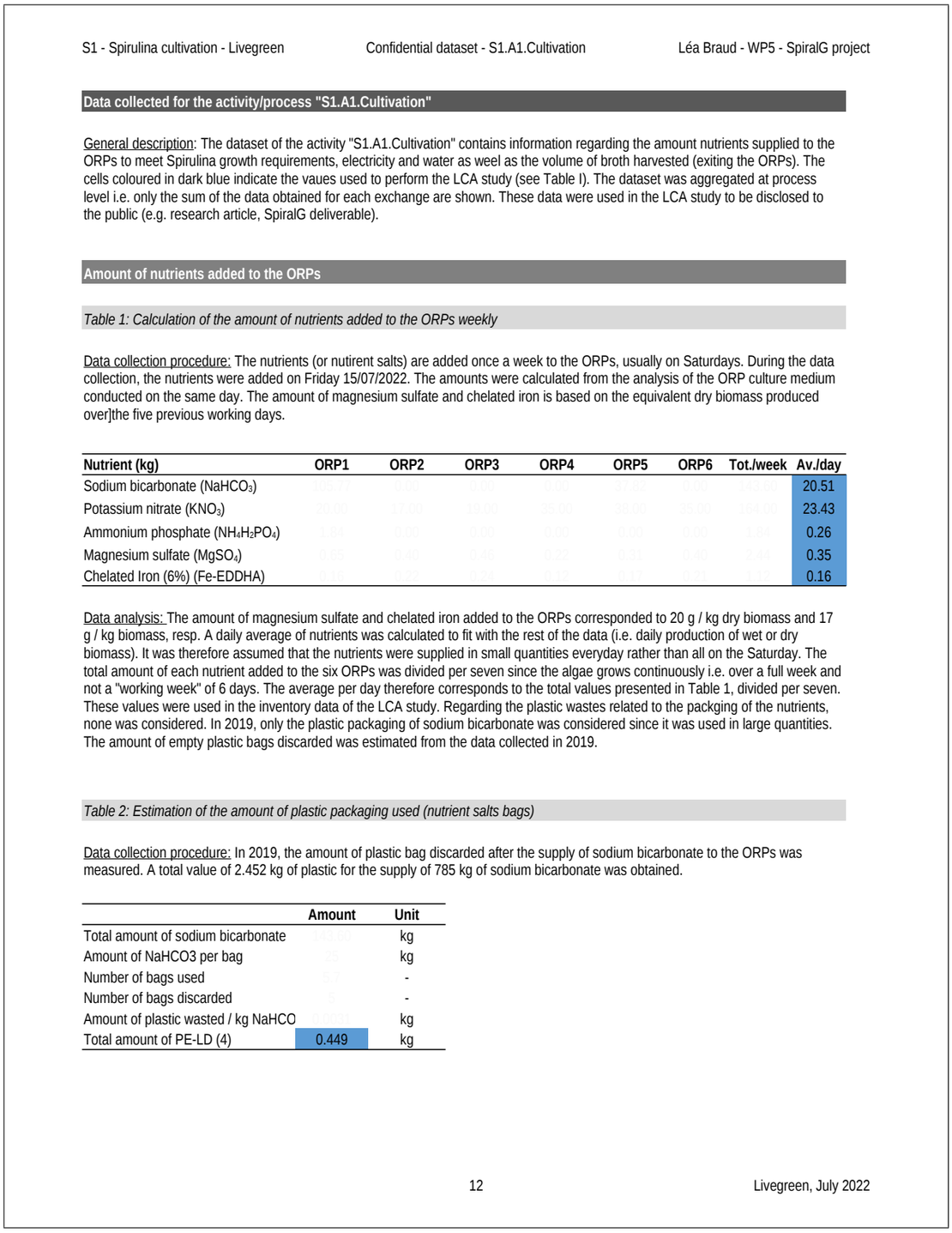
2.2 Aggregation of the raw data at process level

The raw data were aggregated at process level to overcome the issue of confidentiality. The aggregation consists of summing the individual measurements for each exchange in an activity. For instance, the total volume of water used in "S1.A3.Dewatering" was measured by collecting the water exiting the water presses into containers which were weighed at the end of each water press cycle (i.e. each batch). The volume of water used to clean the water presses at the end of the process (e.g. steel structure, cotton bags) was measured using a water meter installed on the hose used for cleaning. The details of each volume measured was noted in the raw dataset. During the aggregation, the total volume used per day was calculated by summing the individual values of the raw datasets to obtain a unique volume of water. The same procedure was applied to electricity, chemicals, materials etc.

In addition, the aggregation procedure allowed the identification of data gaps. Although the data were collected on-site, certain measurements could not be performed due to a malfunctioning or lack of equipment. For instance, only one water meter was available to measure volume of Spirulina broth harvested in S1. The data collected for two ORPs were used as proxy for the four other ORPs. Mass and water balances were conducted at process level to verify the dataset and fill in the data gaps were necessary. The use of Lavoisier's law of mass conservation allowed to calculate missing values. This principle states that for any closed system to transfer of matter and energy, the mass of the system remains constant over time. The quantity of mass

within the system cannot change and therefore nothing can be added or removed.

Several processes were simultaneous, i.e. the biomass output of a process was directly pumped into the next machine and could therefore not be measured. For instance, the hydrolysate obtained from "S3.A1.Extraction" was directly pumped into the tank of the ultrafiltration machine. As a result, the amount of hydrolysate produced was calculated from the mass balance using the principle of conservation (biomass input = biomass output). Ultimately, no losses were accounted for in the process. In addition, certain measurements led to unbalanced processes (e.g. the amount of biomass measured at the end of a process was larger than at the start). In this case, the amounts were adjusted to balance the process by using simple cross multiplications. The mass and water balances of each activity of the three subsystems are detailed in another document.



