Description of the biorefinery unit processes

Beyond phycocyanin: Environmental life cycle assessment of a European pilot scale biorefinery

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Content: This document describes the pilot Spirulina biorefinery analysed in the LCA study. Data were collected on-site in the frame of the SpiralG project. The biorefinery was subdivided into three subsystems which each correspond to an industrial partner of the project. The first subsystem (S1) corresponds to Spirulina cultivation and biomass pre-processing in Arborea (Italy). The second subsystem (S2) corresponds to phycocyanin extraction in Mèze (France). The third subsystem (S3) corresponds to the co-product treatment in Saint-Lô (France). This document provide the details needed to reproduce the LCA study while respecting the confidentiality of the data.

Contents

1 Infrastructures and operation

1.1 Infrastructures

Description

The Spirulina cultivation and biomass pre-processing plant consists of a greenhouse sheltering six open raceway ponds (ORPs) and a processing facility. The processing facility was established in a former cattle stable while the greenhouse was built on a sandy plot surrounded by agricultural fields. The greenhouse covers a total area of 5,184 m^2 and comprises three paddle wheels systems, a heating system, three pumps, and a control panel. Each paddle wheel system consists of a motor gear, pillow block bearings, and plastic paddles. The mixing time is regulated via the control panel. The heating system corresponds to pipes placed under the cover of the ORPs and linked to sensors to maintain the temperature of the culture medium at the optimum for Spirulina growth in winter. The pumps are used to harvest the Spirulina broth and transfer the biomass from the greenhouse to the processing facility. The equipment (i.e. stationary machines) used to dewater, shape, dry, and package the biomass as well as their

Table 1: Lifespan of the materials used to build the greenhouse and the Spirulina biomass pre-processing facility. **Abbreviations.** PE: polyethylene; PVC: polyvinyl chloride; PPR: polypropylene random copolymer; PEXc: cross-linked polyethylene; EPS: expanded polystyrene; HDPE: high-density polyethylene; PC: polycarbonate.

Material	Lifetime (years)	Comments
Nylon	2	Experts advice
Steel	20	Pérez-López et al. [2]
Insulated panels	20	Experts advice
Pumice bricks	20	Experts advice
Concrete bricks	20	Experts advice
Floor tiles	20	Experts advice
Concrete	20	Experts advice
Sand	20	Experts advice
PE pipes	10	Experts advice
PVC pipes	10	Experts advice
PPR pipes	10	Pérez-López et al. [2]
PEXc pipes	10	Experts advice
EPS bricks	10	Experts advice
PVC layer	10	Experts advice
HDPE film	10	Experts advice
PE film	10	Experts advice
PC	20	Experts advice

maintenance was not considered in this study due to a lack of data.

Data collection and aggregation

The dataset for “S1.A0a.Building” contains information regarding the amount of materials used to build the greenhouse, ORPs, and processing facility. The lifetime of each material, ranging from 5 to 20 years, was estimated from experts advice and the literature [2] (see Table 1). The filters of the vibrating filters (VFs) used to harvest Spirulina are made of nylon. The lifetime was estimated at 2 years since the filters are changed regularly. The piping systems were assumed to have a lifespan of 10 years while we attributes a lifespan of 20 years to all other materials (e.g. polycarbonate, steel, concrete). The total amount of each material used was calculated from the dimensions of a sample of the material and its weight. For instance, the total amount of polyethylene used in the piping system was calculated from the dimensions of the pipe (e.g. diameter, length) and the weight of a sample (e.g. 2 cm piece of polyethylene pipe). The length of the piping systems and dimensions of the greenhouse (e.g. length and width of the steel structure) were obtained from the blueprints of the facilities. The total amounts of materials used were divided by the lifetime of the material (e.g. 5, 10, 20 years) and the number of working days in a year (e.g. 330 days) to obtain a daily value. The data were then aggregated per type of material. The disposal of the materials was not considered in the study. Land occupation was included in the inventory data for the greenhouse only (i.e. 5,184 m^2).

Parameterisation

All materials listed in Table 1 were included in the biorefinery model. The inputs of “S1.A0a.Building” consist of the lifetime of the materials mainly. The technological period is only considered for the filters of the VFs since they were replaced between 2019 and 2022. Nylon was only used in period 2. The outputs of the model include the amount of materials expressed as a daily value.

1.2 Operation

Description

The operation of the Spirulina cultivation and pre-processing facilities was included in this study since significant amounts of water and electricity were used daily (e.g. air conditioning, lights). This activity includes all the inputs and outputs that were not associated with specific processes.

Data collection and aggregation

The dataset for "S1.A0b.Operation" contains information regrading the amount of water, electricity, and chemicals used in the general functioning of the Spirulina cultivation and pre-processing facilities.

Ground water: The total volume of ground water consumed in "S1.A0b.Operation" corresponds to the water used in the lavatory, to initially fill up the ORPs, and clean the processing facility (i.e. general cleaning of the floor). The volume of water used to initially fill up the ORPs was calculated from their theoretical volume. The value obtained was then divided per the number of working days (e.g. 330 days) to obtain a daily value. The volumes of water used in the lavatory and for cleaning were measured with a water meter installed outside the processing facility. The values were noted in mornings and evenings during the first data collection campaign in 2019. These data were assumed to be representative of the facility in 2022 as well (i.e. no changes were made in the facilities).

Electricity: The total amount of electricity consumed in "S1.A0b.Operation" corresponds to the electricity used in the processing facility by the lights, air conditioning, refrigerator, freezer, blast chiller, and plugs. The intensity of the current for each equipment listed above was measured in period 1, over three different days. The power was calculated from the intensities and multiplied by the running time of the equipment. In 2019, the freezer was not utilised. Since the data for the two periods were collected in July, the air conditioning was used in the same way. The only difference between 2019 and 2022 is related to the number of working hours per day. In 2022, the processing facility was running for 13.5 h instead of 12.5 h in 2019 which proportionally increased the electricity consumption.

Sodium hypochlorite (NaClO): A solution of NaClO at a concentration below 5% was used to clean the processing facility. Approximately 150 mL of NaClO were used weekly.

Wastewater: The total volume of wastewater generated in "S1.A0b.Operation" corresponds to the water used in the lavatory and for cleaning.

Solid wastes: Three bins were placed in the processing facility to collect the solid wastes during the two data collection campaigns. The plastic, paperboard, and general wastes were weighed on the last day and averaged to obtain a daily value.

Parameterisation

The inputs of the model "S1.A0.Operation" consist of the line (dry or wet), volume of the ORP in cubic meter (m3), number of ORPs, and number of working days. The outputs include the amount of electricity used in kilowatt hour (kWh), volume of ground water in liter (L), amount

Table 2: Schedule of the first data collection campaign for S1 in July 2019. WM: WATER METER.

Category	Data	Monday 22/07/19	Tuesday 23/07/19	Wednesday 24/07/19	Thursday 25/07/19	Friday 26/07/19	Saturday 27/07/19
biomass	broth	x	x	x	x	x	-
biomass	slurry	x	x	x	x	x	-
biomass	paste	x	x	x	x	x	-
biomass	wet spangles	x	x	x	x	x	-
biomass	dry spangles	-	x	x	x	x	x
biomass	humidity	-	-	-	x	x	-
water	general WM	-	-	x	x	x	x
water	lab WM	x	x	x	x	x	x
water	pond WM	-	x	x	x	x	x
electricity	electricity use	x	-	x	-	x	-
wastes	solid wastes	-	-	-	-	-	x
chemicals	nutrients	-	-	-	-	-	x

of sodium hypochlorite in kg, volume of wastewater in m3, and the amount of solid waste, i.e. plastic, paperboard, and general waste in kg.

2 Spirulina cultivation and biomass pre-processing

The subsystem 1 (S1) "Spirulina cultivation and biomass pre-processing" contains 8 activities: "Cultivation" (S1.A1), "Filtration" (S1.A2), "Dewatering" (S1.A3), "Shaping" (S1.A4), "Drying" (S1.A5), "Packaging - dry line" (S1.A6a), "Packaging - wet line" (S1.A6b), "Freezing" (S1.A7), and "Transport" (S1.A8).

The data for S1 were first collected in July 2019, from Monday 22/07/2019 to Saturday 27/07/2019. The schedule of the data collection is shown in Table 2. Spirulina is harvested daily and the biomass processed immediately. Therefore, the entire process chain, from "S1.A1,Cultivation" to "S1.A7.Freezing", fits into a day. Similar data were collected over 6 days which allowed to account for daily variations in the amount of dry Spirulina biomass produced. Due to unforeseen circumstances (e.g., technical issue with the water meters), some data could only be measured certain days over the week. Several assumptions were made to fill in the data gaps. These are described in the following Sections.

2.1 Cultivation

Description

Spirulina is cultivated in six ORPs (dimensions: 90 x 6 x 0.3 m), in a greenhouse. A ventilation system controls the air temperature. The side walls of the greenhouse can be opened or closed to increase or reduce the air flow. A shading net installed under the roof regulates the light incidence. The ORPs are operated continuously for approximately 330 days per year. In winter, the ponds are emptied, cleaned, and refilled to be reinoculated. The central baffle and the paddle wheels circulate the culture medium at a constant velocity 24 h per day. In winter, the temperature of the medium is maintained around the optimum for Spirulina growth (35-37°C) by circulating warm water (45-75°C) in the piping system under the cover of the ORPs. The warm water is provided by a co-located anaerobic digestion (AD) and combined heat and power (CHP) plants. Spirulina is inoculated and first grown using the Zarrouk culture medium [1]. The nutrients are then supplied once a week based on the amount of dry spangles produced as well as the chemical composition of the culture medium. The frequency of nutrient supply varies according to the seasons. A reduced amount of nutrients is required in winter when the growth rate of Spirulina is low. The ORPs are refilled with ground water once a week to compensate for the losses of water per evaporation. A monthly cleaning aims at removing algae and salt residues from the polyvinyl chloride (PVC) layer of the ORPs. A deep cleaning is performed once a year using detergents. The Spirulina broth is harvested 6 days per week using three VFs (i.e. one VFs for two ORPs).

Data collection and aggregation

The dataset for “S1.A1.Cultivation” consists of information regarding the amount of nutrients, ground water, and electricity used in the process as well as the volume of Spirulina broth harvested and the amount of plastic waste generated (e.g., packaging of the nutrients).

Spirulina broth: The volume of broth harvested from the ORPs in “S1.A1.Cultivation” equals the volume of broth entering the three VFs and filtered in “S1.A2.Filtration”. The volume of broth harvested (or filtered) was determined using two different methods during periods 1 and 2. In 2019, a water meter was installed on the pipeline connecting the ORPs 1 and 2 to the VF 1. The volume of broth harvested was measured for one VFs and two ORPs only (i.e. not for VFs 2 and 3 and ORPs 3 to 6). The values measured depended on the percentage of activity of the pump which ranged from 10 to 30%. This percentage is changed daily based on the quality of the broth which is assessed from its visual aspect and ease to be filtered. The relation between the percentage of activity of the pumps and the volume of broth harvested was established from the volumes measured for one VF and two percentages of activity (i.e. 20 and 30%). This relation was then used to determine the volume of broth filtered by the VFs 2 and 3 based on the percentage of activity of the pump used, which was written down at each harvesting time.

Box 1: Percentage of broth harvested

Percentage of broth harvested (%) = volume of broth harvested (or filtered) (m^3) / theoretical volume of the ORPs (m^3)

In 2022, the water meter installed on the VF 1 was moved to the greenhouse and used to measure the volume of culture medium pumped back to the ORPs during the harvesting step. The volume of broth harvested was calculated from the mass and water balance of “S1.A1.Cultivation” and “S1.A2.Filtration” considering that there were no losses of biomass (except the fraction of biomass recirculated to the ORPs). The concentration of Spirulina in the broth (i.e. before harvesting) was measured daily. In addition, the volume of broth harvested was determined from the relation established in 2019. Between the two periods, the filters of the VFs were replaced with nylon filters of a smaller porosity. The percentage of activity of the pumps was reduced from 20-30% to 10% and the harvesting time was doubled (i.e. from 3 h on 2019 to 6 h in 2022).

Box 2: Spirulina broth harvesting efficiency

Harvesting efficiency (%) = amount of Spirulina slurry obtained after filtration (kg DW-eq) / amount of broth harvested (or filtered) (kg DW-eq) x 100

The volumes of broth filtered obtained in 2022 using the two methods described above (i.e. correlation between the percentage of activity of the pump and the mass/water balances) were

Table 3: Nutrient recipes used for Spirulina cultivation in 2019 (period 1) and 2022 (period 2).

Nutrient salt	Period 1	Period 2	Unit
Sodium bicarbonate ($NaHCO_3$)	785	143.6	kg/week
Potassium nitrate (KNO_3)	135.53	164	kg/week
Tetrapotassium pyrophosphate (TKPP)	10.98	0	kg/week
Potassium sulfate (K_2SO_4)	6.28	0	kg/week
Ammonium phosphate ($(NH_4)_3PO_4$)	0	1.84	kg/week
Magnesium sulfate ($MgSO_4$)	3.14	2.44	kg/week
Chelated iron	2.57	1.12	kg/week

significantly different, i.e. 62.24 m^3/day obtained from the correlation and 42.87 m^3/day from the mass and water balance. The value obtained with the second method based on the measure of the volume of culture medium pumped back to the ORPs seemed more appropriate in this study. In fact, the harvesting efficiency, i.e the ratio between the amount of slurry and broth (in kg DW-eq), reached 50% when using the relation and 75% when using the mass and water balance. An harvesting efficiency of 50% seemed low considering the settings of the VFs. Therefore, the second method was selected in the model of "S1.A1.Cultivation". The percentage of broth harvested from the ORPs was calculated from the theoretical volume of the ORPs (see Box 2).

Ground water: The total volume of ground water consumed in "S1.A1.Cultivation" corresponds to the water used to refill the ORPs. The water used to initially fill up the ORPs (i.e. after the annual cleaning) was accounted for in the activity "S1.A0.Operation". The volume of water used to refill the OPRs was measured with a water meter installed in the greenhouse, on the pipe used to refill the ORPs. In 2019, only one ORP was refilled every night. In 2022, one ORP was refilled during the day and another one during the night. The value obtained in 2019 (27.72 m^3/day) corresponds to the average of three measurements. In 2022, the ORPs were refilled with an average flow rate of 1.17 m^3/h and the total volume of water supplied reached 6.61 m^3/day (average of five values).

Nutrients: The nutrients salts are supplied to the ORPs once a week. The analysis of the culture medium performed that day is used to calculate the amount of nutrients needed to meet the growth requirements of Spirulina. The quantities of magnesium sulfate and chelated iron are calculated from the amount of dry spangles produced over the five previous working days. The weekly values obtained are divided per seven to obtain a daily value (i.e. Spirulina grows continuously 7 days/week). As shown in Table 3, the amounts of nutrient supplied to the ORPs were reduced between 2019 and 2022 and the recipe slightly changed.

