CHEERS

Producing novel non-plant biomass feedstocks and bio-based products through upcycling and the cascading use of brewery side-streams

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D1.4

First feasibility study of CHEERS approach at the case study site

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Executive Summary

The present deliverable D1.4, linked to task T1.4, is a report which describes the preliminary feasibility evaluation of the tailored (bio)technological solution for waste streams valorisation CHEERS provides throughout five value chains, within the frame of Lleida brewery site from a technical, environmental and economical point of view.

The estimations are based on compositions and flowrates of the side-streams at the case study site, bioconversion efficiencies of all involved wastes such as bagasse, wastewater, CO_2 and CH_4, biomass productivity and bioproduct yields, already evaluated and identified throughout tasks 1.1, 1.2 and 1.3 and the corresponding deliverables.

This work will serve as baseline for the final feasibility study (D6.5) to be performed in WP6.

The major points of the present document along with preliminary conclusion are described as follow:

Cost-effective value chains:

- Bioconversion of CH_4 into Ectoine with a 3 payback years
- Bioconversion of CO_2 into Chlorine with a 2,5 payback years.

Sustainable value chains:

- High reductions in CO_2 emissions in CH_4 into Ectoine value chain

Efficient transformation:

- Water-scrubbing absorption column prior to BES unit in Chlorine bio-production value chain has the highest efficiency of 96%.
- CH_4 bioconversion into Ectoine value chain with 95% of efficiency

1 Project Overview

CHEERS project has a novel approach and it is the analysis, verification and approval of the possibility to obtain certain products which is currently produced through processes with negative impact on environment and society health.

The brewery of MAHOU paired with WWTP of Lleida are selected as a suitable place for such demonstration, as several waste streams such as biogas, bagasse and yeast as well as several other by-products can be given high added value and produce novel products with lower or zero hazardous ingredients.

The above-mentioned approach is going to be approved through 5 microbial and insect-based bio-processes and technological equipment.

Throughout the project implementation, different side-streams generated at these 5 value chains will be optimized in the most efficient and sustainable possible way and the wastes will be minimized to the lowest achievable value.

The following Figure 1 illustrates the CHEERS solution:

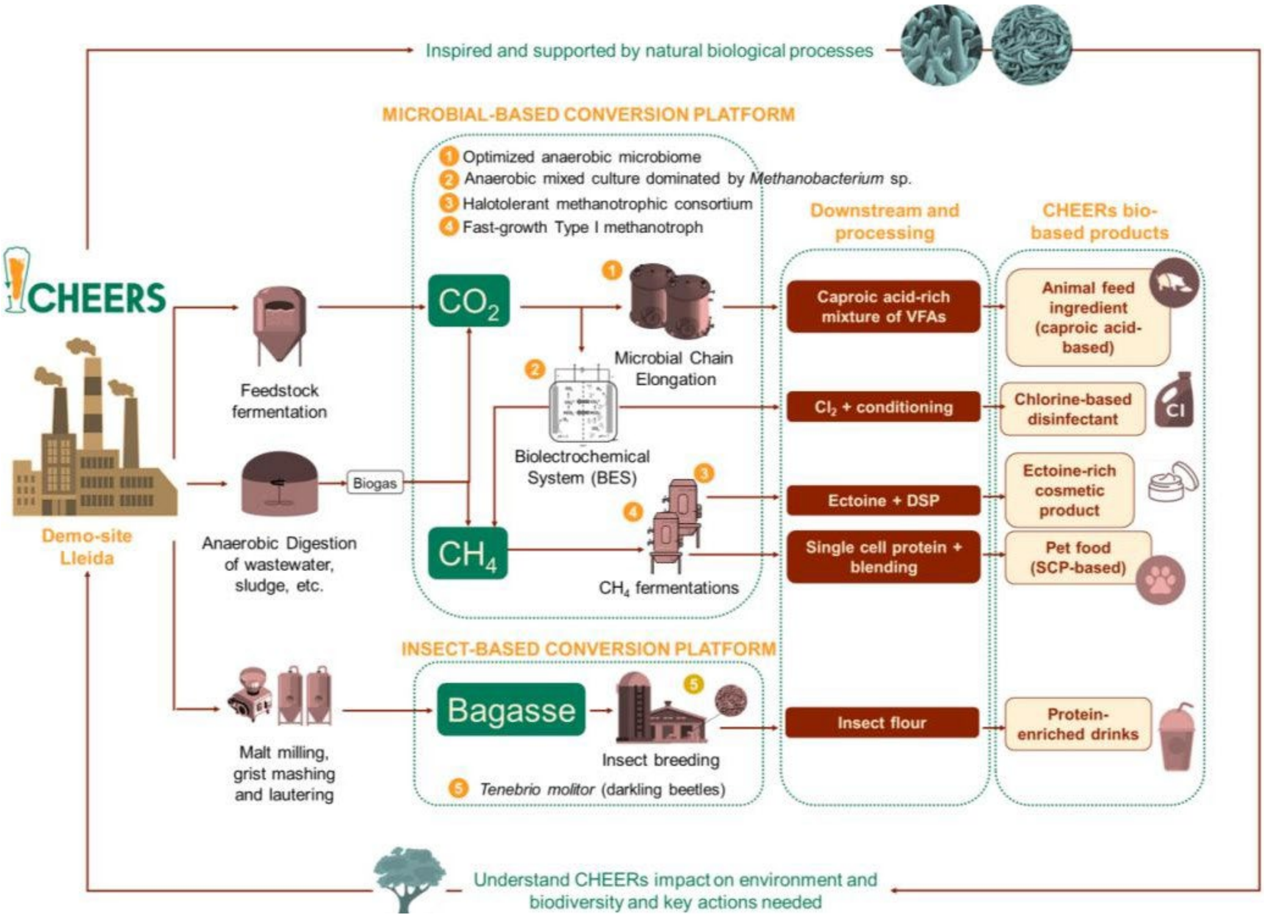


Figure 1. Overall concept of CHEERS multiplatform biorefinery (Grant Agreement)

2 Introduction

The feasibility study here conducted throughout the first seven months of the project's lifespan aims at determining whether CHEERS solution is viable based on the analysis of several aspects, including:

- Economic and financial feasibility
- Technical feasibility
- Environmental feasibility

Mass balances is the basis of the design of a process in early stages. This help determining the requirements of raw material, flows and compositions of the streams involved, equipment dimensioning and manufactured products.