S1 - Spirulina cultivation - Livegreen | Confidential dataset - S1.A1.Cultivation | Léa Braud - WP5 - SpiralG project

Data collected for the activity/process "S1.A1.Cultivation"

General description: The dataset of the activity "S1.A1.Cultivation" contains information regarding the amount nutrients supplied to the ORPs to meet Spirulina growth requirements, electricity and water as weel as the volume of broth harvested (exiting the ORPs). The cells coloured in dark blue indicate the vaues used to perform the LCA study (see Table I). The dataset was aggregated at process level i.e. only the sum of the data obtained for each exchange are shown. These data were used in the LCA study to be disclosed to the public (e.g. research article, SpiralG deliverable).

Amount of nutrients added to the ORPs

Table 1: Calculation of the amount of nutrients added to the ORPs weekly

Data collection procedure: The nutrients (or nutirent salts) are added once a week to the ORPs, usually on Saturdays. During the data collection, the nutrients were added on Friday 15/07/2022. The amounts were calculated from the analysis of the ORP culture medium conducted on the same day. The amount of magnesium sulfate and chelated iron is based on the equivalent dry biomass produced over]the five previous working days.

Nutrient (kg)	ORP1	ORP2	ORP3	ORP4	ORP5	ORP6	Tot./week	Av./day
Sodium bicarbonate ($NaHCO_3$)	19.77	0.00	0.00	0.00	37.62	0.00	143.60	20.51
Potassium nitrate (KNO_3)	20.00	17.00	13.00	35.00	38.00	35.00	164.00	23.43
Ammonium phosphate ($NH_4H_2PO_4$)	1.84	0.00	0.00	0.00	0.00	0.00	1.84	0.26
Magnesium sulfate ($MgSO_4$)	0.65	0.40	0.46	0.22	0.31	0.40	2.44	0.35
Chelated Iron (6%) (Fe-EDDHA)	0.16	0.00	0.00	0.00	0.00	0.00	1.16	0.16

Data analysis: The amount of magnesium sulfate and chelated iron added to the ORPs corresponded to 20 g / kg dry biomass and 17 g / kg biomass, resp. A daily average of nutrients was calculated to fit with the rest of the data (i.e. daily production of wet or dry biomass). It was therefore assumed that the nutrients were supplied in small quantities everyday rather than all on the Saturday. The total amount of each nutrient added to the six ORPs was divided per seven since the algae grows continuously i.e. over a full week and not a "working week" of 6 days. The average per day therefore corresponds to the total values presented in Table 1, divided per seven. These values were used in the inventory data of the LCA study. Regarding the plastic wastes related to the packging of the nutrients, none was considered. In 2019, only the plastic packaging of sodium bicarbonate was considered since it was used in large quantities. The amount of empty plastic bags discarded was estimated from the data collected in 2019.

Table 2: Estimation of the amount of plastic packaging used (nutrient salts bags)

Data collection procedure: In 2019, the amount of plastic bag discarded after the supply of sodium bicarbonate to the ORPs was measured. A total value of 2.452 kg of plastic for the supply of 785 kg of sodium bicarbonate was obtained.

	Amount	Unit
Total amount of sodium bicarbonate	785.00	kg
Amount of NaHCO3 per bag	25	kg
Number of bags used	31.4	-
Number of bags discarded	5	-
Amount of plastic wasted / kg NaHCO	0.0571	kg
Total amount of PE-LD (4)	0.449	kg

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Figure 2: Extract of a data collection sheet used to communicate the results with the industrial partners. The data for S1 were collected for each ORP, every day. The raw data were replaced by blank spaces due to their confidentiality. Only the aggregated data i.e. per day were shown.

3 Spirulina biorefinery model

The Spirulina biorefinery model was developed in the `Python` programming language using the data collected on-site and described in Section 1. The first objective of the model is to facilitate the comparison of the data collected during the different data collection campaigns. The datasets consisting of data collected in 2019 and 2021 correspond to the period 1 while the most up-to-date data, i.e. collected in 2022, correspond to the period 2. The comparison of the datasets of periods 1 and 2 allows to evaluate the effects of the technological improvements made across the value chain on the consumption of energy, water, materials, and chemicals (and to a larger extent on the environmental sustainability of the biorefinery). The second objective of the model is to evaluate the effects of changes in the Spirulina biomass productivity and phycocyanin content on the mass balance of the biorefinery (i.e. proportion of blue extract, CPAc, and CPBc) and the consumption of the energy, water, materials, and chemicals (and to a larger extent on the environmental sustainability of the biorefinery).

The model is divided into the three subsystems described above. Each process (or activity) is computed with a set of functions which typically take as input the amount of biomass to process and the technological period to return the amount of energy, water, materials, and chemicals used in the process plus the waste generated. Therefore, the outputs of the model correspond to datasets for each subsystem with data aggregated at process level. The datasets can be directly used to perform the LCA study of the biorefinery. The parameters of the model were defined based on the research objectives and classified into four categories. The operational parameters include the technological period, number of working days, type of the line (e.g. wet, dry), and material lifespan. In addition, the technical parameters consist of the harvesting efficiency, phycocyanin extraction efficiency, number of ORPs, number of vibrating filters (VFs), and volume of ORP. Finally, the biological parameters include the Spirulina biomass productivity and phycocyanin content. The last parameters correspond to the transportation distances between S1, S2, and S3. In total, 14 parameters were defined among which 6 were used in the environmental LCA studies (see Table 3). The other parameters were considered as constant. The number of working days was set to 330, the total cultivated area to 705 m^2, the number of ORPs to 6, the number of VFs to 3. In addition, the volume of the ORPs was considered as constant (i.e. the volume of water used to fill in the ORPs compensates the losses per evaporation).

3.1 Technological period

The technological period is the main parameter to all activities except for the ones that occurred in period 2 only (e.g. “S2.A5. Ultrafiltration 2”, “S2.A6.Concentration”). This parameter was used to calculate distinctive datasets for periods 1 or 2 based on the data collected for each activity. For instance, the technological period influences the amount of Spirulina paste obtained after dewatering (see Listing 1).

```
def BiomassDewatering (tech_period, slurry_DW):
  biomass_balance_dict_sc1 = {}
  biomass_balance_dict = {}
  if tech_period == '1':
    ## data collected
    slurry_sc1 = 257.71 #amount slurry [kg]
    DM_slurry_sc1 = 14.75 #dry matter content slurry [%]
    slurry_DW_sc1 = slurry_sc1 * DM_slurry_sc1/100 #amount slurry [kg DW-eq]
    paste_sc1 = 91.45 #amount paste [kg]
```

Table 3: Description of the parameters defined in the Spirulina biorefinery model. The underlined parameters correspond to the ones used in the LCA studies. The rest were considered as constant.