Electricity: The total amount of electricity used in "S1.A1.Cultivation" is related to the functioning of the paddle wheels, greenhouse fan, and control panel to open and close the side walls of the greenhouse. The electricity used to pump the Spirulina broth from the ORPs to the VFs was accounted for in "S1.A2.Filtration". The intensity of each equipment was measured in 2019 only (i.e. not in 2022), for three different days. It was assumed that the intensity of the equipment remained the same between the two periods. The power was measured from the value of intensity using two distinct formulas depending on the number of phases (see Box 5). Only one value was measured for the greenhouse fan (two values equaled to zero because the fan was off at the time of the measurement). The paddle wheels mix the culture medium continuously (i.e. 24h/day) at regular speed (which was similar in 2019 and 2022). The control panel was assumed to be used for 5 min per day, approximately. The total electricity consumption for "S1.A1.Cultivation" was identical in 2019 and 2022.

Box 3: From intensity to electricity consumption

Instant power (W) = intensity (A) x 1.73 x 0.85 x 380

Instant power (W) = intensity (A) x 1.73 x 0.85 x 234

Electricity consumption (kWh) = instant power (W) x running time / 1000

Plastic waste: The total amount of plastic waste generated in "S1.A1.Cultivation" corresponds to the nutrient bags discarded after use. In 2019, the amount of plastic wasted was measured for sodium bicarbonate ($NaHCO_3$) only. In fact, a large amount of $NaHCO_3$ was supplied to the ORPs (and therefore several bags were discarded) while only a limited amount of the other nutrients were used (no bags were discarded that week). The amount of plastic waste generated per kilogram of $NaHCO_3$ supplied to the ORPs was used to calculate the value for period 2.

Parameterisation

The inputs of the model "S1.A1.Cultivation" consists of the technological period (2019 or 2022), harvesting efficiency (%), concentration of Spirulina in the culture medium (g/L), daily amount of dry spangles produced (kg DW-eq), and amount of broth harvested (kg DW-eq). The outputs include the amount of electricity (kWh) and volume of ground water (L) used as well as the amount of plastic waste generated (kg). The calculation of the amount of broth harvested is included in "S1.A2.Filtration" since the volume of culture medium recirculated was calculated in this last activity. The quantities of nutrients supplied to the ORPs are calculated from the daily amount of dry spangles produced. Therefore, the model "S1.A1.Cultivation" depends on all the activities from "S1.A2.Filtration" to "S1.A5.Drying". The amounts of electricity, ground water, plastic waste, and nutrients were calculated from the data collected in 2019 and 2022 using linear scaling.

2.2 Filtration

Description

Spirulina biomass is harvested from the ORPs using three VFs as explained in "S1.A1.Cultivation". The volume of broth harvested (i.e. filtered) is calculated in "S1.A2.Filtration" from the mass and water balances. The amount of Spirulina obtained after filtration depends on the harvesting efficiency (see Box 2). The water content of Spirulina biomass was decreased from 90% to 10-15% between the broth and slurry. The culture medium is pumped back to the ORPs during the filtration process.

Data collection and aggregation

The dataset for "S1.A2.Filtration" comprises information regarding the amount of Spirulina broth, electricity, and water used in the process and cleaning as well as the amount of slurry produced and wastewater generated.

Spirulina slurry: The amount of Spirulina slurry produced in "S1.A2.Filtration" was measured by weighing the containers in which the biomass was collected. The data were measured daily for each of the six ORPs and averaged over six and five days in 2019 and 2022, respectively. The dry matter content of the slurry was measured from samples collected at the processing facility.

Electricity: The total amount of electricity consumed in "S1.A2.Filtration" corresponds to the electricity used by the pumps in the greenhouse as well as the pumps and motors of the VFs. The values of intensity for each of the equipment was measured in 2019, for three different days. The instant power was calculated and multiplied by the running time to obtain the total electricity consumed (see Box 5). The intensity of the equipment was assumed to be similar in 2019 and 2022. However, the running time of the VFs was increased from 3 h in 2019 to 6 h in 2022 due to the change of filters. The percentage of activity of the pumps was lowered to 10%. Since the relation between the electricity consumption and the percentage of activity of the pump was not established, we assumed that the VFs consume the same amount of electricity for the different percentages of activity of the pump. Considering this assumption, the electricity used in 2022 was probably overestimated.

Ground water: The total volume of ground water consumed in "S1.A2.Filtration" corresponds to the water used to clean the VFs and pumped back to the ORPs. This volume was measured daily with a water meter installed on the hose used to clean the VFs. The values were noted before and after their respective cleaning. The total volume of water used for cleaning varies according to the operator, from 0.04 to 0.10 m3. Since the water was pumped back to the ORPs, no wastewater was generated from "S1.A2.Filtration".

Parameterisation

The inputs of the model "S1.A2.Filtration" consists of the technological period (2019 or 2022), harvesting efficiency (%), concentration of Spirulina in the culture medium (i.e. before harvesting) (g/L), and amount of broth harvested (kg DW-eq). The outputs include the amount of electricity (kWh) and ground water (L) used as well as the amount of slurry produced (kg DW-eq). The harvesting efficiency corresponds to the ratio between amount of broth harvested and slurry obtained after filtration (see Box 2). This parameter was defined to account for the influence of the percentage of activity of the pump and the nature of the VFs filter (e.g. porosity) on the amount of slurry collected daily. An increase in the harvesting efficiency means that more slurry is collected from the same amount of broth filtered and at a constant percentage of activity of the pump. Since we assumed that the amount of electricity used by the VFs is constant for a same percentage of activity of the pump, an increased harvesting efficiency reduces the amount of electricity used per kilogram of slurry produced. The amount of biomass contained the culture medium pumped back to the ORPs is also reduced.

Box 4: Amount of broth harvested (or filtered)

Volume of broth (m3) = 100 / harvesting efficiency (%) x amount of slurry harvested (kg DW-eq) / concentration of Spirulina in the culture medium (g/L) Amount of broth (kg DW-eq) = concentration of Spirulina in the culture medium (g/L) x volume of broth (m3)

Box 5: Volume of water recirculated

Volume of water in the broth (L) = volume of broth (m3) x 1,000 - amount of broth (kg DW-eq) Volume of water in the slurry (L) = amount of slurry (kg) - volume of water in the slurry (L) Volume of water recirculated (L) = volume of water in the broth (L) - volume of water in the slurry (L)

2.3 Dewatering

Description

The Spirulina slurry obtained from "S1.A2.Filtration" is dewatered using three water presses (WPs). Each WP is equipped with two fabric bags in which the Spirulina slurry is poured and a water bladder filled up with ground water. After each batch, the Spirulina paste is collected and the bladder emptied and refilled. The clarified water exiting the WPs during the process (i.e. coming out of the Spirulina slurry) is discarded to the sewer system and treated was wastewater. In 2022, the water used to fill up the bladder was stored in a tank outside the processing facility and reused (i.e. closed-loop system).

Data collection and aggregation

The dataset for "S1.A3.Dewatering" contains information regarding the amount of slurry, water, and electricity used in the process and cleaning as well as the volume of wastewater generated and amount of paste produced.

Spirulina paste: The amount of Spirulina paste produced in "S1.A3.Dewatering" was measured by collecting the paste exiting the WPs and weighing the containers. Several samples of paste were collected to measure the dry matter content. The data were collected over six and five days in 2019 and 2022, respectively. The mass and water balances of "S1.A3.Dewatering" were used to calculate the amount of biomass lost during the process. The dry matter content of the biomass lost was considered as the average between the dry matter content of the Spirulina slurry and the paste. The volume of water exiting the Spirulina slurry was calculated from the mass and water balances.

Electricity: The total amount of electricity consumed in "S1.A3.Dewatering" corresponds to the electricity used by the three water presses. The electric current was measured in 2019, for three different days. Most of the values equaled zero since the electric current was measured between two batches, when the WPs were turned off. Two values of intensity were used to calculate the electricity consumption. The running times were determined from the number of batches per WP and per day, knowing that a WP cycle lasts for 50 min. The values used in 2019 and 2022 only differ by the number of batches conducted.

Ground water: The total volume of ground water consumed in "S1.A3.Dewatering" corresponds to the water used to fill up the bladders of the WPs at each batch and the water used for cleaning. In 2019, the volume used to fill up the bladder was measured once. The theoretical volume of the bladder (i.e. 80 L) was used to calculate the total volume of water used per day. In 2022, the water used to fill up the bladder was stored in a tank outside the processing facility and reused. Since the water was circulated in a closed-loop system, the volume was not included in the inventory datasets.

Wastewater: The total volume of wastewater generated in "S1.A3.Dewatering" corresponds to the volume of water used to fill up the bladders of the WPs and the water exiting the Spirulina slurry. In 2019, the exact amount of water used to fill up the bladders was considered as wastewater. In 2022, there was no wastewater associated with the WPs, except for cleaning. The water used for cleaning was measured with a water meter plugged to the hose used by the operators at the end of the process. The volume of water exiting the Spirulina slurry was collected in containers and weighed.

Parameterisation

The inputs of the model “S1.A3.Dewatering” consists of the technological period (2019 or 2022) and the amount of slurry produced in S1.A2.Filtration (kg DW-eq). The outputs include the amount of electricity (kWh) and the volume of ground water (L) used as well as the amount of paste produced (kg DW-eq) and the volume of wastewater generated (m3).

2.4 Shaping

Description

The Spirulina paste exiting the WPs was shaped into spangles. The maximum capacity of the shaping machine was 20 kg of paste. However, it was only filled with 5 to 10 kg per batch. Spangles were spread out on trays which were then stacked on trolleys.

Data collection and aggregation

The dataset for “S1.A4.Shaping” corresponds to the amount of paste, electricity, and water used in the process and cleaning as well as the amount of wet spangles produced and volume of wastewater generated.

Spirulina wet spangles: The amount of wet spangles produced in “S1.A4.Shaping” was measured by weighing each tray exiting the shaping machine using a scale with a precision of ± 0.002 kg. The weight of a tray was measured ten times and the average value was then subtracted to the weight of the trays with the wet spangles. The paste which remained in the shaping machine at the end of each batch was collected in a container and mixed with the paste of the next batch. The dry matter content of the wet spangles was not measured since it corresponds to the dry matter content of the paste. The values were averaged over six and two days in 2019 and 2022, respectively. The losses of biomass during the process were calculated from the mass and water balance of “S1.A4.Shaping”.

Electricity: The total amount of electricity consumed in “S1.A4.shaping” corresponds to the electricity used by the shaping machine. The electric current was measured in 2019, for three different days. The instant power was calculated and multiplied by the running time to obtain the total electricity consumed. The running time corresponds to the shaping itself, i.e. 20 sec per tray plus 1 min 45 sec for the preparation of the machine, and was calculated from the number of trays processed daily. No difference was made between the ORPs. The same method was used in 2022, using the intensity measurement from 2019.

Ground water: The total volume of ground water consumed in “S1.A4.shaping” corresponds to the water used to clean the shaping machine. The volume of water used was measured using a water meter. The value was noted before the start and at the end of the process. This value was measured in 2022 only.

Wastewater: The volume of water used for cleaning was considered as wastewater.

Table 4: Adjustement of the values for "S1.A5.Drying" in 2019.

Item	Initial value	Adjusted value	Unit
Amount of dry spangles	27.53	27.25	kg
Dry matter	96.8	96.8	% DW
Mass DW	26.64	26.38	kg DW-eq
Volume water	0.89	0.87	L

Parameterisation

The inputs consists of the technological period (2019 or 2022) and the amount of paste produced in S1.A3.Dewatering (kg DW-eq). The outputs include the amount of electricity (kWh) and the volume of ground water (L) used as well as the amount of wet spangles produced (kg DW-eq) and the volume of wastewater generated (m3).

2.5 Drying

Description

The trays with the wet spangles were stacked on trolleys. The trolleys were placed in the drying chambers and left at 50°C overnight. Each drying chamber can fit up to 120 trays piled on 4 trolleys (i.e. 240 trays on 8 trolleys in total). The trays were sorted per ORP and the dried spangles were not mixed. The dried spangles were packaged the next morning. The spangles left in the drying chamber on Saturday were packaged on Monday morning. They were left in the drying chamber at 40°C from Saturday evening to Monday morning. The dried spangles were weighed while filling in the plastic packaging bags. A small amount of dried spangles was wasted i.e. fell on the floor in the drying chamber or during the packaging step.

Data collection and aggregation

The dataset for "S1.A5.Drying" contains information regarding the amount of wet spangles, electricity, and heat used in the process as well as the amount of water evaporated and dry spangles produced.

Spirulina dry spangles: The amount of dry spangles was measured by weighing the bags filled up with spangles during the packaging step. The mass and water balance of "S1.A5.Drying" for 2019 showed that the amount of dry spangles (kg DW-eq) measured was slightly larger than the amount of wet spangles (kg DW-eq). The masses were balanced by calculating the amount of dry spangles from the mass and water balance, considering the amount of losses measured (i.e. 0.119 kg). The amount of water evaporated was calculated from the mass and water balance.

Electricity: The total amount of electricity consumed in "S1.A5.Drying" corresponds to the electricity used in the drying chamber by the fan and the batteries. The electricity consumption was measured from the values of intensities measured over three different days. In 2022, no electricity was used by the batteries. The wet spangles were dried using the heat from the co-located AD plant. The amount of heat used to replace the electricity was calculated by converting the amount of electricity used in 2019 for the batteries into megajoules.

Parameterisation

The inputs consists of the technological period (2019 or 2022) and the amount of wet spangles produced in S1.A4.Shaping (kg DW-eq). The outputs include the amount of electricity (kWh) used as well has the volume of water vapor emitted (L) as well as the amount of dry spangles produced (kg DW-eq).

2.6 Packaging - dry line

Description

The dry spangles produced in "S1.A5.Drying" are packaged using food grade plastic film. The plastic bags are filled with the dry spangles and closed using a sealer with cutter. The electricity consumption associated with the sealer was not considered in this study. Each bag of dry spangles is weighed and registered for traceability. At the end of the week, the black plastic bags containing the dried biomass are transported to the industrial harbour where they are stored at ambient air temperature in plastic boxes.

Data collection and aggregation

The dataset for the activity "S1.A6a.Packaging" comprises information regarding the amount of dry spangles and plastic packaging used in the process as well as the amount of dry spangles, packaged produced and the plastic waste generated.

Spirulina dry spangles, packaged: The amount of dry spangles, packaged corresponds to the amount of dry spangles plus the weight of the plastic used for packaging.

Plastic packaging: The amount of plastic packaging used in "S1.A6a.Packaging" was measured in 2019 by weighing the roll of plastic film before and after packaging. In 2022, the amount of plastic used was calculated from the length of the bags produced and the weight of a sample of plastic packaging.

Plastic waste: The amount of plastic waste generated in "S1.A6a.Packaging" corresponds to the pieces of plastic remaining after packaging. The pieces were weighed using a scale with a precision of ± 0.002 kg. The data were collected in 2019 and 2022.

Parameterisation

The inputs of the model "S1.A6a.Packaging" consists of the technological period (2019 or 2022) and the amount of dry spangles produced in S1.A5.Drying (kg DW-eq). The outputs include the amount of plastic packaging used (kg) used as well has the amount of dry spangles, packaged produced(kg DW-eq).