Specifically, the D1.4 describes:

- Detailed mass and energy balances (if applicable), efficiencies of pre-treatments and post-treatments, compositions and flowrates of CO_2 and CH_4 streams as well as bio-based products (caproic acid, chlorine, hydroxyectoine, insect-based protein and SCP).
- Economical analysis based on to potential markets, current uses and economic values.

3 Feasibility Study of Value Chains

3.1 Feasibility Study of Value Chain-1: INSECT PLATFORM. Bioconversion of BAGASSE to INSECTS

3.1.1 Introduction

In recent years, the demand for animal protein has been increasing due to the exponential growth of human population and to changes in consumption habits induced by the market globalization. These factors led to the development of industrial production systems resulting in negative effects for the environment. Several authors have pointed out advantages in using insects as an alternative supply of animal protein for human and animal consumption, due to their high feed conversion efficiency, low greenhouse gas emissions and the overall low resources consumed for the production. Moreover, insects contain significant contents of protein (up to 60-70% for species like *Tenebrio molitor*) and essential amino acids, while representing an interesting source of fatty acids and minerals.

Nevertheless, there are some risks associated to insect consumption such as chemical and microbiological hazards. Chemical hazards are mainly associated with the environment where they grow up, the contamination of the feed or toxin presence in a specific development stage. Microbiological hazards are related to rearing conditions and to the insect microbiota. Pathogenic bacteria and viruses for insects are inoffensive for humans due to the phylogenetic differences between insects and humans. Although, according to FAO (The Food and Agriculture Organization), the rules and procedures that are applied to the breeding of other animals must be also implemented in insects farming.

Yellow mealworm (hereinafter *T. molitor*) represents one of the best candidates currently used for insect byproducts production. As other insect species, it entails four stages of development - eggs, larva, pupa and adult.

Several works have demonstrated *T. Molitor* can be used as an important source of protein for animals or humans due to its ease rearing conditions, maintenance and the fact that can be harvested at an earlier stage of their development due to the larvae size, compared to other insects (e.g. black soldier fly). (Costa Sara et al., 2020). The following Figure 2 illustrates *T. molitor* life cycle:

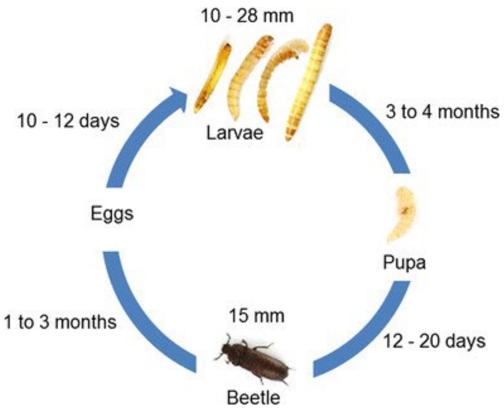
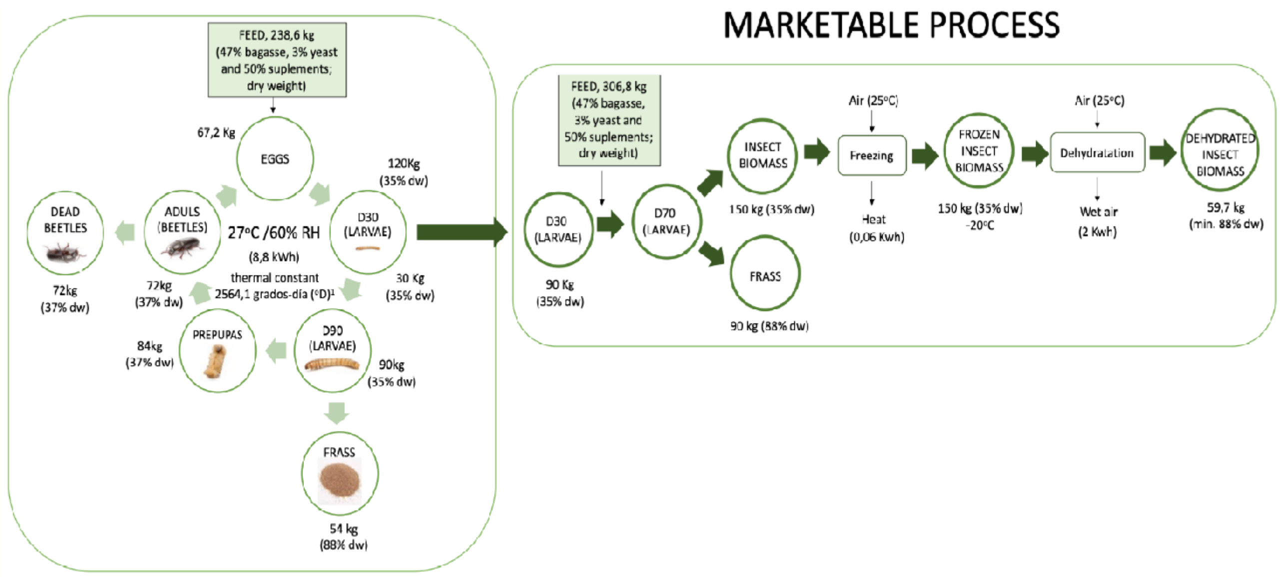


Figure 2. T. Molitor Life cycle. (Su Yean Ong et al., 2018)

In this context, CHEERS proposes a novel application for brewery waste bagasse which in combination with waste yeast and other supplements can be converted into insect-based protein using *T. molitor* (TM) insect.

The resulted insect-protein will be treated to improve its properties and make it a suitable candidate for protein as an ingredient for smoothies and beverages.

A theoretical mass balance of CHEERS idea is described in the following Figure 3:



¹Koo et al., 2013

Figure 3. Bagasse to insect biomass conversion (Koo et al, 2013)

3.1.2 Process design and technical feasibility

The CHEERS insect platform consists of a breeding process unit to reproduce insect followed by a marketable processing unit to obtain the insect protein-rich flour. The breeding process is a productive cycle running in a continuous cycle mode and it takes 100-120 days long to produce larvae for marketable process. This phase of the process is estimated to be fed several times in a year.