Parameter	Description	Unit	Values
Technological period	Period during which the data were collected. Period 1 corresponds to 2019 (S1) and 2021 (S2,S3). Period 2 corresponds to 2022 (S1, S2, S3).	-	'1' or '2'
Productivity	Daily areal dry spaghettini productivity	$g/m^2/day$	[0; 12]
Phycocyanin content	Proportion of phycocyanin in Spirulina biomass	%	[5; 15]
Working days	Number of days during which Spirulina is harvested and the biomass processed	day	[330; 365]
Harvesting efficiency	Efficiency of the filtration step i.e. ratio between the amount of biomass recovered in the slurry and the amount of broth filtered	%	[70; 100]
Line	Dry or wet line for Spirulina biomass preprocessing.	-	'dry' or 'wet'
Distance by car	Distance between the Spirulina cultivation facility and the local industrial harbour	km	[10; 50]
Distance by ship	Distance between the industrial harbours in Italy and France	km	[500; 800]
Distance by truck	Distance between the industrial harbour in France and the phycocyanin extraction facility	km	[50; 200]
Distance by refrigerated truck	Distance between the phycocyanin and the coproduct treatment facility in France	km	[500; 900]
Number of ORPs	Number of open raceway ponds in the Spirulina cultivation facility	unit	6
Number of VFS	Number of Vibrating Filtering Systems in the Spirulina biomass processing facility	unit	3
Volume of ORP	Volume of one open raceway pond	m^3	[150; 250]
Material lifespan	Lifespan of the materials used in the construction of the greenhouse, cultivation facility, and biomass processing facility.	year	[5; 20]

```
    DM_paste_sc1 = 33.07 #dry matter content of paste [%]
    paste_DW_sc1 = paste_sc1 * DM_paste_sc1 /100
    biomass_balance_dict_sc1['slurry'] = {'wet_mass':slurry_sc1, 'DM_content' :
     DM_slurry_sc1, 'dry_mass' :slurry_DW_sc1}
    biomass_balance_dict_sc1['paste'] = {'wet_mass':paste_sc1, 'DM_content':
   DM_paste_sc1, 'dry_mass':paste_DW_sc1}
    ## data modelled
    paste_DW = slurry_DW * paste_DW_sc1/slurry_DW_sc1
    biomass_balance_dict['slurry'] = slurry_DW
    biomass_balance_dict['paste'] = paste_DW
    biomass_balance_dict['losses'] = slurry_DW - paste_DW
 if tech_period == '2':
    ## data collected
    slurry_sc1 = 198.99 #amount slurry [kg]
    DM_slurry_sc1 = 10.39 # dry matter content of slurry [%]
    slurry_DW_sc1 = slurry_sc1 * DM_slurry_sc1/100 #amount slurry [kg DW-eq]
    paste_sc1 = 77.38 #amount paste [kg]
    DM_paste_sc1 = 23.26 #dry matter content of paste [%]
    paste_DW_sc1 = paste_sc1 * DM_paste_sc1 /100
    biomass_balance_dict_sc1['slurry'] = {'wet_mass':slurry_sc1, 'DM_content' :
     DM_slurry_sc1, 'dry_mass' :slurry_DW_sc1}
    biomass_balance_dict_sc1['paste'] = {'wet_mass':paste_sc1, 'DM_content':
   DM_paste_sc1, 'dry_mass':paste_DW_sc1}
    ## data modelled
```

```
    paste_DW = slurry_DW * paste_DW_sc1/slurry_DW_sc1
    biomass_balance_dict['slurry'] = slurry_DW
    biomass_balance_dict['paste'] = paste_DW
    biomass_balance_dict['losses'] = slurry_DW - paste_DW
return biomass_balance_dict_sc1, biomass_balance_dict
```

Listing 1: Extract of the recipe YAML file containing information to run the LCA algorithm.

3.2 Spirulina biomass productivity

3.2.1 Definition of the Spirulina biomass productivity parameter

In this study, the Spirulina biomass productivity was defined as the amount of dry Spirulina spaghettini produced per day per square metre (see Box 1). This definition is different from the one usually used in the literature which considers the amount of algal biomass produced before processing i.e. measured in the culture medium before harvesting. The choice of using the productivity of processed biomass was made due to the lack of data regarding the concentration of Spirulina in the ORPs before harvesting. In contrast, the measurement of the amount of dry spaghettini produced is performed everyday as part of the data collection routine of the operators working at the Spirulina processing facility.

Box 1: Calculation of the dry Spirulina spaghettini productivity

Productivity (g/m^2/day) = Amount of dry Spirulina spaghettini produced (kg DW-eq) x dry matter content (% DW)] / total cultivated area (m^2)] x 1000

In the biorefinery model, the Spirulina biomass productivity is used to determine the concentration of Spirulina in the culture medium before harvesting. The value of concentration obtained is then used to calculate the amount of biomass harvested from the mass balance of the activities "S1.A2.Filtration" and "S1.A1.Cultivation". Although the volume of Spirulina broth pumped to the VFs was measured, the values obtained were associated with a large variability due to numerous assumptions. The concentration of Spirulina in the culture medium was measured in periods 1 and 2. Linear scaling was applied to calculate the concentration of Spirulina in the culture medium (see Box 2).