2.7 Packaging - wet line

Description

In the wet line, the paste produced in “S1.A3.Dewatering” is packaged in transparent plastic film. The data were collected in 2022 only (i.e. the wet line was not performed in 2019). The bags were prepared in advance and sealed using the same machine as in “S1.A6a.Packaging”. Approximately 2 kg of paste were added in each bag, flattened, vacuum sealed, and placed in a freezer. The vacuum bags are made of polyamide and polyethylene (for the inner layer in contact with the food). The proportion of each material is unknown. The packaging film was assumed to be polyethylene according to the datasets available in the ecoinvent 3.6 database. No plastic waste were generated.

Data collection and aggregation

The dataset for “S1.A6b.Packaging” comprises information regarding the amount of wet paste and plastic packaging used was well as the amount of wet paste, packaged produced.

Spirulina paste, packaged: The amount of Spirulina paste, packaged corresponds to the amount of paste plus the weight of the plastic used for packaging.

Plastic packaging: The plastic bags are prepared by the operators in advance. According to their size, the bags either weight 0.016 or 0.024 kg. The bags are filled up with 2 kg of paste each. The amount of plastic used in “S1.A6b.Packaging” was calculated from the weight of plastic per bag of 2 kg of paste (i.e. average of 0.02 kg of plastic per 2 kg of paste). The total amount of plastic was calculated from the total amount of paste produced per day, for the three days during which the wet line was used.

Parameterisation

The inputs of the model “S1.A6b.Packaging” consists of the technological period (2019 or 2022) and the amount of paste produced in S1.A3.Dewatering (kg DW-eq). The outputs include the amount of plastic packaging used (kg) used as well has the amount of paste packaged, produced (kg DW-eq).

2.8 Freezing

The wet paste, packaged obtained from “S1.A6b.Packaging” is stored in a freezer. The amount of electricity used by the freezer was calculated from its theoretical electricity consumption. The value on the label of the freezer is given in kWh/year. The daily amount of electricity was calculated by dividing the value by 365, assuming that the freezer works all year long. We assumed that the electricity consumed by the freezer does not depend on the amount of paste produced. The inputs of the model “S1.A7.Freezing” consists of the amount of paste, packaged produced in “S1.A6b.Packaging”. The outputs include the amount of electricity used by the freezer (kWh) used as well has the amount of paste packaged and frozen produced (kg DW-eq).

2.9 Transport

Description

The end-product of S1, i.e. the Spirulina paste, packaged, frozen or the dry spangles, packaged, are transported from the processing plant in Arborea (39.7829,8.5916) to the local industrial harbour (39.8676, 8.5576). A distance of 12.7 km was estimated from the GPS coordinates of the two geographical locations. This short distance was covered by small van or car. Regarding the dry line, the biomass was stored in the harbour and shipped to the South of France. It was assumed that the Spirulina biomass was transported by ferry from the industrial harbour of Oristano to Sète (43.4171, 3.7282). No information regarding the shipping route was provided. The direct distance between the two GPS coordinates was measured on Google Maps and corresponds to 562 km approximately. We assumed that the biomass was transported by lorry from Sète to Mèze (43.4187, 3.5940) where the phycocyanin was extracted. The distance corresponds to 21 km according to the GPS locations. The transportation distances are uncertain since the shipping routes are unlikely to be direct and usually include some deviations. Due to the lack of data, the distances mentioned above were used in this study. The biorefinery model (S1-2-3) is based on the dry line only. The data regarding the wet line were measured in 2022 with the objective to compare the two lines in a system limited to S1. Hypothetically, the Spirulina paste, packaged, frozen, is transported from the processing facility to the local harbour, shipped to South of France, and transported from Sète to Mèze following the same route as the dry spangles. However, the types of transports (e.g. lorry, ship) are different since the Spirulina paste needs to remain frozen in the wet line.

Data collection and aggregation

In the ecoinvent 3.6 database, the functional unit "ton-kilometer" is used for transport which represents the transport of one metric ton of goods over one kilometer. The data on transport are calculated for an average load factor and include an empty return trip. The amount of wet biomass (i.e. in kg and not kg DW-eq) was considered in the analysis. According to the data collected on-site, a lorry with a load of 7.5-16 metric tons seemed appropriate. Other loads include 3.5-7, 16-32, and >32 metric tons as well as the category "unspecified". In the database, the lorries are also qualified by their EURO level ranging from 3 to 6. They correspond to European Union regulations on emissions[1]. The type "EURO6" was selected in this study since the lorry is likely to be registered after 2015. In the case of the wet line, a 7.5-16 tons lorry equipped with a refrigeration machine to maintain the paste frozen was selected. The vehicles including freezing are only available for the location "GLO". Therefore, the same location was chosen for the dry line. It was assumed that the Spirulina paste and dry spangles were the only goods transported in the lorries and that the total amount of biomass transported does not exceed the load of 7.5-16 metric tons. With this last assumption, the only difference between the dry and the wet line is related to the type of transportation used, i.e. conventional or refrigerated. The Spirulina biomass was shipped in small ferries. If the dataset "market for transport, freight, sea, ferry (GLO)" could be used for the dry line, the equivalent with freezer was not available in the database, only for container ship. Therefore, the transport by container ship was selected for the wet and dry lines, with the only difference that the first container allowed to keep the biomass frozen. No datasets regarding the transport by small van was found. The dataset "market for transport, passenger car with internal combustion engine

[1] https://dieselnet.com/standards/eu/ld.php

Table 5: Ecoinvent 3.6 datasets selected for the transportation of Spirulina biomass.

Trip	Line	Ecoinvent 3.6 dataset
Van/car	Both	Market for transport, passenger car with internal combustion engine
Ship	Dry	Market for transport, freight, sea, container ship
Ship	Wet	Market for transport, freight, sea, container ship with reefer, freezing
Lorry	Dry	
Lorry	Wet	Market for transport, freight, lorry with refrigeration machine, 7.5-16 ton, EURO6, carbon dioxide, liquid refri(...)-15
Lorry	Wet	Market for transport, freight, lorry with refrigeration machine, 7.5-16 ton, EURO5, R134a refrigerant, freezing

(RER)” was used as proxy.

Parameterisation

The inputs of the model “S1.A8.Transportation” consist of the amount of Spirulina paste or dry spangles (kg DW-eq), the amount of Spirulina paste or dry spangles, packaged (kg DW-eq) as well as the car, ship, and lorry distances (km), and the type of the line (i.e. dry or wet). The outputs include the data in ton.km. In the biorefinery model, all the mass flows are expressed in kg DW-eq (i.e. the dry matter contents are omitted and there is no mention of wet mass). In the case of transportation, the wet mass is required to calculate the values in ton.km. The model takes as input the amount of Spirulina paste or dry spangles in kg DW-eq. The wet mass is calculated from the average dry matter content of the Spirulina paste and dry spangles obtained in 2019 and 2022 (included as constants in the model). The amounts of Spirulina paste or dry spangles, packaged, are used to calculate the amount of plastic packaging used (i.e. which corresponds to the difference between the wet and dry biomass). The amounts of wet biomass and plastic packaging are then added to obtain the amount of wet biomass, packaged to be transported. The distance for each section of the transportation route is then multiplied by the amount of biomass transported.

3 Phycocyanin extraction

3.1 Maceration

Description

The maceration step consists of mixing the Spirulina dry spangles obtained in “S1.A5.Drying” with tap water. The product obtained is a mix of Spirulina in water. The equipment used comprises a 2,000 L tank and a mixing system including a paddle, rotor, and motor. In 2021, the content of the tank was mixed overnight (i.e. 25.33 h). In contrast, the mixing time was reduced to 40 min in 2022 which corresponds to the initial mixing of the dry spangles into water and the mixing at the start of the centrifuge to ensure homogeneity.

Data collection and aggregation

The dataset for “S2.A1.Maceration” contains information regarding the amount of dry spangles, water, electricity, and chemicals used in the process and cleaning as well as the wastewater and plastic waste generated and the Spirulina mix produced.

Mix of Spirulina in water: The polyethylene (PE) bags, in which the Spirulina dry spangles were packaged, were selected randomly. The labels (i.e. origin code) were written down for traceability. The content of the bags was added to the maceration tank. The plastic bags were discarded to general waste i.e. were not recycled. The amount of mix obtained after maceration was not measured since it was directly pumped into the centrifuge. According to the mass and water balances, the conservation of mass approach was applied to calculate the amount of mix of Spirulina (see Figure 5). It was assumed that no losses occurred during the process and that the total amount of mix equals the amount of dry spangles plus the volume of water added to the tank. In fact, at the end of the first centrifugation step, the maceration tank was rinsed with water to limit the losses of biomass. The dry matter content of the dry spangles was not measured. It was assumed that it corresponds to the same dry matter content as in “S1.A5.Drying” although in reality, the bags selected did not correspond to this exact batch of Spirulina biomass.

Tap water: The total volume of tap water used in “S2.A1.Maceration” corresponds to the water used for maceration and cleaning. The tank was filled up with tap water before addition of the dry spangles. The volume of water added was measured using a water meter installed on the hose used to fill up the tank (precision: ±1 L). The volume of water used to clean the tank was calculated from the water flow of the hose used and the duration of the cleaning. The volume of water used to rinse the tank after the first centrifugation step was calculated from the mass and water balance of “S2.A2.Centrifugation” and was not accounted for in “S2.A1.Maceration”.

Wastewater: The total volume of wastewater produced in “S2.A1.Maceration” corresponds to the volume of tap water used for cleaning. A negative sign was added to the volume of wastewater following the Opposite Direction Approach (also known as double-negative approach).

Electricity: The total amount of electricity used in “S2.A1.Maceration” corresponds to the electricity used for mixing only. The electric power of the mixing system, which includes a motor, rotor, and paddle, was measured using a power meter plugged to the control panel of the motor (precision of the power meter: ±0.001 kWh). In 2021, the biomass was mixed overnight (i.e. for 25.33h) while in 2022, the biomass was only mixed when adding the dry spangles to the water and when pumping the mix into the centrifuge (i.e. for 46 min). The amount of electricity used in 20121 was considerably higher than in 2022 (i.e. 4.445 vs 0.138 kWh).

Plastic waste: The total amount of plastic waste generated in “S2.A1.Maceration” was calculated from the data obtained in “S1.A6a.Packaging”. The amount of plastic used to packaged one kilogram of dry spangles was used to calculate the amount of plastic used to packaged the dry spangles used in the maceration step. A negative sign was added to the amount of plastic waste following the Opposite Direction Approach (also known as double-negative approach).

Parameterisation

The inputs of the “S2.A1.Maceration” model consists of the technological period (2021 or 2022) and amount of dry spangles, packaged (kg DW-eq). The outputs include the amount of electricity (kWh) and the volume of tap water (L) used as well as the amount of Spirulina mix (kg DW-eq), the volume of wastewater (m3), and the amount of plastic waste (kg) generated. The volume of tap water used in the process was calculated in order to reach a concentration of Spirulina, based on the amount of dry spangles used. The rest of the outputs (e.g. water for cleaning, electricity, wastewater, plastic waste) were calculated from a simple cross-multiplication (rule of three).

3.2 Centrifugation

Description

The centrifugation process allowed to separate the solution of water and soluble molecules (i.e. phycocyanin) from the rest of the biomass. Tap water was first circulated through the centrifuge to initiate the process. The solution of Spirulina in water from “S2.A1.Maceration” was then centrifuged. The pellet and supernatant were stored in distinct tanks. The pellet corresponded to the co-product A (CPA) and had the aspect of a dark green slurry. In contrast, the supernatant had a blue-green colour revealing the presence of phycocyanin.

Data collection and aggregation

The dataset for “S2.A2.Centrifugation” contains information regarding the amount of Spirulina mix, electricity, water, and chemicals used in the process and cleaning as well as the amount of wastewater generated and supernatant and CPA produced.

Supernatant and pellet (CPA): The amount of CPA produced in “S2.A2.Centrifugation” was measured by weighing the container in which it was collected. In 2021 and 2022, the CPA was collected in a refrigerated tank and the dry matter content was measured with a fast moisture analyser. The measurements of dry matter content were performed three times. If the values were significantly different, the measurement was conducted using a oven at 110°C. The permeate obtained after centrifugation was directly pumped into the filtration machine. The amount of permeate produced was calculated from the mass and water balance of “S2.A2.Centrifugation” (see Figure 6). The volume of water used to rinse the tank after the first centrifugation step was calculated from the water balance and added into the product.

Electricity: The total amount of electricity used in “S2.A2.Centrifugation” consists of the electricity used be the refrigerated tank to store the CPA and the electricity used by the centrifuge during the process and cleaning. The refrigerated tank used to store the CPA consumed electricity to maintain the temperature of CPA at 10°C. The cooling and mixing ran for 25.83h in 2022. The electricity used by the refrigerated tank was not measured in 2021. The amount of electricity used by the centrifuge was measured with a power meter plugged to the machine from the start to the end of the process and cleaning, in 2021 and 2022.

Tap water: The total volume of tap water used in “S2.A2.Centrifugation” corresponds to the volume of water used to initiate and clean the centrifuge. The cleaning programme of the centrifuge used water, sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) or nitric acid (HNO_3). The volume of tap water used for cleaning was measured with a water meter or calculated from the water flow of the hose, knowing the duration of the cleaning. The total volume of tap water also included the water used to prepare the solutions of NaOH and H_2O_2) or HNO_3.

Wastewater: The total volume of wastewater generated in “S2.A2.Centrifugation” consists of the water used to initiate the centrifuge (which was discarded to the sewer system) and the water used for cleaning.

Sodium hydroxide (NaOH): In 2021 and 2022, a solution of NaOH was used to clean the centrifuge. In ecoinvent 3.6, the datasets for the production of NaOH correspond to the pure substance. NaOH pearls were used in "S2.A2.Centrifugation" which correspond to a purity close to 100%. Therefore, no calculations were needed to convert real data into data used to model background activities with ecoinvent.

Nitric acid (HNO_3): In 2022, a commercial solution of HNO_3 at 53% was used in the cleaning program. In ecoinvent 3.6, the dataset for nitric acid production is expressed for the pure substance. The amount of HNO_3 at 53% used in the process served as basis for the calculation of the pure amount of HNO_3 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 53%. A value of 1.33 g/cm^3 or kg/L was used for the density of HNO_3 at 53%.

Hydrogen peroxide (H_2O_2): In 2021, a commercial solution of H_2O_2 at 35% was used in the cleaning program. In ecoinvent 3.6, the dataset for hydrogen peroxide production is expressed for the pure substance. The amount of H_2O_2 at 35% used in the process served as basis for the calculation of the pure amount of H_2O_2 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 35%. A value of 1.13 g/cm^3 or kg/L was used for the density of HNO_3 at 35% and 20°C.

Parameterisation

The inputs of the "S2.A2.Centrifugation" model consist of the technological period (2021 or 2022) and the amount of Spirulina mix (kg DW-eq). The outputs include the amount of electricity (kWh) and the volume of tap water (L) used as well as the amount of supernatant and co-product A (CPA) (kg DW-eq) produced. The amount of sodium hydroxide (kg), hydrogen peroxide (kg), nitric acid (kg), and ultrapure water (L) used were included as well as the volume of wastewater (m3) generated.