The next stage followed by this breeding process is estimated to take 70 days long which leads to an approximately 5 production times in a year (an approx. 750 kg biomass/year). However, such rate might be modified based on insect production requirements or bagasse consumption as they are replicable modules. (Information provided by PROTE)

The CHEERS demonstrative plant has been specifically designed to transform bagasse and other similar wastes of MAHOU brewery to insect biomass, by means of TM (*Tenebrio molitor* insect). Mealworm larvae used in this tailored design, were reared in a constant climate chamber, at 27 ºC with a relative humidity of 60% fed with brewery bagasse, yeast and supplements.

Table 1 includes list of the raw materials available at Alovera case study site and used for the five different value chains included in CHEERS project.

Table 1. Main waste and by-products mass flows available for CHEERS technologies deployment at Alovera case study site.

Description	Unit	Measurement
Spent yeast extract (89.4% humidity)	ton/y	1 566
Bagasse (76.3% humidity)	ton/y	28 381
Biogas (65% CH_4)	Nm^3/y	303 231

CO_2 fermentation surplus (100% CO_2)	ton/y	3 928
Wastewater (99.6%humidity)	m^3/y	520 000
Expired ethanol and dealcoholized products inlet (95.7% humidity)	ton/y	3 410.4

According to laboratory-scale experiments carried out by PROTEINSECTA (PROTE) and AINIA (at upstreaming and downstreaming levels) within M1-M8, and based on the demo-size of value chain nr. 1 (insect biomass production and processing) and the available substrates at MAHOU case study site (see Table 1), the following operational mass balances of the process at the case study site has been calculated (see Table 2). Scale factors were applied for both upstreaming and downstreaming considering lab and demo sizes to calculate streams, dimensions and energy (thermal/electricity) demands.

As it can be observed at the following mass flow diagram, both bagasse and spent yeast extract (SYE, 13.9 ton/y) are used for both breeding and production (or the so-called marketable) phases. Based on the optimum formula definition provided by PROTE, the breeding unit will include 18 insect modules and require SYE, bagasse, and wheat bran for D30 larvae generation within the 120 days-cycle developed per year. About 5% of the larvae produced will be used for the generation of D90 and adult individuals, thus producing eggs and maintaining the colony.

An electricity consumption of about 77.100 kWh/y has been estimated for the upstreaming unit (both comprising breeding and fattening units), mainly associated to the air handler required for insect temperature maintenance. On the other hand, the remaining 95% larvae will pass to the production unit, composed of 54 working modules operating at 5 cycles/y with a duration of 70 days/cycle. The diet at this phase has been also optimized by PROTE, thus requiring about 13,3 ton bagasse/y, 9,9 ton SYE/y and 66,5 ton wheat bran. Along this phase, D30 larvae will be converted into D70, which will result in frass and insect biomass, the later calculated as 13.5 ton/y (35%TS) (see Table 2).

Table 2. Main waste and by-products mass flows of the CHEERS case study site within the Insect Platform (value chain nr. 1).

Description	Unit	Measurement
SYE inlet (89.4% humidity)	ton/y	9,9
Bagasse inlet (76.3% humidity)	ton/y	13,3
Wheat bran (10.1% humidity)	ton/y	66,5
Insect biomass produced (65% humidity)	ton/y	13.5
Fat and other non-water particles produced	ton/y	11.5
Insect defatted flour produced after processing	ton/y	2.0

According to preliminary tests at AINIA, the insect biomass produced at the demo size will be further processed through cold press extractor (for defatting), particle size-defined mill (for size reduction), extraction decanter (for protein extraction), spray drying (for size optimization) and high-pressure homogenizer for further formulation, which will be assisted by pasteurization and liquid mixer by MAHOU). The energy demand associated to DSP, 6 times higher than that of upstreaming, has been estimated considering activity for 260 days/y in a batch mode, based on the possibility to store the biomass produced once frozen. The insect defatted flour obtained prior formulation has been estimated at 2.0 ton/y (88% TS,
<2% fat, 70% protein), based on the yields reported by AINIA in previous trials.

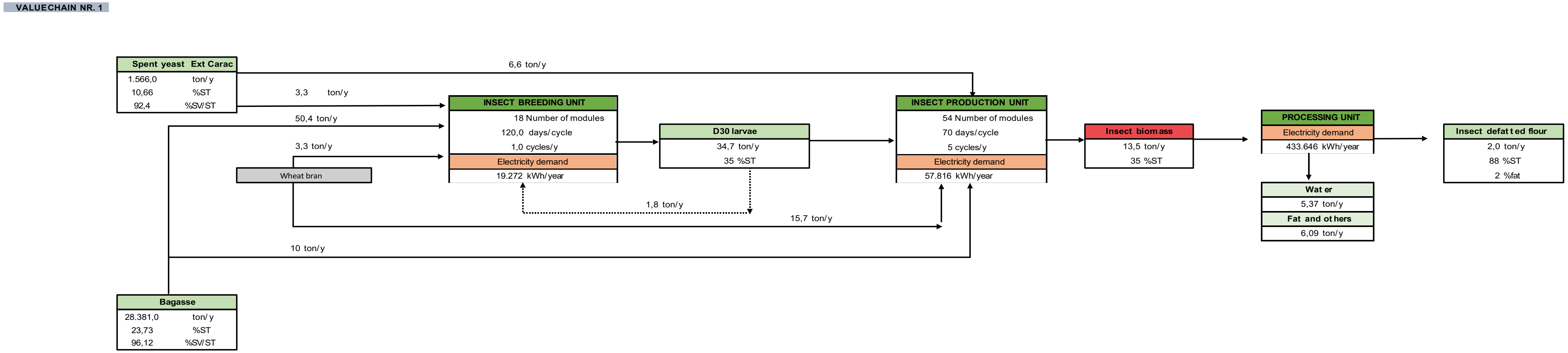


Figure 4. Overall mass balance overview of insect-based value chain for the selected CHEERS demo size.

Color legend: The colors used in all process flow diagrams:

Light green: feedstock and by-products

Dark green: process units

Red: Final product

3.1.3 Financial projection and environmental perspective

The preliminary cost-benefit analysis of the CHEERS demo for Insect-protein production plant included in D6.1 was correspondingly updated, considering current bagasse and spent yeast potential at Lleida case study site. The total available amount of bagasse is partially valorized through Insect platform and partially through value chain2 (Caproic Acid production).