Box 2: Calculation of Spirulina concentration in the culture medium

Concentration of Spirulina in scenario B (g/L) = [Spirulina biomass productivity in scenario B (g/m^2/d) x Concentration of Spirulina in scenario A (g/L)] / Spirulina biomass productivity in scenario A (g/m^2/d)

3.2.2 Calculation of the initial values

The Spirulina biomass productivity was calculated for periods 1 and 2 based on the data collected on site. The total cultivated area corresponds to 4,230 m^2 (i.e., the surface of six ORPs of 705 m^2 each) and is identical for both periods. The amount of dry Spirulina spaghettini produced and their dry matter content was measured daily during the two data collection campaigns and averaged. The calculated values of Spirulina biomass productivity varied considerably between periods 1 and 2 (see Table 4). The cause of the reduction of productivity

from 6.3 to 3.5 g/m^2/day is unknown due to a lack of data regarding Spirulina growth (i.e. "healthiness") at the time of the measurements.

The processes from "S1.A3.Dewatering" to "S1.A6.Packaging" remained unchanged between the two data collection campaigns. Regarding "S1.A2.Filtration", the filtration net used in the VFs to harvest Spirulina from the ORPs was replaced several weeks before the second data collection campaign in 2022. The new filter, made of nylon, has a lower porosity which limits the percentage of activity of the pump to 10% (instead of 20-30% in 2019). Since the flow rate of broth pumped from the ORPs was considerably reduced, the duration of the harvesting was multiplied per two i.e. from 3 hours per ORP in 2019 to 6 hours in 2022. Therefore, we assumed that the reduction of Spirulina biomass productivity between 2019 and 2022 was not related to a reduction of harvesting efficiency (i.e. measures were implemented to maintain the daily amount of biomass processed constant after the change of filter).

The reduction of Spirulina biomass productivity was considered to be related to the "healthiness" of the biomass which influences the concentration of the culture medium. At a low Spirulina biomass growth rate, less biomass is present in the ORPs and "available" for harvesting. The reduction of biomass productivity is in this case associated with changes in the meteorological conditions, contamination, nutrient supply etc. In this study, the Spirulina biomass productivity was considered to be associated with biological parameters and independent from the processes (since they were identical between the two data collection campaigns).

Table 4: Calculation of the initial values of the SpiralG biorefinery model.

	Period 1	Period 2	Unit	Comment
Number of ORPs	6	6	-	Value measured on site.
Surface of one ORP	705	705	m^2	Area based on the blueprint of the cultivation facility.
Amount of dry spagh. produced	27.53	15.59	kg	Average of measured values
Dry matter content of the biomass	96.8	94.99	%	Average of measured values
Amount of dry spagh. produced	26.65	14.81	kg DW-eq	
Biomass productivity	6.3	3.5	g/m^2/day	Calculated using (Eq. 1)

3.2.3 Variations of Spirulina biomass productivity

In the biorefinery model, Spirulina biomass productivity is used to calculate the concentration of the cyanobacterium in the culture medium (see Box 2). The concentration serves as a basis for the calculation of the amount of broth filtered and slurry produced in "S1.A2.Filtration". As shown in Figure 3, the activities "S1.A2.Filtration" to "S1.A5.Drying" are sequentially linked by biomass flows. The calculation of the inputs and outputs of each activity is interdependent (e.g. the slurry exiting "S1.A2.Filtration" is used to calculate the amount of paste produced in "S1.A3.Dewatering"). Variations in Spirulina biomass productivity correspond to variations in the daily amount of Spirulina dry spaghettini at the end of the pre-processing chain. The calculation of the concentration allows to evaluate the effects on the upstream part of the chain. The outputs of "S1.A1.Cultivation" were calculated at the end (i.e. sixth position) due to a lack of data regarding the amount of broth harvested from the ORPs.

As explained above, we assumed that variations in Spirulina biomass productivity were associ-

ated with changes in algal growth related to meteorological conditions, contamination etc. The harvesting efficiency was considered constant due to the measures implemented by the manager of the plant to maintain the amount of biomass processed per day steady (e.g. augmentation of the duration of harvesting). Since linear scaling is applied to all the activities to calculate the outputs of each activity (e.g. biomass, water, electricity), variations in Spirulina biomass productivity do not affect the amount of electricity, water, and materials used for the production of 28.78 kg DW-eq of dry Spirulina spaghettini. In "S1.A2.Filtration", we assumed that the electricity used by the VFs was not proportional to the amount of biomass filtered. During the second data collection campaign, Spirulina biomass was harvested 6 hours per day (i.e. 6 hours per ORP). The electricity consumption was assumed constant during harvesting and independent from the amount of biomass filtered. This included the electricity used for the pumps and motors. Therefore, the amount of electricity used in "S1.A2.Filtration" varies according to the productivity. In addition, the "infrastructures" and "operation" activities were assumed constant for any productivity. The amount of water and electricity used in the daily operation of the Spirulina pre-processing facility was constant (i.e. values for a day).

In the LCA studies, we compare the data collected and results obtained for periods 1 and 2. We assume that the Spirulina biomass productivity is related to the growing conditions only (and is independent from the pre-processing processes). The comparison between the values obtained was reported per kilogram of dry spaghettini produced. A value of 28.78 kg DW-eq of spaghettini was used a reference to compare the data obtained over the whole biorefinery. The data reported per kilogram of dry spaghettini produced were linearly increased to reach the target of 28.78 kg DW-eq. We also assumed that the activities "infrastructures" and "operation" were independent from the amount of biomass produced per day. In other words, the amount of materials used and wastes generated in "infrastructures" were identical for periods 1 and 2. The amount of electricity and water used in "operation" were different but evaluated and expressed per day.

In the scenario LCA, we consider a reduction of Spirulina biomass productivity due to a change of nutrient supply: nitrogen from digestate is used in place of nitrogen from potassium nitrate. According to Attene et al. [1], the use of ammonium salts reduces Spirulina biomass productivity in the culture medium (i.e. before harvesting). A productivity of 0.390 g DW-eq/L/day is reached with potassium nitrate as source of nitrogen while values of 0.344 g DW-eq/L/day and 0.246 g DW-eq/L/day are reached using ammonium sulfate and ammonium citrate as source of nitrogen, respectively. In the model, we considered that ammonium salts are directly assimilable by Spirulina and supplied in quantities sufficiently low to remain below the toxicity level for the cyanobacterium (i.e. ammonia can be toxic). Following the experiments of Attene et al. [1], we considered that the growth of Spirulina using ammonium salts as a source of nitrogen reduced the productivity by 11.8% and 36.9% for ammonium sulfate and ammonium citrate, respectively (see Table 4). In the biorefinery model, we assumed that the reduction of biomass productivity before harvesting was applicable for the biomass productivity after processing (since the pre-processing is assumed independent from the biomass productivity).