3.3 Filtration

Description

The supernatant from "S2.A2.Centrifugation" was pumped through a series of cellulose filters with decreasing porosities to isolate the molecules smaller than 0.2 μm. The larger particles accumulated on the filters at each filtration step. They were considered as losses of biomass. If the cellulose filters were initially recycled (i.e. discarded to the paperboard waste), the cellulose filters recently underwent experimental analyses to evaluate their potential use as feedstock for anaerobic digestion. The losses of supernatant during the process were limited by recirculating the product accumulated on the bottom tray of the filtration machine, at the end of each filtration batch. The filtration machine was cleaned with detergent and water between two batches when the difference of porosities was large.

Data collection and aggregation

The dataset for "S2.A3.Filtration" contains information regarding the amount of supernatant, electricity, water, and cellulose filters used in the process and cleaning as well as the amount of filtrate produced and wastewater generated.

Filtrate and biomass accumulated on the filters: The filtration produced by "S2.A3.Filtration" contained molecules smaller than 0.2 μm, including phycocyanin and other small proteins. Since the filtrate was directly pumped into the ultrafiltration machine, the amount was calculated from the mass and water balance (see Figure 7). It was assumed that no losses of product occurred during the process. The volume of water contained in the filtrate was calculated for the water balance. In 2022, the amount of biomass accumulated on the cellulose filters was measured by drying all the filters in the sun for two days and comparing the weight of the novel filters with the dry filter with biomass. It was assumed that drying the cellulose filters in the sun was effective to reduce the water content of the filters and the biomass. In 2021, only one piece of 5 x 5 cm of a wet filter was used to perform a dry matter content measurement. The results obtained for the specific type of filter were used to calculate the amount of biomass accumulated on the other filters. It was assumed that the amount of biomass accumulated on the different types of filters was similar. However, the visual aspect of the filters after use show that the amount of biomass accumulate vary according to the porosity of the filters.

Cellulose filters: The amount of cellulose filters used in “S2.A3.Filtration” was measured by weighing the new filters in their packages, before use. The measurements were conducted for each porosity and type of filter. The number of filters used varies considerably between 2019 and 2022, using 42 and 380, respectively.

Electricity: The total amount of electricity used in “S2.A3.Filtration” corresponds to the electricity used by the pump which circulates the supernatant through the cellulose filters. The intensity of the pump was measured in 2021 and used to calculate the amount of electricity used from the duration of each filtration step. In 2022, no electricity was used since the pump worked with compressed air. The amount of compressed air used was not measured since the specific amount used by the pump could not be isolated from the total consumption of compressed air in the facility (in which processes other than PC extraction were also performed).

Tap water: The total volume of water used in “S2.A3.Filtration” corresponds to the water used to rinse the filtration machine between the filtration steps (i.e. when changing the filters) and at the end of the process, as well as the water used to rinse the containers in which the filtrate was stored after each filtration step. The volumes were all measured with a water meter installed on the hose used for cleaning.

Wastewater: The total volume of wastewater generated in “S2.A3.Filtration” corresponds to the water used to clean the filtration machine. The water used to rinse the filters at the end of each filtration step was added in the product and not discarded to the sewer system.

Detergent: A diluted solution with detergent was used to clean the filtration machine between the filtration steps. In 2021 and 2022, the volume of detergent used was measured for the whole process. The bottle was weighed on the first day of data collection and on the last day. The difference between the two values corresponded to the volume of detergent used.

Parameterisation

The inputs of the “S2.A3.Filtration” model consists of the technological period (2021 or 2022) and the amount of supernatant (kg DW-eq) produced in S2.A2.Centrifugation. The outputs include the amount of electricity (kWh) and the volume of tap water (L) used as well as the amount of filtrate (kg DW-eq) produced. The amount of cellulose sheets (kg) used, the volume of wastewater (m3) and paperboard waste (kg) generated were also accounted for.

3.4 Ultrafiltration 1

Description

The first ultrafiltration (UF1) process consists of isolating the fraction of the molecules smaller than 0.2 μm and larger than 20 kDa. The retentate from UF1 was concentrated in the internal tank of the machine up to a volume of 70 L. This fraction corresponds to the product of interest i.e. the blue extract. The volume of permeate, a colourless solution containing small molecules, was measured from the data logger. This fraction constitutes the co-product D (CPD).

Data collection and aggregation

The dataset for “S2.A4.Ultrafiltration1” contains information regarding the amount of filtrate, electricity, water, chemicals used in the process and cleaning as well as the amount of permeate and retentate produced and the volume of wastewater generated.

Permeate and retentate from UF1: The retentate from UF1 (or blue extract 1), which contains the large molecules including phycocyanin, was directly used in the second ultrafiltration machine. The amount of blue extract 1 was calculated from the mass and water balance (see Figure 8). The amount of permeate (or CPD) containing small molecules was noted from the data logger of the ultrafiltration machine. CPD is colourless (or has a light blue colour). It was stored in a 2,000 L tank attached to the ultrafiltration machine while the blue extract was concentrated in the internal tank.

Electricity: The total amount of electricity used in “S2.A4.Ultrafiltration1” corresponds to the electricity used by the ultrafiltration machine in the process and cleaning. The electricity consumption was measured using a power meter plugged to the ultrafiltration machine from the start to the end of the process and cleaning. The values were expressed in kWh.

Tap water: The total volume of tap water used in “S2.A4.Ultrafiltration1” corresponds to the water used in the cleaning programme and to rinse the equipment at the end of the process. The volume of water used in the cleaning programme was noted from the data logger of the ultrafiltration machine. The volume of water used to rinse the equipment was calculated from the water flow of the hose and the duration of cleaning.

Sodium hydroxide (NaOH): A commercial solution of NaOH at 30% was used in the cleaning program. In ecoinvent 3.6, the dataset for sodium hydroxide production is expressed for the pure substance. The amount of NaOH at 30% used in the process served as basis for the calculation of the pure amount of NaOH (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 30% A value of 1.36 g/cm^3 or kg/L was used for the density of NaOH at 30%.

Hydrogen peroxide (H_2O_2): A commercial solution of H_2O_2 at 4% was used in the cleaning program. In ecoinvent 3.6, the dataset for hydrogen peroxide production is expressed for the pure substance. The amount of H_2O_2 at 4% used in the process served as basis for the calculation of the pure amount of H_2O_2 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 4% A value of 1.04 g/cm^3 or kg/L was used for the density of H_2O_2 at 4% and 20°C.

Phosphoric acid (H_3PO_4): A commercial solution of H_3PO_4 at 75% was used in the cleaning program. In ecoinvent 3.6, the dataset for phosphoric acid production is expressed for the pure substance. The amount of H_3PO_4 at 75% used in the process served as basis for the calculation of the pure amount of H_3PO_4 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 75% A value of 1.11 g/cm^3 or kg/L was used for the density of H_3PO_4 at 75% and 20°C.

Parameterisation

The inputs of the "S2.A4.Ultrafiltration1" model consists of the technological period (2021 or 2022) and the amount of filtrate (kg DW-eq) produced in S2.A3.Filtration. The outputs include the amount of electricity (kWh) and the volume of tap water (L) used as well as the amount of blue extract and co-product D (CPD) (kg DW-eq) produced. The amount of sodium hydroxide (kg), hydrogen peroxide (kg), nitric acid (kg), and ultrapure water (L) used were included as well as the volume of wastewater (m3) generated.

3.5 Ultrafiltration 2

Description

The second ultrafiltration (UF2) allows to concentrate the blue extract from "S2.A4.Ultrafiltration1" in a volume of 20 L, using the same membrane porosity in an ultrafiltration machine with a smaller dead volume. This process was only performed in 2022. Due to technical issues, the second ultrafiltration of the blue extract 1 was interrupted in 2021. The retentate from UF2 corresponds to the final product of interest, the blue extract 2. The permeate contains small molecules and corresponds to the CPD.

Data collection and aggregation

The dataset for "S2.A5.Ultrafiltration2" contains information regarding the amount of retentate from UF1, electricity, water, and chemicals used in the process and cleaning, as well as the volume of wastewater generated and permeate and retentate of UF2 produced.

Permeate and retentate from UF2: The amount of retentate (or blue extract 2) and permeate from UF2 were measured by weighing the containers in which they were stored (precision of the scale ±0.5 L). In reality, the permeate from UF2 was discarded to the sewer system and treated as wastewater. However, this volume could be added to the CPD produced in "S1.A4.Ultrafiltration1". The dry matter content of the CPD was too low to be measured accurately. The dry matter content of the blue extract 2 was measured and a value of 6.55% DW was obtained. The mass and water balance of "S1.A5.Ultrafiltration2" showed that the amount of biomass distributed between the CPD and blue extract 2 was larger than the biomass content of the blue extract 1. The mass flows were balanced by considering that the dry matter content of the CPD was 0.1% DW, keeping the same amount of CPD produced (see Figure 9). The amount of blue extract 2 produced was reduced by 10.3% between the original and adjusted values. In addition, the concentration of phycocyanin in the blue extract 2 was calculated from the measurement of the absorbance of the solution at 616 nm. The absorbance was also measured for a sample of mix of Spirulina in water obtained after "S2.A1.Maceration" and treated in the laboratory (i.e. extraction of PC at laboratory scale via centrifugation and filtration). The value obtained was compared with the concentration calculated form the sample of blue extract 2. The comparison between the two values was meant to show the efficiency of extraction at pilot scale. However, it seems that the data obtained are not reliable. The dry matter content of the blue extract 2 was measured. There was 50% different in the amount of biomass between the blue extract 2 and the pure phycocyanin calculated from the concentration. The blue extract contains 50% of PC and 50% of other molecules. The density of the blue extract was measured as well.

Calculation of the concentration of phycocyanin in the blue extract 2.

$$C_{PC} = \frac{A_{616nm} \times d}{7} \tag{1}$$

with: C_{PC} the concentration of phycocyanin in the blue extract 2, A_{616nm} the absorbance of the blue extract at 616 nm, and d the dilution rate used to measure the absorbance.

Electricity: The total amount of electricity used in “S2.A5.Ultrafiltration2” consists of the electricity used by the ultrafiltration machine during the process and cleaning. The electricity used during the process was measured using a power meter plugged to the control panel of the ultrafiltration machine. The values were collected in kWh directly. The intensity of the machine was measured to calculate the electricity consumed during the cleaning. The value of power obtained was multiplied by the duration of the cleaning.

Tap water: The total volume of tap water used in “S2.A5.Ultrafiltration2” consists of the water used for cleaning which was measured with a water meter. The water used to prepare the solution of sodium hydroxide (NaOH) and to rinse the equipment was calculated from the water flow of the hose and the duration of the cleaning.

Wastewater: The total volume of wastewater generated in “S2.A5.Ultrafiltration2” corresponds to the water used for cleaning.

Sodium hydroxide (NaOH): A commercial solution of NaOH at 30% was used in the cleaning program. In ecoinvent 3.6, the dataset for sodium hydroxide production is expressed for the pure substance. The amount of NaOH at 30% used in the process served as basis for the calculation of the pure amount of NaOH (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 30% A value of 1.36 g/cm^3 or kg/L was used for the density of NaOH at 30%.

Nitric acid (HNO_3): In 2022, a commercial solution of HNO_3 at 53% was used in the cleaning program. In ecoinvent 3.6, the dataset for nitric acid production is expressed for the pure substance. The amount of HNO_3 at 53% used in the process served as basis for the calculation of the pure amount of HNO_3 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 53% A value of 1.33 g/cm^3 or kg/L was used for the density of HNO_3 at 53%.

Parameterisation

The inputs of the “S2.A5.Ultrafiltration2” model consists of the amount of blue extract (kg DW-eq) produced in S2.A4.Ultrafiltration 1. The outputs include the amount of electricity (kWh) and the volume of tap water (L) used as well as the amount of blue extract and CPD (kg DW-eq) produced. The amount of sodium hydroxide (kg), nitric acid (kg), and ultrapure water (L) used were included as well as the volume of wastewater (m3) generated. The technological period was not included since the second ultrafiltration step was only conducted in 2022 (i.e. during the period 2).

3.6 Concentration

Description

The CPD obtained from the activity "S2.A4.Ultrafiltration 1" was transferred to an evaporator and concentrated under vacuum conditions up to a volume of 70 L. The CPD from UF2 was not used in "S2.A6.Concentration" although it was included in the model (see section "parameterisation"). The volume of distillate collected into 1,000 tanks was measured as well as the concentrate produced. Samples of the fractions were taken to measure the dry matter content of two fractions and estimate the losses of product in the distillate.

Data collection and aggregation

The dataset for "S2.A6.Concentration" contains information regarding the amount of CPD, gas, electricity, and water used in the process and cleaning as well as the amount of CPD concentrate produced and volume of wastewater generated.

CPD concentrate and distillate: The amount of concentrate obtained after evaporation was measured by weighing the containers in which it was collected. The distillate had a light blue colour showing that there were some losses of product during evaporation. The dry matter contents of the concentrate and distillate were measured from samples collected at the end of the process. The volume of distillate was measured by weighing the contained in which it was stored. The mass and water balance of "S2.A6.Concentration" led to a total dry biomass larger in the outputs than the input. The system was balanced by calculating the amount of dry biomass in the distillate from the CPD and CPD concentrate. The corrected values were used in this study (see Figure 10). The difference in the water balance between the inputs and outputs was modelled as a volume of water added to the product (i.e. 38.5 L).

Natural gas: The total amount of natural gas used in "S1.A6.Concentration" corresponds to the natural gas used by the evaporator. The gas consumption was calculated from the difference of the values measured at the start and the end of the process. No natural gas was used for cleaning. In fact, the amount of natural gas used to start the evaporator corresponds to half of the consumption over the entire process. The amount of natural gas used to evaporate the CDP from UF1 and UF2 was calculated using linear scaling.

Electricity: The total amount of electricity used in "S2.A6.Concentration" corresponds to the electricity used by the evaporator during the process. No electricity was used for cleaning. The electricity consumption was measured with a power meter plugged to the control panel of the evaporator from the start to the end of the process. The amount of electricity used to evaporate the CDP from UF1 and UF2 was calculated using linear scaling.

Tap water: The total amount of tap water used in "S1.A6.Concentration" corresponds to the water used for cleaning and the water added into the product. The water added into the product was calculated from the water balance (see Figure 10). The volume of water used for cleaning was calculated from the water flow of the hose used and the duration of the cleaning.

Wastewater: The distillate from evaporation was considered as wastewater as well as the water used for cleaning. A negative sign was added to the volume of wastewater following the Opposite Direction Approach (also known as double-negative approach).

Parameterisation

The inputs of the “S1.A6.Concentration” model consists of the amount of CPD (kg DW-eq) produced in S2.A4.Ultrafiltration 1 and S2.A5.Ultrafiltration 2. The outputs include the amount of electricity (kWh), natural gas (m3), and the volume of tap water (L) used as well as the amount of CPD concentrate (kg DW-eq) produced and the volume of wastewater (m3) generated. The technological period was not included since the concentration step was performed in 2022 only (i.e. during the period 2).

3.7 Packaging of the blue extract

Description

The blue extract 2 obtained after “S2.A6.Ultrafiltration2” was packaged in HDPE containers and stored in a freezer.

Data collection and aggregation

The dataset for the activity “S2.A7a.Packaging” comprises information regarding the amount of plastic used to package the blue extract from “S2.A5.Ultrafiltration2”. The data were collected in 2022 only.