Operational costs (OPEX) associated to power demands were calculated assuming 0.18€/kWh. Also, a scale factor for operational and investment costs was applied for lab-scale to demo-scale data conversion. To estimate the revenue, selling price of 5 500 €/ton of insect-defatted flour is considered (see Table 3). The most significant operational costs were found to be associated to energy demands mainly at DSP unit prior to obtain the final product ready to access the market. The revenue estimated belongs to the demo size dimension which may not be enough to cover the operational cost, however, there will be an improvement in the process to reduce the costs and increase the revenue, which will be implied throughout the implementation of the project.

Table 3. Cost-benefit analysis of the Insect-protein production value chain (nr. 5) under full biogas valorisation.

Description	Unit	Measurement
Insect-protein	ton/y	2
Investment costs	€	110 000
Operational costs	€/y	131 212
Insect-protein price	€/ton	3 500-5 500
Insect-protein selling revenues	€/y	11 000
Pay back	y	>7

Annual cash flow has been obtained from the difference between total income, depreciation and operational costs. Payback was calculated by dividing the investment costs by the annual cash flow.
Operational costs considered include raw materials, electrical power, personnel and maintenance costs.
Depreciation period = 10 years..

In terms of GHG emissions, an emission factor of 0.38 kgCO$_2$-eq/kWh (Spanish National Markets and Competition Commission (CNMC)) was considered for further calculations *. The insect platform depicted does not lead to the production of tonCO$_2$/y due to wastes. The environmental impact is affected by the 194.08 tonCO$_2$-eq/y associated to emissions resulted from power consumption (510 734 kWh/y), might be expected. However, further environmental assessment within WP6 will be deployed in D6.4 at different bio-based industries scale to further confirm this first approach.

* Tier 1 Calculation Method: GHG emission = 0.001 * Fuel Usage * High heat value *Emission factor. CO2 e = GWP*GHG emission (tons)

This process, also adds value to bagasse and spent yeast, which are currently being used as animal feed.

To this positive impact, lower land and water consumption can be added.

3.2 Feasibility Study of Value Chain-2: MICROBIAL PLATFORM. Bioconversion of CO_2 to CAPROIC ACID

3.2.1 Introduction

The brewery sector faces significant management issues related to their main waste side-streams, either in the form of wastewater (WW), expired ethanol by-products, bagasse or even biogas, the last two being currently valorized as low-cost animal feed and heat/electricity, respectively.

A biotechnological process comprising the conversion of reduced waste into volatile fatty acids (VFA) and further medium chain fatty acids (MCFA), named as microbial chain elongation (MCE), has been lately described and scaled-up as an environmentally-friendly approach for the production of n-caproic acid when compared with its petrochemical counterpart production pathway. Such technology allows for the conversion of WW especially, though extrapolated to other organic waste susceptible to produce VFA and entering the process. This novel technology has demonstrated to be enhanced by the addition of ethanol (either synthetic or in the form of ethanol-rich byproducts) as electron donor and CO2 as inorganic carbon source (De Araujo et al., 2016; Roghair et al., 2018), and is being optimized within the frame of CHEERS project by AINIA (see deliverable D1.2 for further details).

Based on the availability of potential substrates for MCE at MAHOU's site location at Lleida (Table 1), CHEERS aims at exploring and up-scaling it at demo size while valorizing the WW, expired ethanol by-products, bagasse and the CO2 of the biogas towards the production of n-caproic acid, an added-value product of the chemical and feed sectors (4-10 €/kg depending on the purity grade).

3.2.2 Process design and technical feasibility

This bio-process consists of an acidogenic reactor, similar to a mixing anaerobic digestor, tailored to develop the reaction to reach VFA (volatile fatty acids) and lead this VFA toward the following microbial chain elongation reactor (MCE), prior to allowing methanation occur which is the scenario of an anaerobic digestor. The short-chain VFAs will be transformed to longer chain compounds through the latter reactor.

According to laboratory-scale experiments carried out by AINIA within M1-M8 (see deliverable D1.2 for further details), and based on the demo-size of value chain nr. 2 (caproic acid production) and the available substrates at MAHOU brewery, the following operational mass balances of the process at the case study site have been calculated (Table 4). Scale factors were applied for both upstreaming and downstreaming units considering lab and demo sizes to calculate streams, dimensions and energy (thermal/electricity) demands.

As it can be observed through the process flow diagram below, both bagasse (9.5 ton/y) and wastewater (6.7 ton/y) are initially mixed together in a mixing tank to adjust the percentage of total solids (%TS) below 15% and pump it to the first unit, the acidogenic reactor. The digestate produced after a hydraulic retention time (HRT) of 8 days, enriched in VFA, will be pumped to the microbial chain elongation reactor, with an HRT of 6.4 days.

Both reactors, of 0.5 m^3, operate at mesophilic conditions with its energy demands satisfied through heating pipes/coils.

Specifically, the MCE will be designed to be also fed with expired ethanol products and dealcoholizing extracts (5-7% ethanol, 4.2 ton/y) and CO_2 from the fermentation surplus at the brewery plant (100% CO_2, 0.175 ton/y), in order to promote the elongation of the VFA already produced in the acidogenic unit implementing a gas recycling rate (R) of 15x.

In this regard, electricity demands at upstreaming level have been estimated to be almost double for the MCE reactor compared to the acidogenic one, due to the significant power consumption reported for the blower used throughout gas recycling.

A total of 88.6 m^3 biogas (enriched with CH_4 up to 80% v/v) could be produced through hydrogenotrophic activity, while reducing about 80% of the inlet CO_2 feeding concentration. It must be highlighted that such stream will be susceptible for valorisation towards the methanotrophic platforms within CHEERS (value chains nr. 4 and 5) or even be deviated to upgrading units, if applicable.

Table 4. Main waste and by-products mass flows of the CHEERS case study site to be used in the caproic acid value chain (nr. 2).