Table 5: Variations in Spirulina biomass productivity according to the source of nitrogen. According to Attene et al. [1].

Culture medium	g DW-eq/L/day	Reduction (%)
Potassium nitrate as source of N	0.39	-
Ammonium citrate as source of N	0.246	36.9
Ammonium sulfate as source of N	0.344	11.8

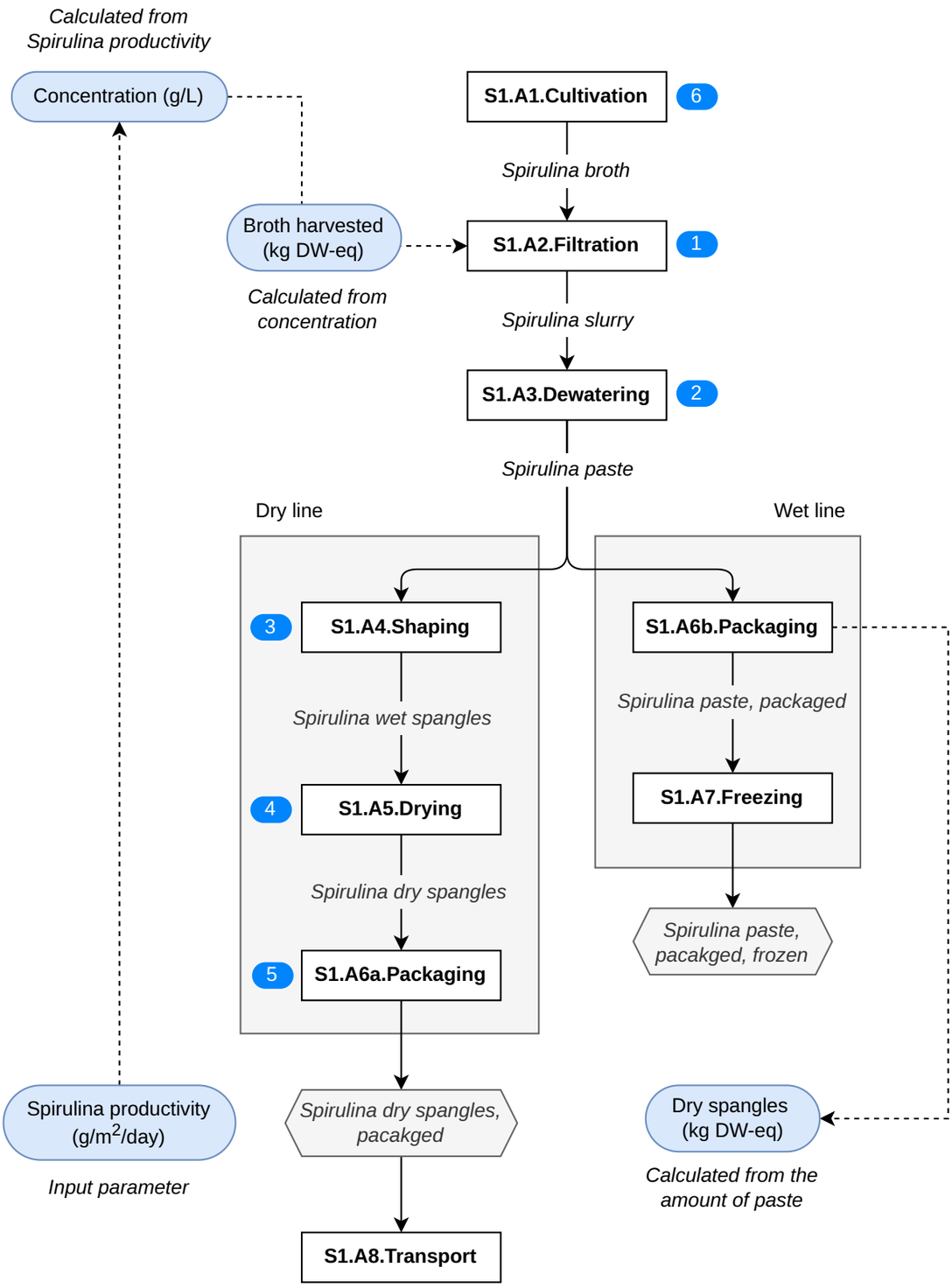

Figure 3: Definition of the Spirulina biomass productivity parameter.

3.3 Phycocyanin extraction efficiency

In this study, the phycocyanin extraction efficiency corresponds to the amount of phycocyanin recovered at the end of the pilot process compared to the amount of phycocyanin in the Spirulina mix obtained after maceration (and sampled before the centrifugation step). The extraction efficiency is calculated using the equations described in Box 3.

Box 3: Calculation of the phycocyanin extraction efficiency

Amount of phycocyanin recovered:

Amount of PC recovered (kg PC/kg DW-eq spagh.) = Amount of PC after UF2 (kg DW-eq)/Amount of dry Spirulina spagh. used for extraction (kg DW-eq)

Phycocyanin content of the blue extract:
PC content of the blue extract (%) = Amount of PC in the blue extract (kg DW-eq)/ [Amount of blue extract (kg DW-eq) x dry matter content of the blue extract (%)] x 100

Amount of phycocyanin obtained after UF2:
Amount of PC after UF2 (kg DW-eq) = [concentration of PC in the blue extract (g/L) x volume of blue extract obtained after UF2 (L)]/1000

Volume of blue extract obtained after UF2:
Volume of blue extract obtained after UF2 (L) = Amount of blue extract obtained after UF 2 (kg) x density of the blue extract (g/L)

Amount of phycocyanin in the mix:
Amount of PC in the mix (kg DW-eq) = Volume of mix (L) x concentration of PC obtained in the sample after PC extraction in the lab (g/L)/1,000

Volume of mix obtained after maceration:
Volume of mix (L) = Volume of water used for maceration (L) + Amount of dry spaghettini (kg DW-eq)

Phycocyanin extraction efficiency at pilot scale:
Extraction efficiency at pilot scale = Amount of PC obtained after UF2 (kg DW-eq)/Amount of PC in the mix (kg DW-eq) x 100

Table 6: Calculation of the phycocyanin extraction efficiency for period 2.