Blue extract 2, packaged: The amount of blue extract 2, packaged produced was calculated from the mass balance assuming that there were no losses during the process. The amount of blue extract 2, packaged corresponds to the amount of blue extract 2 plus the weight of the packaging.

Plastic packaging: The total amount of plastic packaging used was measured in two different ways. In 2022, the plastic containers used to package the phycocyanin were weighed empty, with the lid. Five containers of each size were weighed using a scale with a precision of ± 0.01 g. The containers were weigthed at the end of the packaging and the weight of the plastic containers (empty) was subtracted.

Parameterisation

The inputs consists of the amount of blue extract (kg DW-eq) produced in S2.A5.Ultrafiltration 2. The outputs include the amount of plastic packaging used (kg)and the amount of blue extract packaged produced (kg DW-eq).

3.8 Packaging of CPA

The inputs consists of the amount of the CPA (kg DW-eq) produced in S2.A2.Centrifugation. The outputs include the amount of plastic packaging used (kg)and the amount of CPA packaged produced (kg DW-eq). The CPA was stored in a 200 L containers and sent to the North of France for further processing.

3.9 Packaging of CPDc

The inputs consists of the amount of the CPD concentrate (kg DW-eq) produced in S2.A6.Concentration. The outputs include the amount of plastic packaging used (kg)and the amount of CPD packaged produced (kg DW-eq).

3.10 Transport

Description

The CPA produced in "S2.A2.Centrifugation" and packaged is transported from Mèze (43.4187, 3.5940) to Saint-Lô (49.1103, -1.05608). A distance of 985 km was estimated from the GPS coordinates of the two geographical locations. This distance was covered by refrigerated lorry.

Data collection and aggregation

Similarly as "S1.A8.Transport", the transportation was modelled with ecoinvent 3.6 datasets. The amount of wet CPA was calculated from the average of the dry matter contents obtained in 2021 and 2022. A lorry with a load of 7.5-16 metric tons was selected, with a EURO6 level. The CPA was frozen during the transportation. Therefore, a refregirated lorry with freezing was selected for the "GLO" location. It was assumed that the CPA was the only good transported in the lorry and that the total amount of biomass does not exceed the load of 7.5-16 metric tons.

Parameterisation

The inputs of the "S2.A8.Transport" model consists of the CPA (kg DW-eq), CPA, packaged (kg DW-eq), and the transportation distance. The outputs include the data in ton×km.

4 Co-product A treatment

4.1 Extraction

Description

The CPA from S2 was transported by refrigerated lorry, frozen during the shipping, and received thawed. The extraction consists of an acid hydrolysis of the proteins in presence of sulfuric acid (H_2SO_4) at 80°C. This method targets the production of a nitrogen-rich amino acid fraction with heterogeneous molecular weights. The CPA was transferred into a thermo-regulated tank and brought up to 80°C before addition of H_2SO_4. Five samples of the mixture were collected to follow the kinetics of the chemical reaction. The product obtained is called "hydrolysate".

Data collection and aggregation

The dataset for "S3.A1.Extraction" contains information regarding the amount of CPA, electricity, tap water, and chemicals used in the process and cleaning of the equipment as well as the volume of wastewater generated and amount of hydrolysate produced.

Hydrolysate: The CPA was packaged in plastic containers (e.g. one 200 L container in 2021 and several 25 L containers in 2022). The containers were rinsed and reused in the facility. Therefore, they were not considered as plastic waste in the LCA study. The amount of CPA was measured by weighing the content of the container(s) previously poured in a bucket on a scale (precision: ±0.001 kg). A sample of CPA was collected before the addition of H_2SO_4 to measure the dry matter content from an homogeneous mixture. Four samples were collected during the acid hydrolysis to follow the kinetics of the reaction. Each sample corresponded to approximately 200 g. The total amount of biomass sampled was not accounted for in the inventory data since the samples were not discarded but frozen for further laboratory analyses. However, the samples were included in the mass and water balance of "S3.A1.Extraction" to calculate the amount of hydrolysate obtained after extraction. Indeed, the hydrolysate was directly pumped into the tank of the filtration machine and could not be measured. It was assumed that the mass was conserved in the extraction process and that no losses (other than the samples) occurred. This last assumption is arguable since CPA residues remained in the extraction tank at the end of the process. However, these losses of biomass were not measured nor estimated. The losses of biomass per evaporation are certainly negligible. The dry matter content of the hydrolysate corresponded to the dry matter content of the last sample collected. Finally, the amount of hydrolysate obtained after extraction was calculated using the equations 2 and 3 and the volume of water evaporated, the equation 4 (see Figure 11).

Mass and water balance of "S3.A1.Extraction".

$$m_{hydrolysate_{DW}} = m_{CPA_{DW}} - m_{samples_{DW}} - m_{losses_{DW}} \tag{2}$$

$$m_{hydrolysate} = \frac{m_{hydrolysate_{DW}} \times 100}{DM_{hydrolysate}} \tag{3}$$

$$V_{water,evapo.} = (V_{water_{CPA}} + V_{water_{\mathrm{H_2SO_4}}}) - (V_{water_{hydrolysate}} + V_{water_{samples}}) \tag{4}$$

with: $m_{hydrolysate_{DW}}$ the amount of hydrolysate produced (kg DW-eq), $m_{CPA_{DW}}$ the amount of CPA used (kg DW-eq), $m_{samples_{DW}}$ the amount of biomass sampled (kg DW-eq), $m_{losses_{DW}}$ the amount of losses (kg DW-eq), $DM_{hydrolysate}$ the dry matter content of the hydrolysate, $m_{hydrolysate}$ the mass of hydrolysate (kg), $V_{water,evapo.}$ the volume of water evaporated during the acid hydrolysis, $V_{water_{CPA}}$ the volume water contained in CPA (L), $V_{water_{\mathrm{H_2SO_4}}}$ the volume of water in the solution of H_2SO_4 (L), $V_{water_{hydrolysate}}$ the volume of water contained in the hydrolysate (L), and $V_{water_{samples}}$ the water contained in the samples.

Sulfuric acid (H_2SO_4): A solution of H_2SO_4 at 3M was prepared by mixing a commercial solution of H_2SO_4 at 96% (18M) with water, in a beaker filled with ice to control the exothermic reaction. The solution of H_2SO_4 was produced in Europe (i.e. France for the solution used in 2021 and Belgium in 2022). In ecoinvent 3.6, the activity "market for sulfuric acid (RER)" presents data for the production of pure H_2SO_4 in kilogram. The amount of H_2SO_4 at 96% used in the process served as basis for the calculation of the pure amount of H_2SO_4 (i.e. with a concentration of 100%) and volume of ultrapure water used to dilute the solution from 100% to 96%. A value of 1.83 g/cm^3 or kg/L was used for the density of H_2SO_4 at 20°C.

Calculation of the amount of pure sulfuric acid (H_2SO_4)

1) Calculation of the volume of water required to dilute the commercial solution used:

$$V_{water} = \frac{C_{\mathrm{H_2SO_4},comm.solution,tech_a} \times V_{\mathrm{H_2SO_4},tech_a}}{c_{\mathrm{H_2SO_4},dilutedsolution,tech_a}} - V_{\mathrm{H_2SO_4},tech_a} \tag{5}$$

with: V_{water} the volume of water used to dilute the commercial solution of H_2SO_4 (L), $C_{\mathrm{H_2SO_4},comm.solution,tech_a}$ the concentration of the commercial solution (M), $V_{\mathrm{H_2SO_4}}$ the volume of H_2SO_4 used in the process, $C_{\mathrm{H_2SO_4},dilutedsolution,tech_a}$ the targeted concentration to dilute the solution of H_2SO_4.

2) Calculation of the equivalent amount of pure H_2SO_4 (for the use of the ecoinvent 3.6 dataset):

$$V_{\mathrm{H_2SO_4},ei36} = \frac{C_{\mathrm{H_2SO_4},tech_a} \times V_{\mathrm{H_2SO_4},techa}}{C_{\mathrm{H_2SO_4},ei36}} \tag{6}$$

$$m_{\mathrm{H_2SO_4},ei36} = V_{\mathrm{H_2SO_4},ei36} \times d_{\mathrm{H_2SO_4}} \tag{7}$$

with: $V_{\mathrm{H_2SO_4},ei36}$ the volume of pure H_2SO_4 equivalent to the volume of H_2SO_4 used in the process (L), $C_{\mathrm{H_2SO_4},tech_a}$ the concentration of H_2SO_4 from the solution used in the process during $tech_a$ (i.e. period 1 or 2) (%), $V_{\mathrm{H_2SO_4},techa}$ the volume of H_2SO_4 used in the process during $tech_a$ (L), $C_{\mathrm{H_2SO_4},ei36}$ the concentration of the solution of H_2SO_4 in ecoinvent 3.6 (i.e. 100%) (%), $d_{\mathrm{H_2SO_4}}$ the density of H_2SO_4 at 20°C (kg/L), and $m_{\mathrm{H_2SO_4},ei36}$ the mass of pure H_2SO_4 (kg).

3) Calculation of the volume of ultrapure water added in the inventory data:

$$V_{water,ultrapure} = V_{\mathrm{H_2SO_4},tech_a} - V_{\mathrm{H_2SO_4},ei36} \tag{8}$$

with: $V_{water,ultrapure}$ the volume of ultrapure water (L), $V_{\mathrm{H_2SO_4},tech_a}$ the volume of H_2SO_4 used in the process during $tech_a$, and $V_{\mathrm{H_2SO_4},ei36}$ the volume of pure H_2SO_4 equivalent to the volume of H_2SO_4 used in the process.

Electricity: The total amount of electricity used in “S3.A1.Extraction” comprises the amounts of electricity used to mix and regulate the temperature of the CPA in the extraction tank. Both values were measured using a power meter plugged to the each of the equipment, i.e. the motor/rotor and the temperature regulation system, from the start to the end of the process. The extraction tank itself did not require any electricity. The values were read in kWh directly with a precision of ±0.001 kWh. Most of the electricity consumption is related to temperature regulation system since the CPA mixture is maintained at a temperature of 80°C. In 2021, the extraction was conducted over 24h. In 2022, the reaction time was reduced to 4h which resulted in a large decrease in the electricity consumption. In addition, the cleaning of the extraction tank did not require any electricity in 2022 since it was rinse with water at the end of the process. In 2021, the CPA residues on the tank of the walls dried. The cleaning required to fill up the tank with water and warm it up at a temperature of 50°C for a day.

Tap water: The volume of tap water used in “S3.A1.Extraction” corresponds to the water used to regulate the temperature of the tank, prepare the solution of H_2SO_4, and clean the tank at the end of the process. The volume of water used to regulate the temperature was measured by collecting the water contained in the double layer of the tank, piping system, and in the temperature regulation machine. The container was then weighed (precision: ±0.01 kg). The volume of water used to prepare the solution of H_2SO_4 was measured with a graduated cylinder (precision: ±0.01 mL). Regarding the cleaning, the volume of water used to fill up the tank was measured with a graduated ruler on the wall of the tank. The additional water used to rinse the tank and the containers was calculated by multiplying the duration of the cleaning with the water flow of the hose used.

Wastewater: The volume of wastewater generated in "S3.A1.Extraction" corresponds to the volume of water used for cleaning. In fact, the volume of water used to prepare the solution of H_2SO_4 was incorporated to the product and not considered as wastewater. A negative sign was added to the volume of wastewater following the Opposite Direction Approach (also known as double-negative approach).

Parameterisation

The inputs of the "S3.A1.Extraction" model consists of the technological period (2021 or 2022) and the amount of CPA, packaged used (kg DW-eq). The outputs include the amount of electricity (kWh) and pure sufuric acid (kg) used as well as the volume of tap water and ultrapure water (L) used. The amount of hydrolysate produced (kg DW-eq) as well as the volume of wastewater (m^3) and water vapor (L) generated were also included. The output values were calculated from a simple cross-multiplication (i.e. rule of three) based on the real data collected on-site.

4.2 Diafiltration

Description

The diafiltration (DF) of the hydrolysate was conducted using 0.2 µm ceramic membranes. After an initial separation, the permeate was stored in an external tank. The retentate was diluted with water in the internal tank of the machine and underwent a second filtration step. This allowed to increase the fraction of molecules smaller than 0.2 μm transferred to the permeate. The retentate obtained after DF corresponded to the co-product enriched with carbohydrates and further analysed at laboratory scale. The permeate from DF was ultrafiltered at 15 kDa.

Data collection and aggregation

The dataset for "S3.A2.Diafiltration" contains information regarding the amount of permeate from DF, electricity, water, and chemicals used in the process and cleaning of the equipment as well as the volume of wastewater generated and the amount of permeate and retentate produced.

Permeate and retentate from DF: In 2021, the amount of permeate from DF (< 0.2 μm) was measured by weighing the container in which the product was stored. In 2022, the volume of permeate from DF was measured by using the graduated ruler of hte tnk in which the product was collected. Alternately, the volume of retentate (> 0.2 μm) was obtained from the data logger of the filtration machine. Samples of each fraction were collected to measure their dry matter content. In 2022, a volume of 370 L of permeate at 1.54% DW was obtained. Since the dry matter content is low, it was assumed that the permeate had a density of 1 kg/L. Alternately, 88 L of retentate were collected in 2022 with a dry matter content of 10.09%. This the density of the retentate was not measured, its amount (in kg) could not be calculated. The mass and water balance was used to calculate the amount of retentate obtained in 2022 (see Figure 12). It was considered that no losses, other than the loss of permeate measured and the samples collected, occurred during the diafiltration. In 2021, the amount of permeate and retentate were both measured. However, the mass balance resulted in a difference of 3.99 kg DW-eq between the inputs and outputs of "S3.A2.Diafiltration". Since the losses of biomass represented 0.071 kg DW-eq in 2022, 3.99 kg DW-eq seem too high for only being losses. This value could be explained by the overestimation of the amount of dry biomass in the hydrolysate, which was assumed from the conservation of mass in "S3.A1.Extraction". Another possibility is that the measurements of the amounts and dry matter contents of the permeate and retentate could be biased. In this work, it was assumed that the 3.99 kg DW-eq corresponded to losses of product with a dry matter content corresponding to the average between permeate and retentate (see Figure 12, values in red).

Tap water: The total amount of tap water used in "S3.A2.Diafiltration" corresponds to the water added to the product during the diafiltration and the water used for cleaning. The volume of water used to clean the filtration machine was obtained from the data logger in 2021. In 2022, the volume of water used for cleaning, i.e. to prepare the solution of NaOH and rinse the machine afterwards, was measured by collecting the water exiting the machine into containers and weighing them. The volume of water used in the diafiltration (i.e. in the process) was not measured but calculated from the water balance (see Figure 12).

Electricity: The total amount of electricity used in "S3.A2.Diafiltration" corresponds to the electricity used by the filtration machine. The electricity consumption was measured with a power meter plugged to the machine from the start to the end of the process and cleaning steps (precision: ±0.001 kWh). The amount of electricity used was higher in 2022 than in 2021 since the filtration machine ran overnight, without interruption.

Sodium hydroxide (NaOH): At the end of the process, the filtration machine was cleaned with a commercial solution of NaOH at 30% diluted in water. For the same reason as H_2SO_4 in "S3.A1.Extraction" the amount of pure NaOH was calculated to use the ecoinvent 3.6 dataset in the LCA analysis.