Description	Unit	Measurement
Bagasse inlet (76.3% humidity)	ton/y	9.51
Waste Water inlet (99.6% humidity)	m^3/y	19.58
Expired ethanol and dealcoholized products inlet (95.7% humidity)	m^3/y	4.65
CO_2 fermentation surplus inlet (100% CO_2)	Nm^3/y	97.4
CH_4-enriched biogas produced	Nm^3/y	0
MCE-based digestate produced	m^3/y	25,1
DSP-based retentate produced	ton/y	13.91
Caproic-rich VFA mixture salt produced	ton/y	0.250

The raw digestate produced within MCE, estimated as 25,1 ton/y (11,4/L caproic acid and 3% ST) based on an HRT of 6 days, will be subjected to a downstreaming process (DSP) including centrifugation, solid filtration, micro- and nanofiltration, thus ending up with a spray drying module leaving 0.3 ton caproic acid-rich VFA mixture/y at a concentration of over 414,8 kg VFA/ton, which is aligned with its corresponding KPI (minimum production of 50 kg/y of chlorine) (see Figure 5).

The highest energy demands at this level will be represented by the centrifugation step and spray drying, based on the period of operation considered to avoid digestate spoilage (260 days/y) and quantity of water to be removed, respectively.

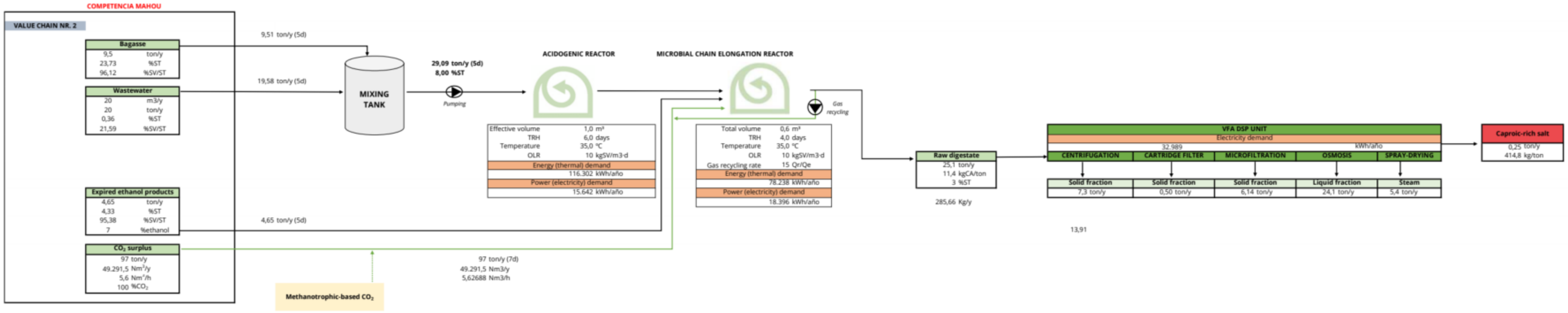


Figure 5. Overall mass balance overview of caproic acid-based value chain for the selected CHEERS demo size.

3.2.3 Financial projection and environmental perspective

The preliminary cost-benefit analysis of the CHEERS demo caproic acid production plant included in D6.1 was updated considering the maximum valorisation of substrates (waste/by-products) available on-site given the current potential at Lleida (9,51 ton bagasse/y, 19,58 m3WW/y, 4,64 ton expired ethanol products/y, 0,19 tonCO2/y) and the main limiting substrate due to its lower availability, which are the expired ethanol products and dealcoholizing extracts (hereinafter called as EEPDE). Under such conditions, the complete consumption of EEPDE waste will imply the co-valorisation of 9,51 ton bagasse/y, 19,58 tonWW/y and 0,19 $tonCO_2/y$. According to the preliminary information provided by AINIA, a yield of 3 kg caproic-rich VFA/kg CO_2 can be used for further calculations. Operational costs (OPEX) associated to power demands were calculated assuming 0.18€/kWh. Also, scale factors for operational and investment costs were applied considering existing data at CHEERS scale (see Table 5). As observed, the financial projection slightly differs from the theoretical one initially presented in D6.1, with a payback of 6-7 years considering a VFA salt price of 5 €/kg. The most significant operational costs were found to be associated to energy demands at DSP level.

Table 5. Cost-benefit analysis of the caproic acid production value chain (nr. 2) under maximum EEPDE, CO_2, WW and bagasse valorisation.

Description	Unit	Measurement
Available EEPDE (7% ethanol)	ton/y	4,65
Bagasse consumed (76.3% humidity)	ton/y	9,51
WW consumed (99.6 %humidity)	m^3/y	19,58
CO_2 surplus consumed (100% CO_2)	ton/y	0,19
Caproic-rich VFA salt potential production	ton/y	0,250
Investment costs	€	407.519,98
Operational costs	€/y	126.305,45
Caproic-rich VFA salt price	€/kg	5
Caproic-rich VFA salt selling revenues	€/y	1.250,65
Payback	y	6-7

Annual cash flow has been obtained from the difference between total income, depreciation and operational costs. Payback was calculated by dividing the investment costs by the annual cash flow.

Operational costs considered include raw materials, electrical power, personnel and maintenance costs.

Depreciation period = 10 years..

*Investment costs= USP unit investment costs + DSP Unit investment costs/Ratio investment; Operational costs= USP operational costs+ DSP operational costs/opex ; Revenues= Ton AGVs/y*5*1000*

In terms of GHG emissions, an emission factor of 0.38 $kgCO_2$-eq/kWh (Spanish National Markets and Competition Commission (CNMC)) was considered for further calculations *. Also, the direct microbial CO_2 conversion of this value chain leads to the reduction of 211.9 $tonCO_2/y$ along MEC; whereas the intrinsic use of the aforementioned waste quantities may lead to CO2 savings of 71 645,9 $tonCO_2/y$ (emissions associated to waste =-2.89*(ton WW/y+ton bagasse+ ton EEPDE/y). Taking into account the emissions associated to power consumption 378.9 tonCO2-eq/y ((0.38/1000)*kWh/y/opex), final negative 7 1478.9 $tonCO_2$-eq/y might be expected. Nonetheless, further environmental assessment within WP6 will be deployed in D6.1 at different bio-based industries scale to further confirm this first approach.