	Amount	Unit
Phycocyanin concentration of the sample of Spirulina mix obtained after maceration and before centrifugation (obtained after phycocyanin extraction in the lab)	2.78	g/L
Volume of water used for maceration	500	L
Amount of dry Spirulina spaghettini used for extraction	12.2	Kg DW-eq
Volume of Spirulina mix obtained after maceration	512.2	L
Amount of phycocyanin in the mix before centrifugation	1.42	Kg DW-eq
Amount of phycocyanin obtained after the pilot scale extraction	1.31	Kg DW-eq
Phycocyanin extraction efficiency of the pilot scale process (compared to the lab scale extraction)	92.25	%

In the biorefinery model, variations of phycocyanin extraction efficiency affects the activity "S2.A2.Centrifugation". In fact, an increase in phycocyanin extraction efficiency was modelled as an increase in phycocyanin content of the supernatant based on a 1:1 ratio. An increase or decrease of the phycocyanin extraction efficiency can be linked to the pre-treatment method applied to Spirulina biomass (e.g. drying, freezing/thawing). For instance, drying increases the phycocyanin extraction efficiency. The freezing and thawing method is even more efficient since the molecules are not affected by the pre-treatment (while drying affects the properties of the pigment). No pre-treatment results in lower extraction efficiency.

4 Environmental life cycle assessment

The environmental LCA of the SpiralG biorefinery was performed using the `Brightway` open source framework computed in the Python programming language [**mutel2017brightway**]. This Section describes how the foreground databases are built, what background database is used, and how the LCA calculations are performed using the different `Brightway` libraries (e.g. `bw2io`, `bw2data`, `bw2calc`).

4.1 Foreground and background databases

We developed one foreground database per subsystem based on the outputs of the biorefinery model described in Section 3. The datasets obtained for the activities "S1.A0.Building" and "S1.A0.Operation" were removed from S1 and added to the databases "infrastructures" and "operation", respectively. In addition, the activities "S1.A8.Transport" and "S2.A8.Transport" were both transferred into the foreground database "transport". The inputs and outputs of each activity were calculated from the biorefinery model based on a specific combination of parameter values. For instance, the foreground databases used in *Chapter 6* to model the seventeen scenarios are based various combinations of Spirulina biomass productivity and phycocyanin extraction efficiency values (see Table **??**). The datasets generated by the biorefinery model are normalised per main output. For S2 and S3, the main output corresponds to the blue extract obtained after ultrafiltration and the CPA concentrate. Regarding S1, the main output is either the dry Spirulina spaghettini obtained after drying or the paste obtained after dewatering (see articles). The datasets are then exported in the `bw2package` format and used as foreground databases for the LCA analyses. The background database used was ecoinvent version 3.6 cut-off.

4.2 LCA calculations using Brightway

This Section describes the `Brightway` libraries used to perform the LCA analyses of the SpiralG biorefinery i.e. to import and export databases in the `bw2` format, perform LCA calculations, and analyse the results. The libraries were integrated in a `Python`-based framework consisting of four main building blocks: (1) initialisation, (2) database import, (3) LCA calculations, and (4) interpretation. Each of those is described in the following Sections and summarised in Figure 4. This novel framework corresponds to a flexible structure that can be used via `Jupyter notebooks` or using `Spyder` to perform LCA analyses, similar to any LCA Software (e.g. OpenLCA, SimaPro, Activity Browser). Such frameworks, based on `Brightway`, are expandable i.e. other building blocks can be added to the structure to perform sensitivity and scenario analyses (see *Chapters 5 and 6*), prospective LCA etc.

4.2.1 Initialisation

The information used to run the algorithm is stored in a `YAML` file based on a human-readable data serialisation language. The document, called "`recipe`" is imported in the algorithm and copied (see Listing 3.1). The information contained in the file concern the foreground databases to import and analyse, the type of contribution analyses to perform, the activities to include in the contribution analyses, the functional unit, and the LCIA methods are used throughout the LCA study. Once the recipe is imported, a `bw2` project is selected or created. Projects