Wastewater: The total volume of wastewater generated in "S3.A2.Diafiltration" corresponds to the volume of tap water used for cleaning. A negative sign was added to the volume of wastewater following the Opposite Direction Approach (also known as double-negative approach).

Parameterisation

The inputs of the "S3.A2.Diafiltration" model consists of the technological period (2021 or 2022) and the amount of hydrolysate (kg DW-eq) produced in "S3.A1.Extraction". The outputs include the amount of electricity (kWh) and sodium hydroxide (kg) used as well as the volume of tap water and ultrapure water (L) used. The amount of retentate and permeate produced (kg DW-eq) as well as the volume of wastewater (m3) generated were also included. The output values were calculated from a simple cross-multiplication (i.e. rule of three) based on the real data collected on-site.

4.3 Ultrafiltration

Description

The permeate from DF was transferred into the tank of the filtration machine which was equipped with 15 kDa ceramic membranes after a thorough cleaning. The ultrafiltration of the retentate from DF produced a permeate with all the molecules smaller than 15 kDa and a retentate with the molecules smaller than 0.2 μm and larger than 15 kDa. In 2021, the entire volume of permeate from DF was ultrafiltered. Only 72% of the volume were processed in 2022 due to technical issues and time constraints. The permeate from DF was stored in the extraction tank (previously cleaned) and the volume was measured with a ruler before and after UF. A volume of 104 L remained after UF, i.e. was not ultrafiltered at 15 kDa. The filtration flow (e.g. flow of permeate exiting the filtration machine) was low (13.9 L/h), probably due to the clogging of the membranes. In this study, it was considered that the total amount of permeate from DF was ultrafiltered (i.e. 370 L instead of 266 L). The quantities of permeate and retentate obtained from UF were calculated from the values measured for 266 L using a simple cross-multiplication (or rule of three). The density of the permeate was considered to equal 1 kg/L at such low dry matter contents. Therefore, 370 L of permeated from DF would generate 244 L of retentate and 126 L of permeate. The electricity consumption was calculated from these values while the water consumption remained constant.

Data collection and aggregation

The dataset for "S3.A3.Ultrafiltration" contains information regarding the amount of permeate from DF, electricity, water, and chemicals used in the process and cleaning of the equipment as well as the amount of permeate and retentate produced and wastewater generated.

Permeate and retentate from UF: The amount of permeate obtained after UF was measured by weighing the container in which the product was collected. The volume of retentate was obtained from the data logger of the filtration machine. In 2022, the volume of permeate from DF corresponded to 72% of the total volume produced in "S3.A2.Diafiltration" (i.e. only 266 L were ultrafiltered over the 370 L produced). The data measured for the processing of 266 L of permeate from DF were up-scaled using a simple cross-multiplication (rule of three). The difference in the dry mass between the permeate from DF and the retentate and permeate from UF was low (i.e. 0.04 kg DW-eq). This amount of biomass was modelled as a loss (see Figure 13). The difference in the volumes of water between the input and outputs was added as a loss of water in the process. In 2021, the mass and water balance of "S3.A3.Ultrafiltration" showed that there was an important difference between the dry mass of the permeate from DF and the dry mass of the two products, i.e. the retentate and permeate from UF. NO losses were measured on-site. The amount of each of the three products and their dry matter contents were measured (and not calculated). No issue occurred during the data collection. The method used to balance the three amounts of dry mass consists of scaling down the output values using a simple cross-multiplication. Using the corrected values, the sum of the dry mass of the retentate and permeate from UF equal the amount of dry mass in the permeate from DF (see Figure 14). The adjustment of the mass values led to an increase in the difference between the input and output of water. The difference was modelled as a loss of water in the process.

Electricity: The total amount of electricity used in "S3.A3.Ultrafiltration" consists of the electricity used by the filtration machine. The electricity consumption was measured with a power meter plugged to the machine from the start to the end of the process and cleaning. In 2022, the amount of electricity was measured for the processing of 266 L of permeate from DF. The theoretical electricity used to process 370 L was calculated with a simple cross-multiplication based on the running time of the filtration machine. The value measured for 266 L corresponded to 6.5 h of ultrafiltration at 15 kDa. The filtration of 370 L would have taken 9.04h. The instant power of the filtration machine was calculated and the value obtained was multiplied by the running time of the filtration machine. The amount of electricity used for cleaning is independent from the amount of permeate from DF processed. The value measured for 266 L was also used for 370 L. The total amount of electricity used for UF corresponded to the measured value for cleaning and the calculated value for the processing of 370 L.

Tap water: The total amount of tap water used in “S3.A3.Ultrafiltration” consists of the water used for cleaning only. No water was added into the product during the ultrafiltration step. In 2022, it was assumed that the volume of water used for cleaning does not depend on the volume of permeate ultrafiltered. Therefore, the input of tap water remained unchanged for the processing of 266 and 370 L of permeate from DF. Since the filters were clogged with algal biomass residues, they were let overnight with NaOH. The filtration machine was then flushed and new water was recirculated until the pH of the water passing through the membranes reached a value of 7-8. The volume of tap water used to clean the filtration machine was measured by collecting the water exiting the machine and weighing the containers. The storage tanks and pipes were rinse with a high-pressure hose. The volume of water was calculated from the water flow of the hose and the use time. In 2021, the volume of water used for cleaning was obtained from the data logger.

Wastewater: The volume of wastewater generated corresponded to the volume of water used for cleaning.

Sodium hydroxide (NaOH): The filtration machine was cleaned at the end of the process using a solution of NaOH in water. A volume of 1.1 L of a solution of NaOH at 30% was mixed with water in the tank of the filtration machine.

Parameterisation

The inputs consists of the technological period (2021 or 2022) and the amount of permeate (kg DW-eq) produced in S3.A2.Diafiltration. The outputs include the amount of electricity (kWh) and sodium hydroxide (kg) used as well as the volume of tap water and ultrapure water (L) used. The amount of retentate and permeate produced (kg DW-eq) as well as the volume of wastewater (m3) generated were also included.

4.4 Concentration

Description

The concentration was performed in an evaporator, under vacuum conditions, and at medium temperature (15-25°C) to preserve the product of interest. The reservoir of the evaporator was filled up with water to initiate the evaporation. The distillate was discharged at regular intervals, per volumes of 3 L, from the start to the end of the process. The evaporator was rinsed with water and no chemicals were used for cleaning. In 2021, the evaporation was performed using the permeate from UF. The process was interrupted due to an excessive foaming. The dataset for the period 1 is incomplete and was not included in the study. In 2022, the aim was to concentrate the retentate from UF up to a dry matter content of 6%. This value was targeted to meet the requirements from the formulators who would mix the concentrate with other compounds (e.g. organic nitrogen, phosphorous, and potassium) to produce a foliar and/or soil biostimulant. This extract could also be used as an ingredient for animal health products. This last option was not investigated in this work. In 2022, the process was interrupted close to the end. The dry matter content of 6% was not reached and the volume of concentrate obtained was larger than expected.

Data collection and aggregation

The dataset for “S3.A4.Concentration” contains information regarding the amount of retentate from UF, electricity, water, and chemicals used in the process and the cleaning of the equipment, as well as the amount of CPA concentrate produced and wastewater generated.

Concentrate and distillate: The volume of concentrate obtained after evaporation was measured by emptying the tank of the evaporator into containers and weighing them. The precision of the scale used was ±0.01 kg. The amount of distillate generated during the concentration was read on the data logger of the evaporator. The volume of distillate depends on the discharges and has a precision of ±3 L. The volume of water contained in the reservoir was measured by emptying its content in a container and weighing it. Since the evaporator was interrupted before the end of the process, the product was not concentrated at a dry matter content of 6%. The amount of concentrated obtained if the dry matter content of 6% was reached was calculated from the water and mass balance of “S3.A4.Concentration” (see Figure 15). The conservation of mass approach was used i.e. the amount of retentate equals the amount of condensate in kg DW-eq. It was assumed that there were no losses of biomass during evaporation.

Electricity: The electricity consumption was measured using a power meter plugged to the evaporator from the start to the end of the process, for the interrupted concentration of 165.75 L of retentate from UF (see Figure 15). The theoretical amount of electricity used to evaporated 230.57 L of retentate from UF into a 6% DW concentrate was calculated from the running time of the evaporator. It was assumed that the electricity required to further concentrate the product was proportional to the volume of retentate. Indeed, starting the evaporator would consume more energy before the machine reaches a steady state during which the electricity consumption is constant. The precision of the power meter was ± 0.001 kWh.

Tap water: The tap water used during the concentration process is related to the use of water to fill in the reservoir of the evaporator and for cleaning. The reservoir was filled in a first time and cleaned twice during the process due to excessive foaming. In total, the reservoir was filled up three times. and rinsed. The evaporator was cleaned at the end of the process, part of the water was reused from the tank of the ultrafiltration machine. The volume of water used to fill in the reservoir was measured by emptying the content in a container and weighing it. The volume of water used to rinse the tank of the evaporator and reservoir was calculated from the measurement of the water flow from the hose and the time required for cleaning. The volume of water used to fill up the tank for cleaning was read on the data logger. It was assumed that the volume of water used in “S3.A4.Concentration” is independent from the volume of retentate from DF evaporated. The total amount of water used was similar for the real and theoretical scenarios.

Wastewater: The volume of wastewater generated equaled the volume of tap water used to fill up the reservoir of the evaporator and clean the equipment.

Parameterisation

The inputs of the “S3.A4.Concentration” model consists of the amount of retentate (kg DW-eq) produced in “S3.A3.Ultrafiltration”. The outputs include the amount of electricity (kWh) and water (L) used as well as the volume of wastewater generated and the amounts of concentrate produced. The technological period was not considered as an input since the concentration step was only performed and completed in 2022. The amounts of electricity, water, wastewater, and concentrate were calculated by a simple cross-multiplication.

4.5 Packaging

The CPA concentrate obtained from “S3.A4.Concentration” was packaged in 10 L HDPE containers. The dataset for “S3.A5.Packaging” contains information regarding the amount of plastic used in the process. The total amount of HDPE used was calculated from the weight of an empty container (with the lid) considering the number of containers used to package the total amount of CPA concentrate obtained. Regarding the amount of CPA, packaged obtained, a conservation of mass approach was applied. The amount of CPA, packaged obtained was calculated by summing the weight of CPA and the amount of plastic used for packaging. The input of the “S3.A5.Packaging” model include the amount of CPA concentrate (kg DW-eq). The outputs include the amount of plastic packaging (kg) used as well as the amount of CPA concentrate, packaged (kg DW-eq). The technological period was not included as input since only the data collected in 2022 were used. A simple cross-multiplication was used to calculate the amount of $C_6H_7KO_2$.

4.6 Stabilisation

The CPA concentrate was stabilised after packaging, by adding potassium sorbate ($C_6H_7KO_2$) in the 10 L containers. The dataset for “S3.A6.Stabilisation” contains information on the amount of $C_6H_7KO_2$ stabilised which was measured by weighing the containers before and after addition of the preservative. The containers were then stored at room temperature. The theoretical amount of $C_6H_7KO_2$ used to stabilise the total volume of CPA concentrate obtained was calculated by considering a targeted concentration of 0.2% $C_6H_7KO_2$ in the containers. Regarding the amount of CPA, packaged, stabilised obtained, a conservation of mass approach was applied. The amount of CPA, packaged, stabilised obtained was calculated by summing the weight of CPA packaged and the amount of preservatives used. The dataset consists of the data collected in 2022 only. The fraction of the CPA concentrated changed between 2021 and 2022 (initially the permeate from UF and lately the retentate). In 2022, the rentate from DF was not stabilised but stored in a freezer for further analyses. The input of the “S3.A6.Stabilisation” model consists of the amount of CPA concentrate, packaged (kg DW-eq). The outputs include the amount of $C_6H_7KO_2$ used (kg) as well as the amount of CPA, packaged, stabilised produced (kg DW-eq). The technological period was not included as input since only the data collected in 2022 were used. A simple cross-multiplication was used to calculate the amount of $C_6H_7KO_2$.

5 Mass and water balances of the whole biorefinery

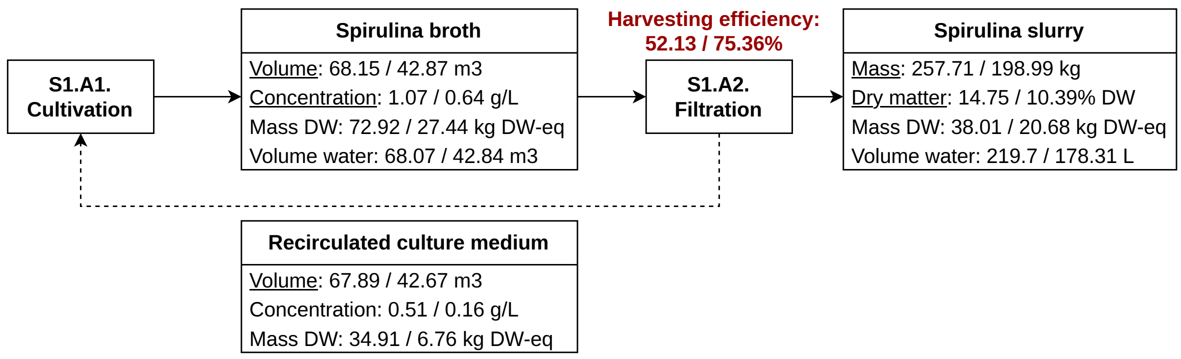

Figure 1: Mass and water balance of the activity “S1.A.Cultivation” and “S1.A2.Filtration”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

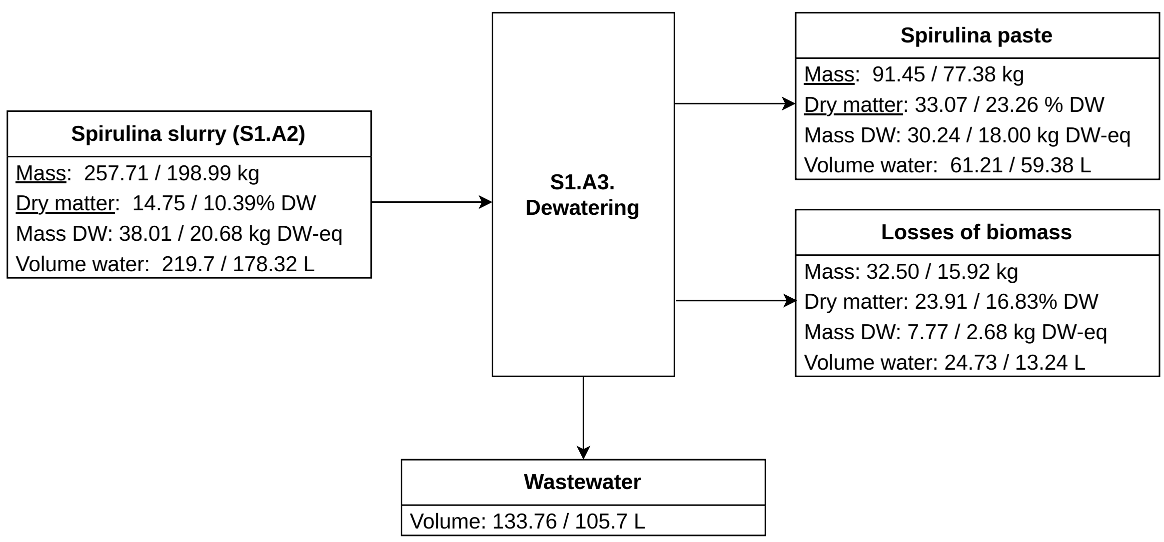

Figure 2: Mass and water balance of the activity “S1.A3.Dewatering”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