* Tier 1 Calculation Method: GHG emission = 0.001 * Fuel Usage * High heat value *Emission factor. CO2 e = GWP*GHG emission (tons)

3.3 Feasibility Study of Value Chain-3: MICROBIAL Platform. Bioconversion of CO_2 to CHLORINE-based Disinfectant

3.3.1 Introduction

Wastewater Treatment Plants (WWTP) are clear examples of facilities which use energy-intensive processes to treat water. Anaerobic Digestion (AD) of wastewater treatment sludge and other organic wastes, reduces energy needs of own WWTP while resolving waste management issues related to sludge/wastes disposal. Biogas is the main product of AD of organic wastes and WWTPs sludge, which typically contains about 65% CH_4, 35% CO_2 and limited quantities of H_2S, N_2, H_2 and other impurities.

Valorisation of biogas in WWTPs is achieved in CHP (Combined Heat Power) engines to produce heat and electricity for WWTP self-consumption while the excess of electricity is being fed into electrical grid.

Although such valorisation is interesting from economic point of view, low efficiency of CHP units, approx. 33%, for electricity, and approx. 45%, for thermal recovery, is pushing the users towards alternative valorisation options for biogas produced, which leads to resource efficiency and environmental benefits.

Meanwhile, biogas and more specifically, biomethane are attracting interest as Green House Gases (GHG) emissions reduction while easing a sustainable energy supply development. Biomethane, as an affordable and sustainable biofuel and like natural gas, is obtained through a costly Upgrading process.

Prior to introducing biogas into an Upgrading system, impurities such as H_2S, need to be removed. There are, currently, four Upgrading technologies being the most widely used. One of them, water scrubbing, is a simple process based on solubility differences of CH_4 and CO_2. It consists of an absorption column followed by a stripping column where CO_2 is released into atmosphere which is a negative contribution to GHG emissions.

This value chain of CHEERS project improves the mentioned system, by replacing stripping column with Bio-Electrochemical System (BES) which not only reduces CO_2 emissions, but also increases bio-CH_4 production. This novel system is tailored by partner AQUALIA (hereinafter, AQUA) and is going to be optimized throughout the implementation of the project. (Batlle-Vilanova, 2019)

The potential impacts of applying this technology are as follow:

- Increasing biomethane production
- Decreasing CO_2 emissions
- Producing chlorine compounds, which are capable of disinfecting all the treated wastewater of the WWTP of Lleida.

3.3.2 Process Design and technical feasibility

This process consists of an absorption column where biogas is being fed and its CO_2 content is dissolved in water, which is necessary to enter the column as a feedstock. This water will be wastewater produced at WWTP of the case study which leads to reduced water consumption.

This effluent emerged from absorption column is a CO_2-rich aqueous effluent feeding cathode chamber of BES (Bio Electrochemical System), trace amount of clean water as well as biomethane with high content of CH_4. Biocathode chamber contributes to additional CH_4 production, as its output is bioCH_4 with a CH_4 in content over 90%.

The BES bioanode chamber is being fed with the brine (6% NaCl) resulting from methanotrophic ectoine production Down Stream Process (DSP) which Cl_2-rich water, through the oxidation of the brine Cl- and furtherly is converted into chlorine disinfectant through an evaporation unit. (Batlle-Vilanova, Pau et al., 2019)

According to laboratory-scale experiments carried out by AQUALIA (hereinafter AQUA) within M1-M8 (partially reported in deliverable D1.2) and based on the demo-size of value chain nr. 3 (chlorine-based disinfectant production) and the available substrates at Lleida, the following operational mass balances of the process at the case study site has been calculated (see Table 6). Scale factors were applied for both upstream and downstream units considering lab and demo sizes to calculate streams, dimensions and energy (thermal/electricity) demands.

As depicted in Table 6, both wastewater (29.2 m^3/y) and biogas (14.6 m^3/y) can be valorised into the system at the scale proposed for CHEERS project. Both streams are mixed in countercurrent mode in the absorption column for wastewater (WW) saturation at 40% CO_2.

As a result of the absorption step, a biomethane stream with less than 2%CO_2 might be recovered upwards at 14.6 m^3/y. The liquid stream is later pumped into the cathode chamber of the BES system (0.5 m^3 equally divided into two compartments, biocathode and anode) for the bioelectrochemical conversion of CO_2 to CH_4 at a rate of 35%.

As a result, about 7.1 m^3 biomethane/y (85% CH_4 can be additionally produced by hydrogenotrophic activity and mixed with the biomethane produced along absorption, which leads to an average CH_4 content of 94.5%). The biomethane sum could be either employed for gas grid injection (if a polishing step is applied) or even integrally valorized within the CHEERS methanotrophic platforms (value chains nr.4 and 5) if properly mixed with air, as indicated later.

The clean water resulting from the cathode compartment may be returned to the wastewater treatment plant line for further polishing steps.

The HRT indicated for both cathode and anode were calculated based on the flows indicated by AQUA, though might be significantly reduced under optimized demo conditions, thus leading to a higher WW treatment capacity and biomethane production potential.

On the other hand, at the anode compartment, the unit is able to valorised the brine produced in the first step of the ectoine DSP unit ($NaCl_2$-rich broth, at 6%, 29.2 m^3/y) through the operating current towards HClO (10.4 kg HClO/$m^3 CH_4$ produced) and further Cl_2 in the range of ppm (100-300 ppm depending on the chlorine form) (Figure 6).

Table 6. Main waste and by-products mass flows of the CHEERS case study site to be used in the chlorine production value chain (nr. 3).

Description	Unit	Measurement
Biogas inlet (65% CH_4)	m^3/y	14.6
Wastewater inlet	m^3/y	29.2
Brine inlet from Ectoine DSP	m^3/y	29.2
Clean water produced	m^3/y	29.2
Biomethane produced (94.5% CH_4)	m^3/y	7.1
HClO produced	kg/y	73
Cl_2-rich water (0.03% Cl_2)	m^3/y	29.2
DSP-based chlorine concentrated stream (11% Cl_2)	kg/y	892.5

According to the Cl_2 concentrations reported by AQUA in this stream, a DSP process consisting on both a nanofiltration and an evaporation step is required to increase purity and concentration of Cl_2 to the 11% targeted, thus resulting in 892.5 kg chlorine concentrated/y. Based on the production rates of Cl_2-rich water at the anode outlet, a removal of 29.2 m^3/y of water from such stream is needed to achieve the indicator mentioned, the biggest power consumption at this point being attributed to the evaporator (130 kWh/m^3 water).