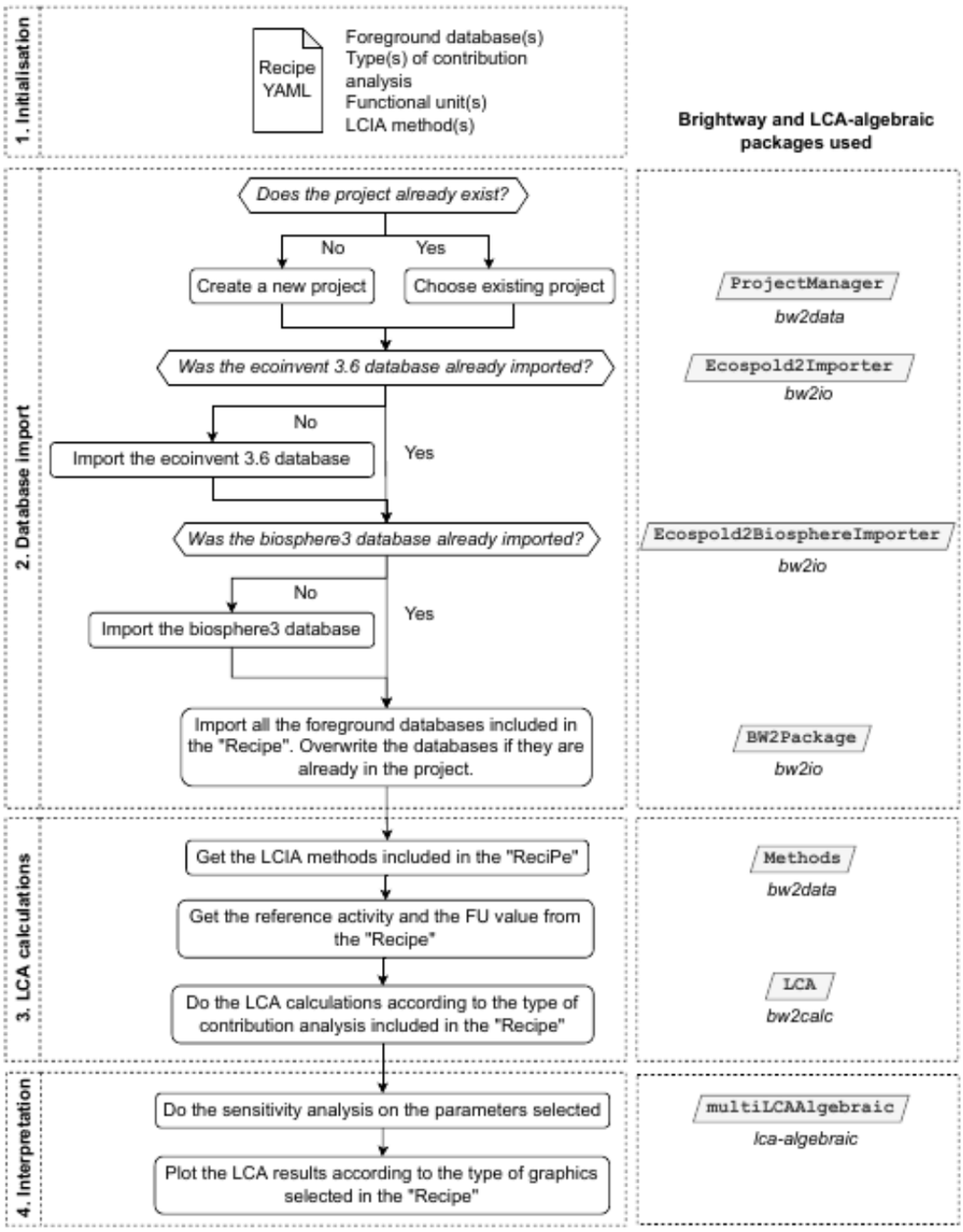

Figure 4: Structure of the LCA framework based on Brightway.

correspond to directories containing a copy of the foreground and background databases studied as well as the LCIA methods used to perform the LCA calculations. We created a novel project per analysis so that changes made in the databases do not interfere with other work.

```
input:
  analyses:
    CA_subsystems:
      relative_stacked_bar_plot: True
      bar_chart_one_ic: False
    CA_processes:
      relative_stacked_bar_plot: False
      pie_chart_stacked_bar_plot: False
      stacked_bar_plot_comparison: False
    CA_input_category:
      stacked_bar_plot: False
      heatmap: False
  CA_processes:
    relative_stacked_bar_plot:
      databases:
        - db_infrastructures
        - db_operation
```

```
18        - db_S1
19        - db_S2
20        - db_S3
21        - db_transport
22      lcia_methods:
23        - !!python/tuple [ReCiPe Midpoint (H) V1.13, fossil depletion, FDP]
24        - !!python/tuple [ReCiPe Midpoint (H) V1.13, climate change, GWP100]
25        - !!python/tuple [ReCiPe Midpoint (H) V1.13, metal depletion, MDP]
26        - !!python/tuple [ReCiPe Midpoint (H) V1.13, water depletion, WDP]
```

Listing 2: Extract of the recipe YAML file containing information to run the LCA algorithm.

4.2.2 Database import

Once a project is selected, the background and foreground databases are imported following the `recipe`. First, the background databases `biosphere3` and `ecoinvent 3.6 cut-off` are imported using the `Ecospold2Importer`. The database `biosphere3` contains elementary flows for which the names have been normalised to `eocinvent 3`. The function `bw2setup()` imports biosphere3, LCIA methods, and additional metadata required to import other databases. The datasets are differentiated using unique identifiers which include the name of the database and a code such as a number, UUID, and name. For instance, the code ("biosphere", "f66d00944691...") is a valid identifier. LCIA methods corresponds to tuples with the general name of the method (e.g. ReCiPe Midpoint (H) V1. 13), the name of the impact category (e.g. climate change), and its abbreviation (e.g. GWP100). The foreground databases are imported in the `bw2package` format and are linked to the `ecoinvent 3.6 cut-off` database. The linking between the datasets obtained from the biorefinery model described in Section 3 is performed using an Excel file containing the information regarding the `ecoinvent 3.6 cut-off` datasets used (e.g. name of the exchange, location, unit).

4.2.3 LCA calculations

The LCA calculations are performed according to the type of contribution analysis selected in the `recipe`. The environmental impacts can be calculated at three different levels: subsystems, processes, and exchanges. The basic structure for LCA calculation was used at each of the three levels.

Subsystem level: The LCA scores are calculated for each subsystem to analyse the contribution of infrastructures, operation, S1, S2, S3, and transport to the overall environmental impacts of the biorefinery. Two types of graphs can be generated. The relative stacked bar plot shows the contribution of each subsystem to the overall environmental impacts of the biorefinery for as many impact categories as indicated in the `recipe`. The second graph consists of a bar plot that shows the impacts of different foreground database scenarios for a specific impact category.

Process level: The LCA scores are calculated for each process (or activity) to evaluate the contribution of each activity to the environmental impacts of a specific subsystem (e.g. S1, S2, S3). Two types of graphs can be generated. The relative stacked bar plot shows the contribution of each activity (e.g. “S1.A1.Cultivation”, “S2.A1.Maceration”) to the environmental impacts of a specific subsystem for as many impact categories as indicated in the `recipe`. The second

graph consists of a stacked bar plot that shows the impacts of different scenarios of a same subsystem (e.g. S1 in 2019 versus S1 in 2022) for a specific impact category.

Exchange level: The LCA scores are calculated per category of exchanges across the subsystems (i.e. for the entire biorefinery). The categories include chemicals, energy, transport, equipment, construction, nutrient, packaging, water, and waste (see Table 7). The graph generated corresponds to a heat map which shows the relative contribution of each category of exchange to the impact categories selected.