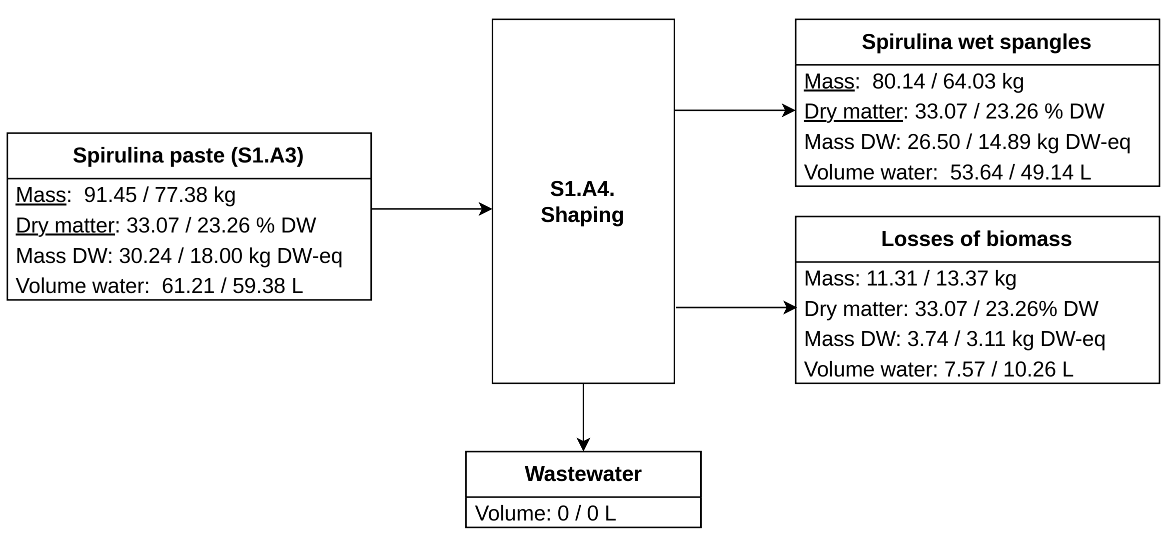

Figure 3: Mass and water balance of the activity “S1.A4.Shaping”. The values for period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

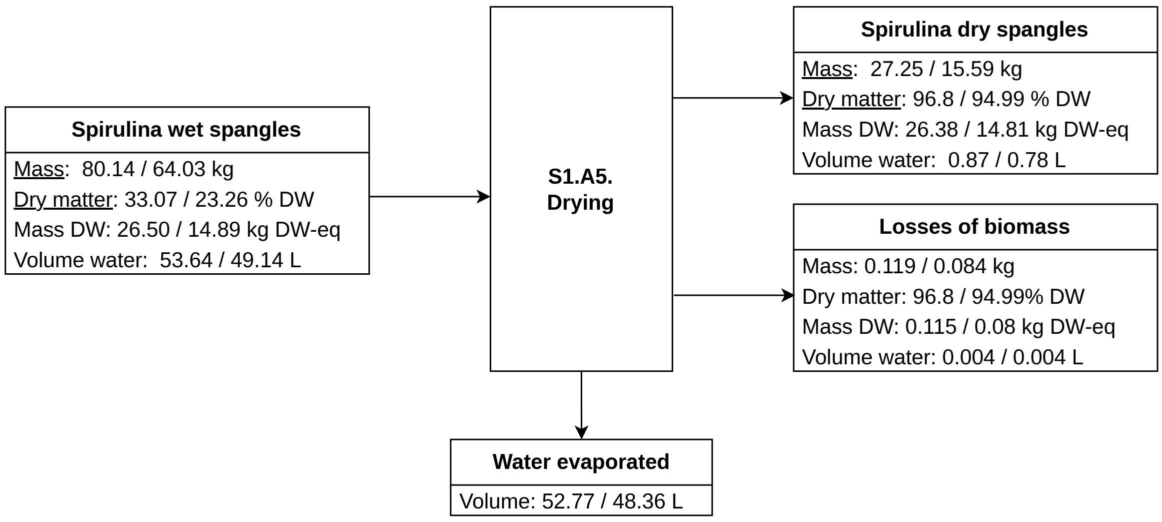

Figure 4: Mass and water balance of the activity “S1.A5.Drying”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

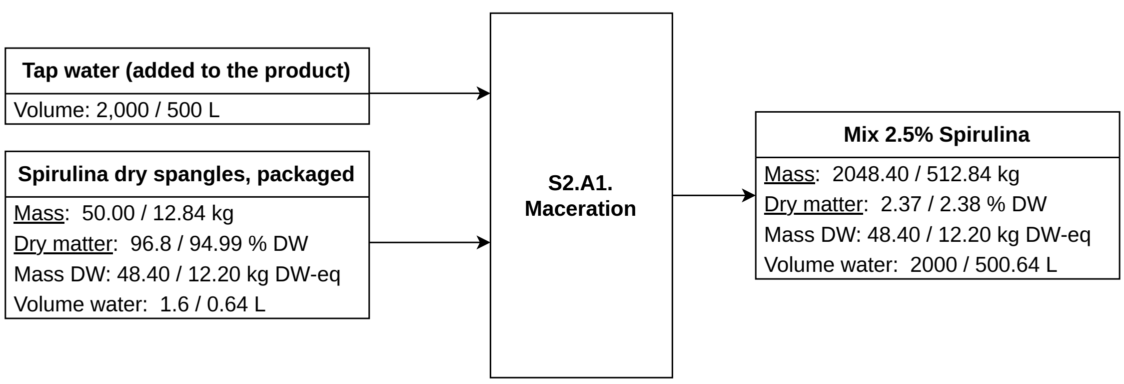

Figure 5: Mass and water balance of the activity “S2.A1.Maceration”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

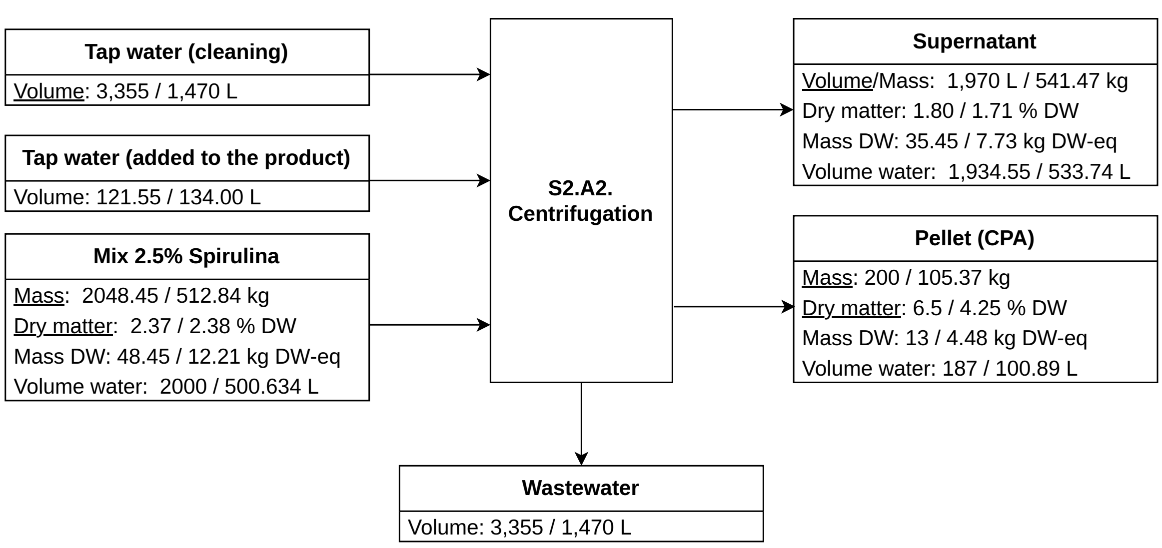

Figure 6: Mass and water balance of the activity “S2.A2.Centrifugation”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

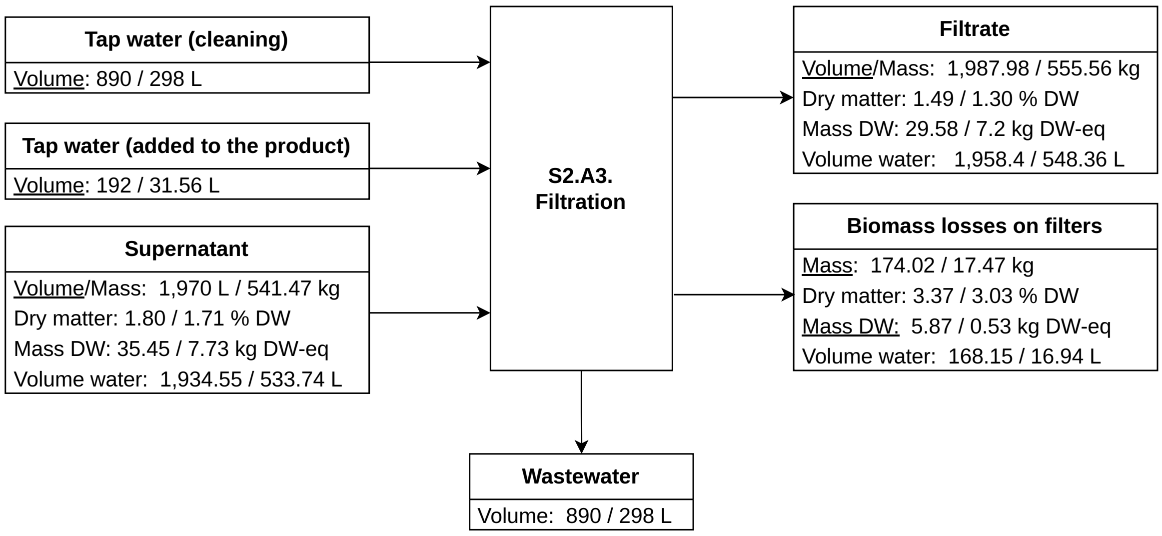

Figure 7: Mass and water balance of the activity "S2.A3.Filtration".. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

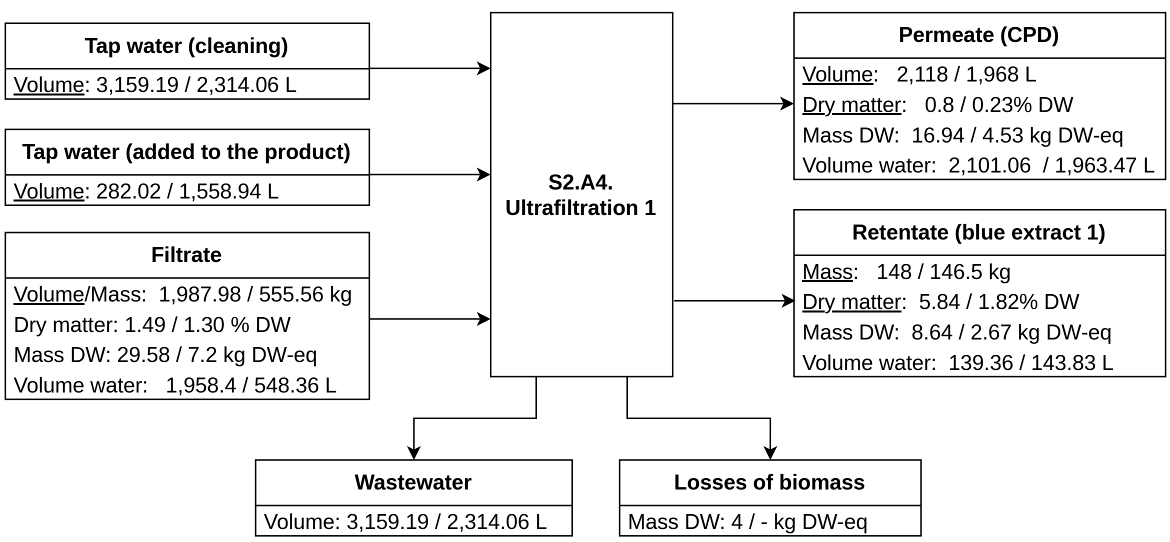

Figure 8: Mass and water balance of the activity "S2.A4.Ultrafiltration1". The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

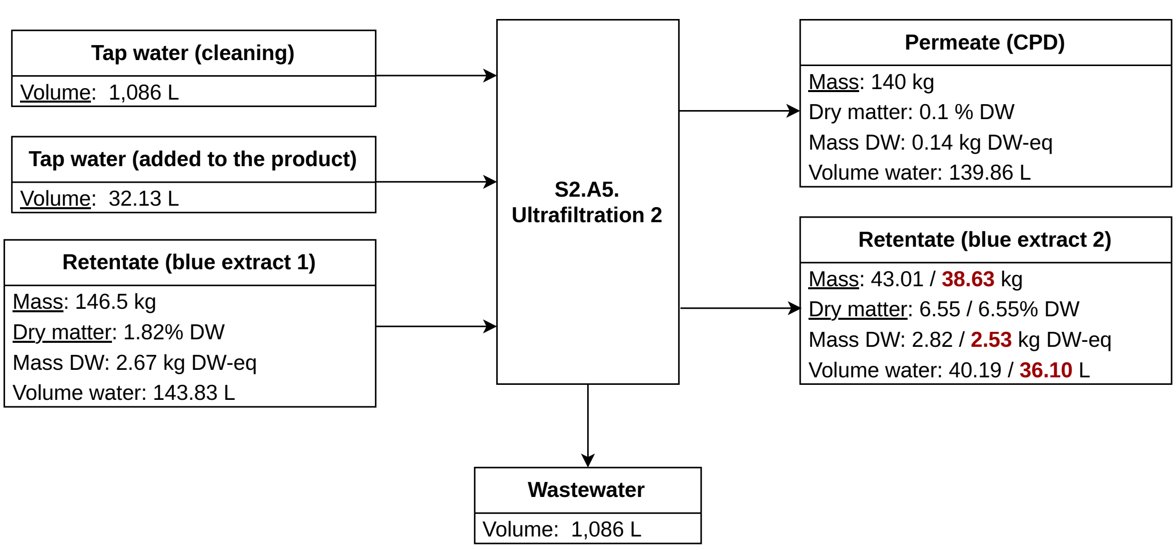

Figure 9: Mass and water balance of the activity "S2.A5.Ultrafiltration2". The corrected values appear in red. The underlined values were measured on-site, the rest are calculated.