Moreover, considering disinfection practices of WWTPs at 1 mgCl_2/L, the overall disinfection capacity of the amounted product can be up to 98 465 m^3/y. It must be highlighted that the quantity of chlorine disinfectant produced surpasses the minimum quantities reported for the associated KPI, and can be even improved at higher NaCl contents (depending on the brine stream used) and optimizing L/G ratios in the absorption column constructed at demo scale.

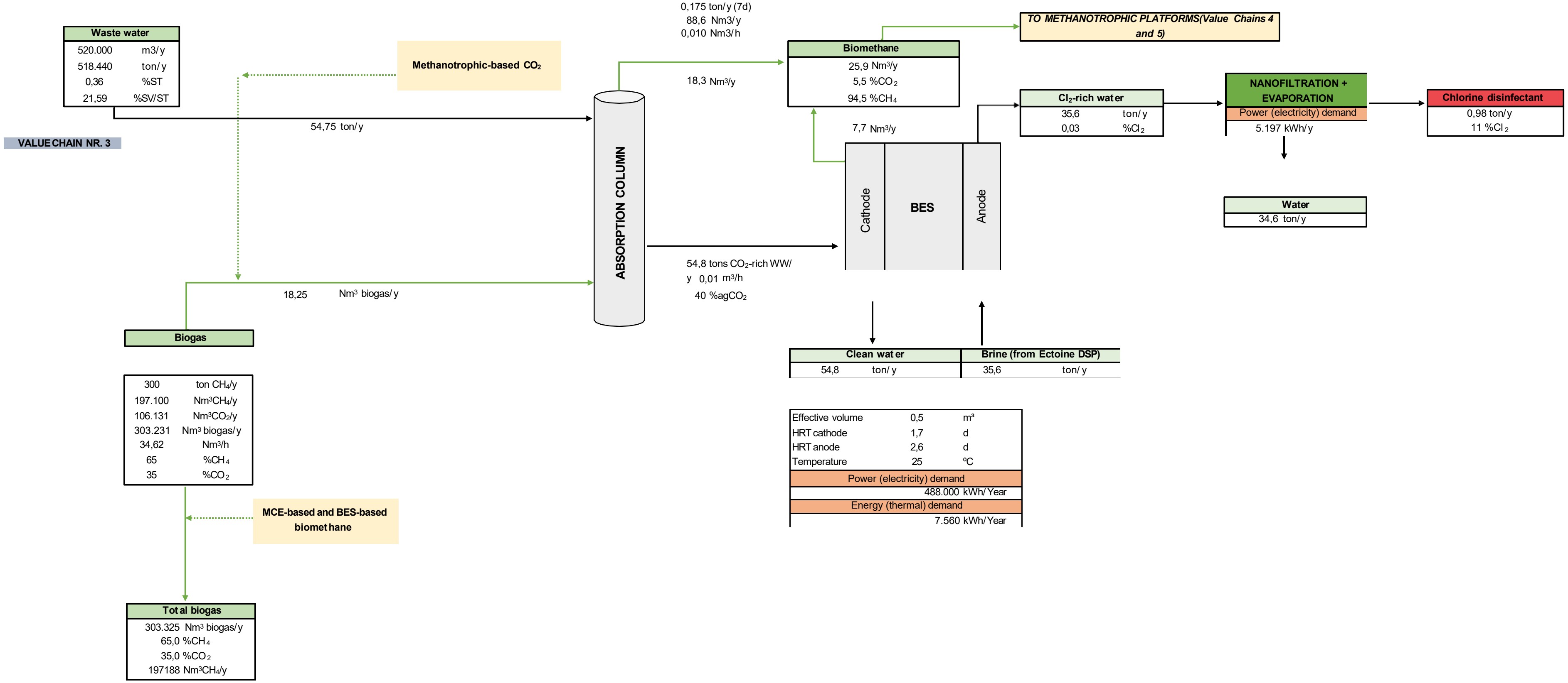


Figure 6. Overall mass balance overview of Chlorine production value chain for the selected CHEERS demo size.

3.3.3 Financial projection and environmental perspective

The preliminary cost-benefit analysis of the CHEERS demo-scale plant for Chlorine production, included in D6.1, was correspondingly updated, considering current biogas potential at Lleida case study site (303 231 Nm^3 biogas/y) is partially valorized through the Cl_2-disinfectant value chain.

The yield observed by AQUA at demo-scale, given as 21 $kgCl_2/kgCO_2$, has been considered for further calculations.

Operational costs (OPEX) associated with power demands were calculated assuming 0.18€/kWh. No scale factor is considered for the evaluation, as the information provided by AQUA was adapted to demo-scale. The revenue obtained in demo-scale is estimated for the Chlorine-based disinfectant of 0.50 €/kg (see Table 7). The most significant operational costs were found to be associated with energy demands.

Table 7. Cost-benefit analysis of the Chlorine production value chain (nr. 5) under full biogas valorisation.

Description	Unit	Measurement
Available biogas (35% CO_2)	Nm^3/y	303 231
CO_2 availability	Nm^3/y	106 131
Cl_2 production	ton/y	668
Disinfectant production	ton/y	6 072
Investment costs	€	798 600
Operational costs	€/y	56 932
Cl_2-based disinfectant selling revenues for demo-scale	€/y	166 988
Payback	y	2-3

Annual cash flow has been obtained from the difference between total income, depreciation and operational costs. Payback was calculated by dividing the investment costs by the annual cash flow.

Operational costs considered include raw materials, electrical power, personnel and maintenance costs.

Depreciation period = 10 years.

In terms of GHG emissions, an emission factor of 0.38 $kgCO_2$-eq/kWh (Spanish National Markets and Competition Commission (CNMC)) was considered for further calculations*. The environmental impact is affected by the tCO_2-eq/y saving resulting from the conversion of CO_2 to $bioCH_4$ at the inlet, which exhibits an emission factor of 28 tCO_2-eq/tCH_4. Taking into account the emissions associated to power consumption (187 tCO_2-eq/y), final 186 tCO_2-eq/y might be expected. However, further environmental assessment within WP6 will be deployed in D6.1 at different bio-based industries scale to further confirm this first approach.