Table 7: Categories of inputs used to perform the contribution analysis per exchange.

Exchange categories	Exchanges included
Chemicals	Sodium hydroxide, hydrogen peroxide, phosphoric acid, sulfuric acid, potassium hydroxide, nitric acid, sodium hypochlorite
Energy	Electricity (French mix), electricity (Italian mix), natural gas, heat from anaerobic digestion
Materials	Galvanised steel, polypropylene pipes, sand, propylene pipes, polyvinylchloride cover, polypropylene random copolymer (PPR) pipes, polyethylene film, mosquito net made of polyethylene, polycarbonate walls and ceiling, concrete, EPS bricks, ceramic floor tiles, wall pumice bricks, wall concrete bricks, insulated panels made of polyurethane, polyisocyanurate, rock wool, solar shading net made of high density polyethylene, PEX pipes (HDPE), cellulose filters, nylon, food grade packaging (polyethylene).
Nutrients	Sodium bicarbonate ($NaHCO_3$), carbon dioxide (CO_2), chelated Iron (6%) (Fe−EDDHA), TKPP ($K_4P_2O_7$), potassium sulfate (K_2SO_4), magnesium sulfate ($MgSO_4$), ammonium phosphate ($NH_4H_2PO_4$), potassium nitrate (KNO_3), ammonium phosphate ($NH_4H_2PO_4$)
Transport	Transport by truck, transport by refrigerated truck, transport by ship, transport by refrigerated ship,transport by car
Wastes	Wastewater, waste polyethylene, plastic waste, paperboard waste, general waste
Water	Ground water, tap water, ultrapure water

4.3 Visualisation of the LCA results

Specific attention was given to the visualisation of the LCA results. According to the level at which the contribution analysis was performed (e.g. subsystem, process, exchange), several types of graphs can be plotted (see Fig. 5).

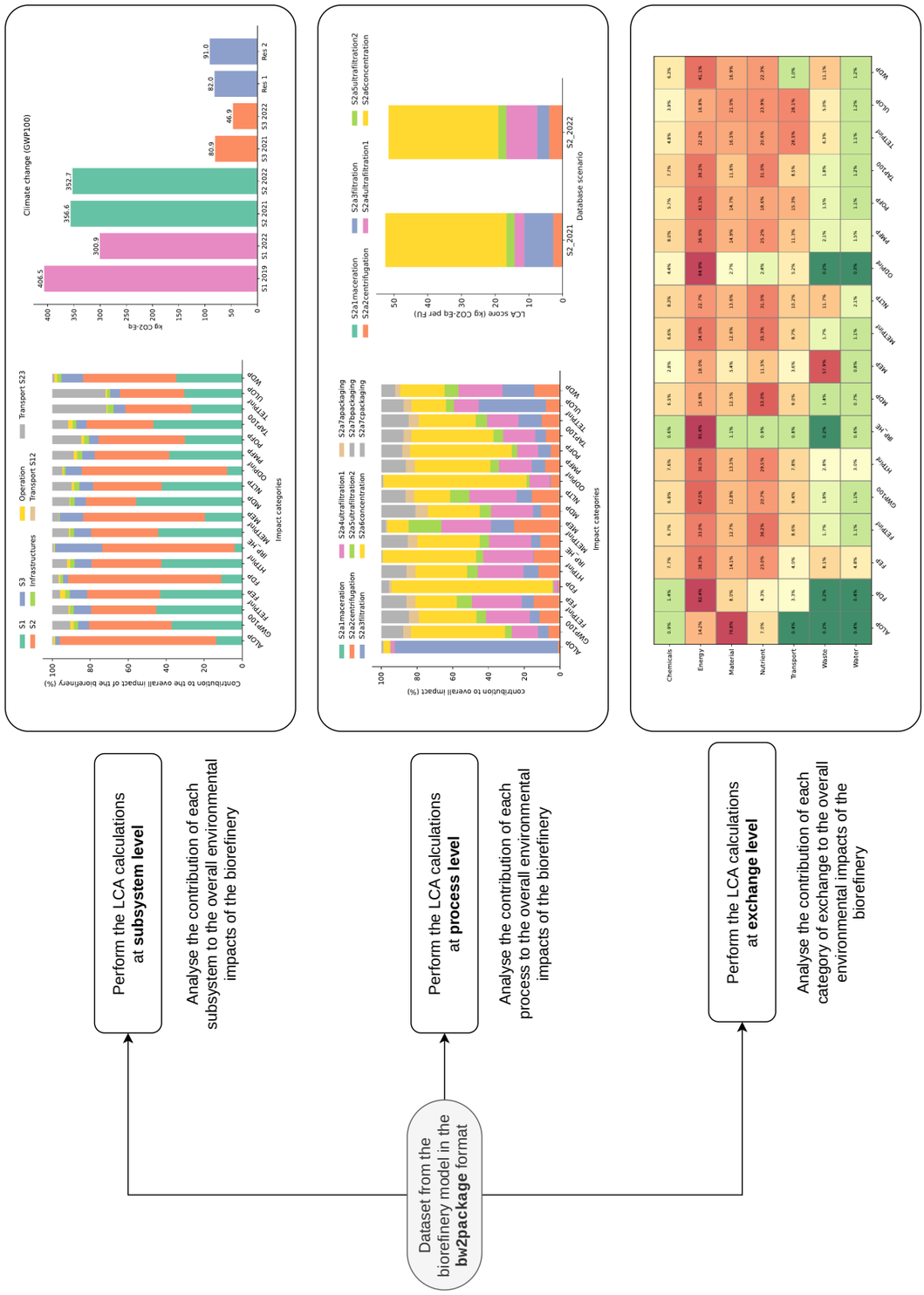

Figure 5: Three different types of visualisation according to the contribution analysis performed

References

[1] Luca Attene et al. “Efficient Nitrogen Recovery from Agro-Energy Effluents for Cyanobacteria Cultivation (Spirulina)”. In: *Sustainability* 15.1 (2023), p. 675.