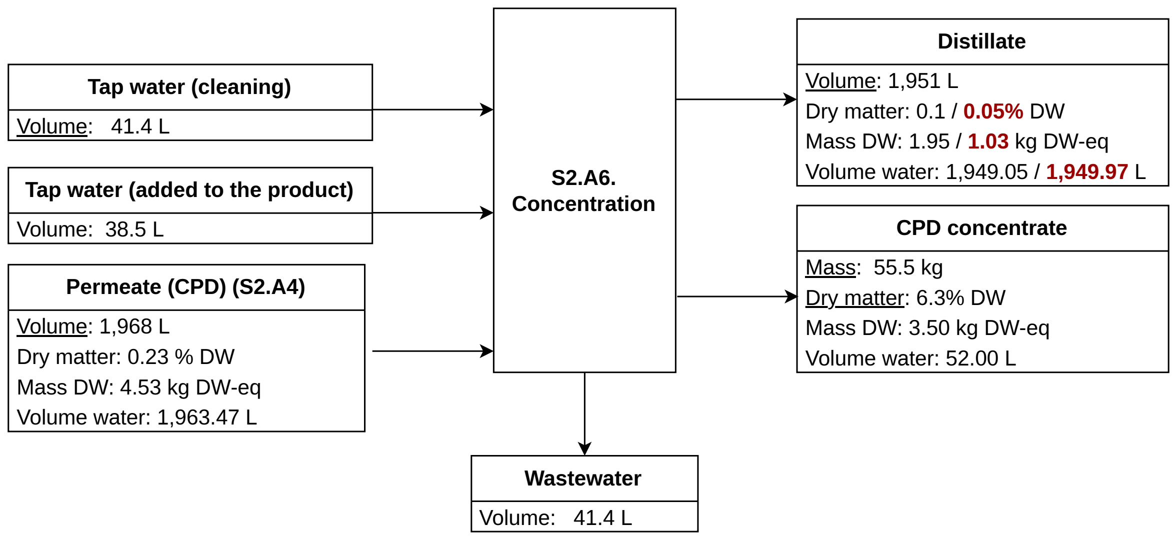

Figure 10: Mass and water balance of the activity "S2.A6.Concentration". The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

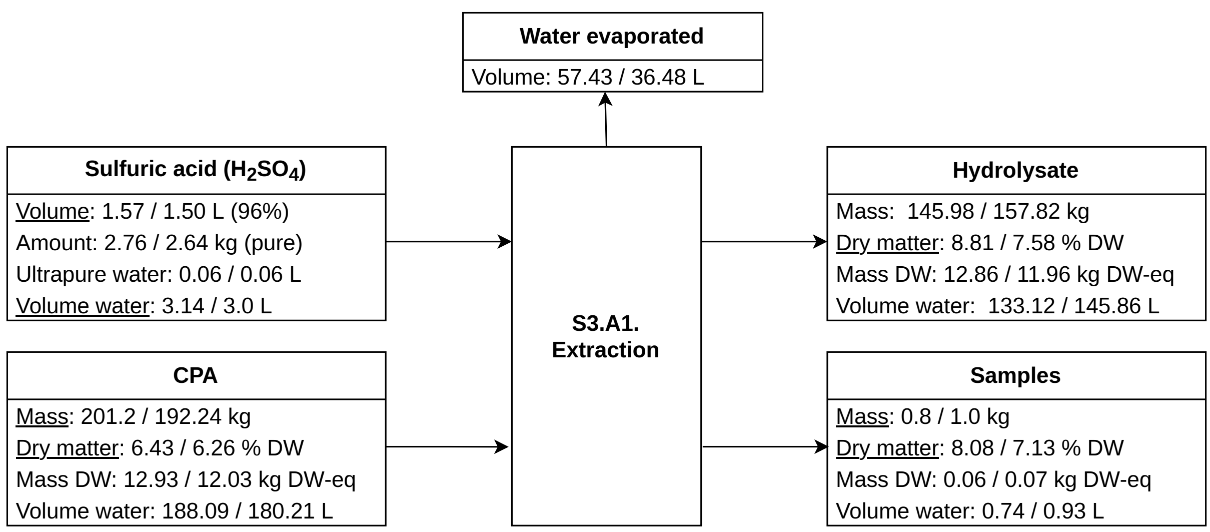

Figure 11: Mass and water balance of the activity "S3.A1.Extraction". The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

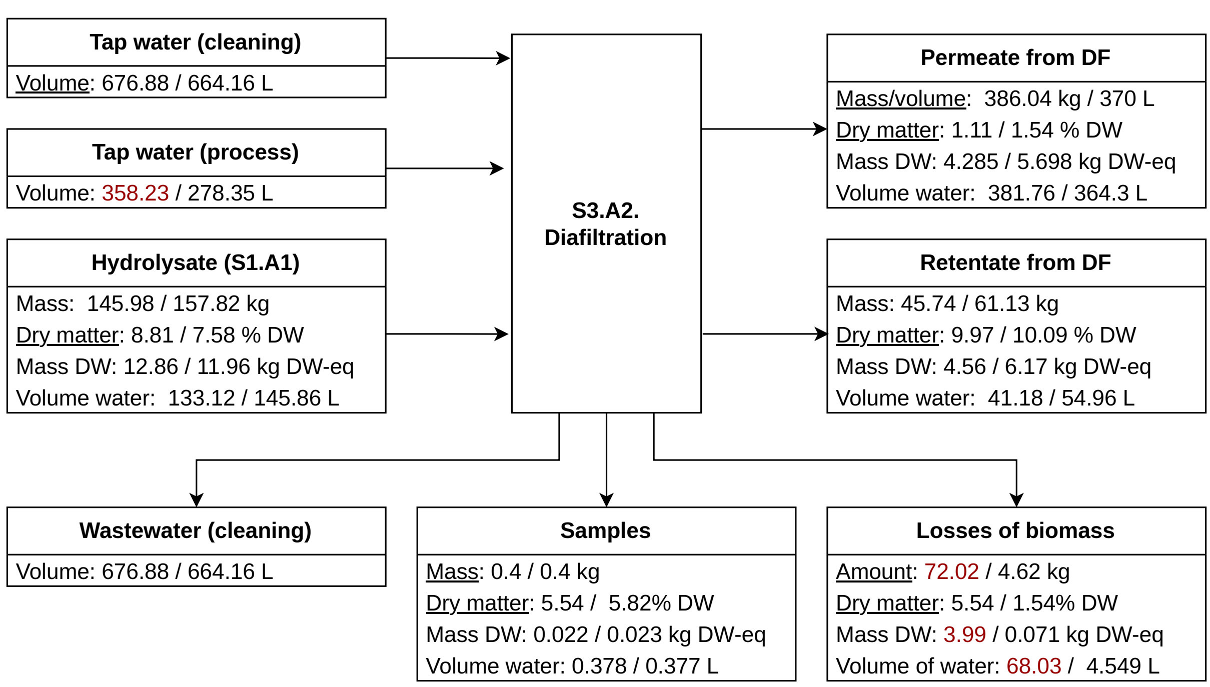

Figure 12: Mass and water balance of the activity "S3.A2.Diafiltration". The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

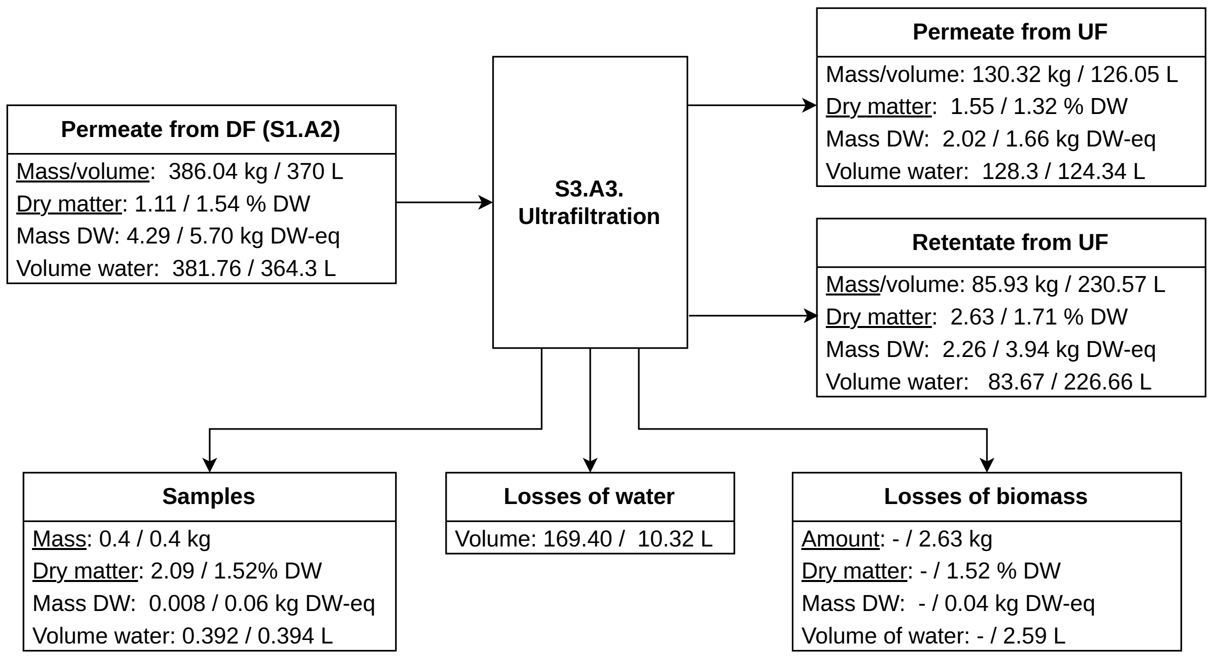

Figure 13: Mass and water balance of the activity “S3.A1.Extraction”. The values for the period 1 are on the left and the values for period 2 on the right. The underlined values were measured on-site, the rest are calculated.

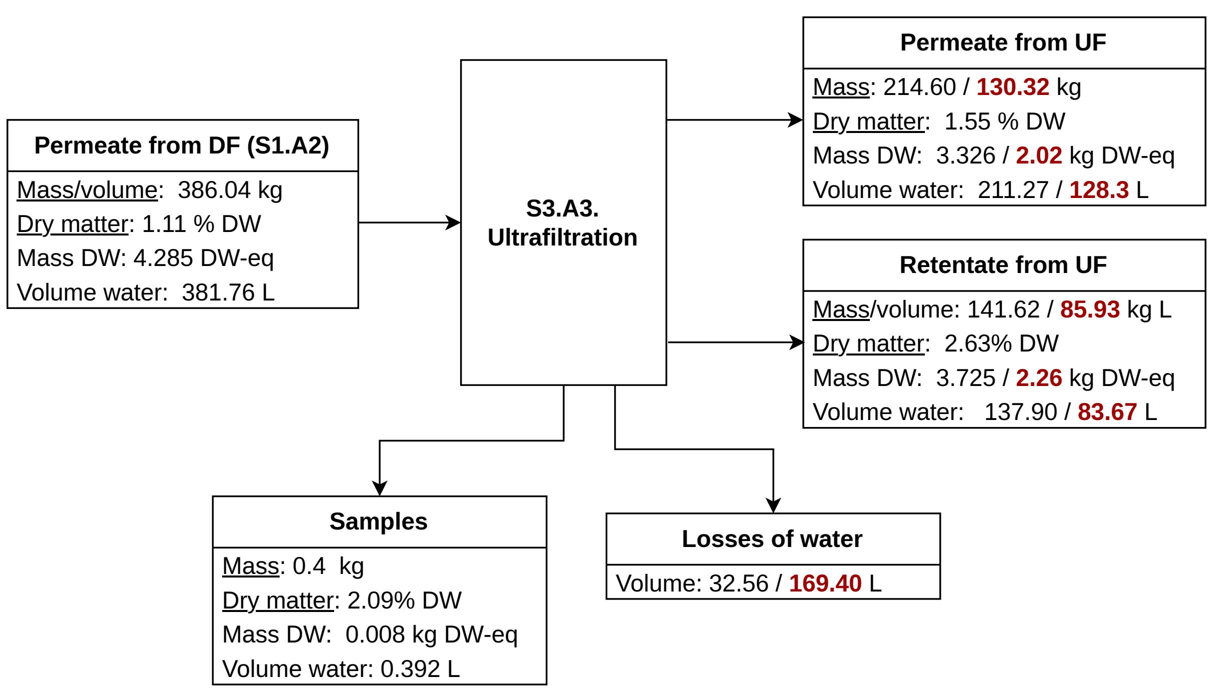

Figure 14: Correction of the mass and water balance of the activity “S3.A1.Extraction” for the period 1. The original values collected for the period 1 are on the left. The values colored in red and on the right side correspond to the adjusted values.

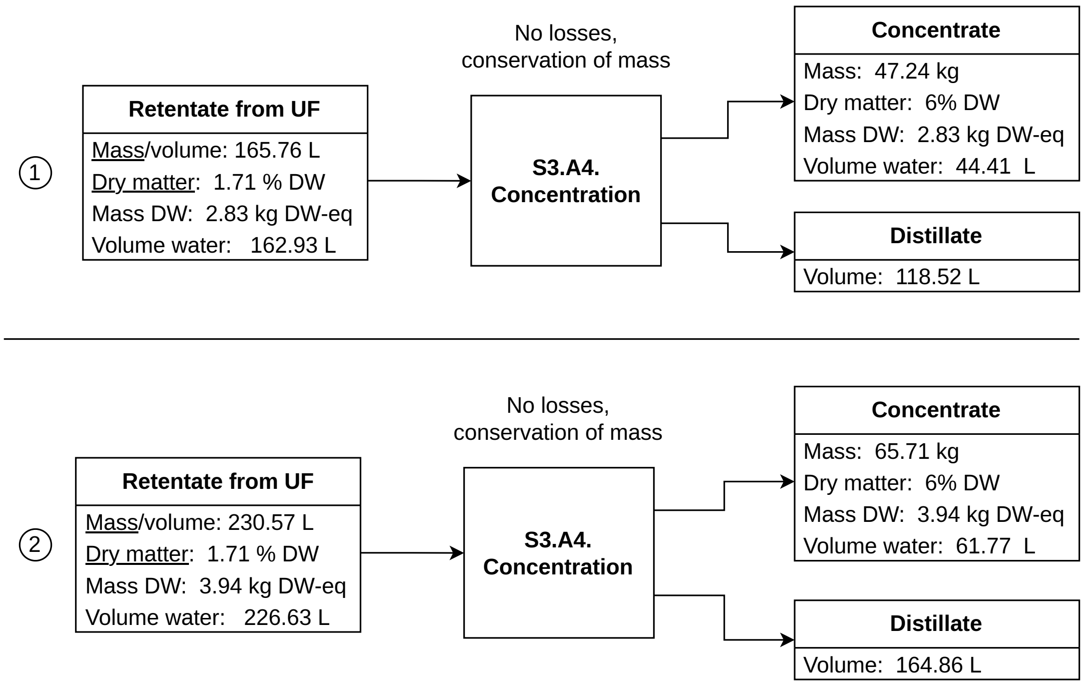

Figure 15: Mass and water balance of the activity "S3.A4.Concentration". (1) Real data collected on site for the concentration of 165.76 L of retentate from UF. (2) Calculation of the amount of concentrate obtained from the targeted dry matter content of 6% DW. (3) Theoretical data calculated from the processing of 230.57 L of retentate from UF (from the 370 L of permeate from DF ultrafiltered). The underlined items correspond to the data measured on-site. The rest correspond to calculated data.

References

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

[2] Paula Pérez-López et al. "Comparative life cycle assessment of real pilot reactors for microalgae cultivation in different seasons". In: *Applied energy* 205 (2017), pp. 1151–1164.