* Tier 1 Calculation Method: GHG emission = 0.001 * Fuel Usage * High heat value *Emission factor. CO2 e = GWP*GHG emission

(tons)

3.4 Feasibility Study of Value Chain-4: MICROBIAL PLATFORM.Bioconversion of CH_4 to ECTOINE

3.4.1 Introduction

The decreasing profitability of biogas valorization into heat and electricity caused by the high capital and maintenance costs of cogeneration engines, as well as the rapid decrease in production costs of solar and wind energy productions, has reduced incomes associated to biogas valorization through CHP (Combined Heat Power) engines. In this regard, creating additional value out of biogas produced by waste treatment AD has become a priority throughout recent past years. (Rodríguez et al., 2020)

In this context, alternative biogas valorization, such as the production of biomethane and chemicals such as methanol, polyhydroxyalkanoates (PHA), or single cell protein and ectoine from biogas components have rapidly developed and attracted significant research efforts from academia and industry.

Moreover, the transition from the current linear anaerobic digestion plants to novel concepts of circular waste biorefineries might help in adapting the processes align with highly restrictive environmental policies, the European Green New Deal and Circular Economy Directives, while improving the economic feasibility of waste treatment plants.

In this regard, haloalkaliphilic methanotrophic bacteria capable of accumulating high amounts of ectoine (up to 230 mg ectoine/g biomass) in waste treatment plants have demonstrated a great potential to upgrade the traditional use of biogas. Bioconversion of biogas into ectoine, an osmolyte with high interest in cosmetic industry, emerged as a promising strategy. (Pérez et al., 2021)

3.4.2 Process design and technical feasibility

Prior to biogas valorization into ectoine, a desulfurization stage will be designed for preventing corrosion in downstream piping and equipment. Also, a mixed culture of haloalkaliphilic methanotrophic bacteria was selected for the production of ectoine from CH_4 and biogas-CH4. The reason for this selection is supporting long-term operation and process robustness.

An NaCl concentration of 6%w/w has been applied as the optimal salinity for accumulation of ectoine in methanotrophic culture. The bioreactor will operate under continuous mode and with a certain dilution rate per day in order to avoid total organic carbon accumulation in the system. Moreover, to support methanotrophic bacteria growth and ectoine synthesis, a mineral medium solution containing $NaNO_3$ and NaCl as well as trace micronutrient concentrations is intended to be added to the reactor.

The mentioned Biogas or CH_4, from CO_2 methanation in the BES, will be converted into intracellular ectoine using a novel Taylor flow biofermentor.

According to laboratory-scale experiments carried out by University of Valladolid (UVa) within M1-M8 (partially reported in deliverable D1.2), and based on the demo-size of value chain nr. 4 (ectoine production) and the available substrates at Lleida, the following operational mass balances of the process at the case study site has been calculated (see Table 8). Scale factors were applied for both upstreaming and downstreaming considering lab and demo sizes to calculate streams, dimensions and energy (thermal/electricity) demands.

As it can be observed in the following mass balance flow diagram, biogas (2 803.2 m^3/y) can be valorized in the sized CHEERS demo through the two methanotrophic platforms hereby developed. Such biogas stream requires mixing with air (32 237 m^3/y) to both ensure safety conditions below CH_4 explosive limit (5% v/v) and adequate methane bioxidation conditions with O_2 excess (CH_4:O_2 ratio ≥1.5) within gas feeding.

The resulting mixed gas (35 040 m^3/y) will be equally divided to feed both methanotrophic platforms (17 520 m^3/y or 2 m^3/h; 5% CH_4). Specifically, for the ectoine process line (value chain nr. 4), methane is valorized through the mentioned Taylor flow biofermentor of 1 m^3 with an HRT of 10 days and a working temperature of about 30 ºC, based on the conditions indicated by UVa for *M. alcaliphilum* 20Z.

The microbial diet will be completed with the provision of a nutrient solution (including industrial grade salts for cell growth and maintenance, NaCl being the most significant), at a rate of 36.5

The overall gas residence time (GRT) applied will be tentatively about 30 min, a recycling rate of 15x being implemented to ensure a minimum of 95% CH_4 bioconversion. However, in order to maximize ectoine production, both GRT and R will be reduced and increased, respectively, along demo operation. The GRT applied will result in the generation of an exhausted biogas with about 0.25 %CH_4 and 5.1 %CO_2, as a result of a 95% CH_4 oxidation and a carbon mineralization to CO_2/biomass of 50% according to the laboratory results provided. The HRT will produce a culture broth including 6% NaCl, 3g/L biomass (exhibiting about 7%

intracellular ectoine), which is later deviated to the ectoine DSP unit (see Figure 7). In terms of energy requirements, the blowers associated to gas feeding to the fermenter (biogas and air) have been identified among the equipments with the highest electricity consumption along the prototype.

Table 8. Main waste and by-products mass flows of the CHEERS case study site to be used in the ectoine production value chain (nr. 4).

Description	Unit	Measurement
Biogas inlet (65% CH_4)	Nm^3/y	1 401.6
Air inlet (21% O_2)	Nm^3/y	116 508
Nutrient solution inlet (6 %NaCl)	m^3/d	0.1
Culture broth outlet (3 g/L, 7% intracellular ectoine)	m^3/y	36.5
Brine fraction produced (6% NaCl)	ton/y	3.
M. alcaliphilum-based SCP produced (50% protein content)	ton/y	0.7
Ectoine (<5% humidity, >95% purity)	kg/y	6.8

The ectoine DSP unit require d for the purification of the cosmetic ingredient will consist of a sequential process of centrifugation, hyposmotic shock, ultrafiltration, electrodialysis, ion exchange (IE), spray-drying, solubilization in methanol solution, ultrafiltration, and crystallization, thus finally end up in a highly purified product at a minimum rate of 4.27 kg/y (see Figure 7). Mass balances at DSP level have been carried out in direct collaboration with UVa assuming an 85% ectoine secretion, a demand of 10 kg methanol/kg ectoine and a maximum humidity of 5% in the final product.

Among DSP units, electrodialysis and centrifugation were clearly identified as the units with the highest energy consumptions (16 and 12%, respectively). Despite, the aforementioned ectoine quantities do not match yet the specified KPI (20 kg/y), this value is expected to be achieved once the fermenters' TRH is optimized along demo operation.

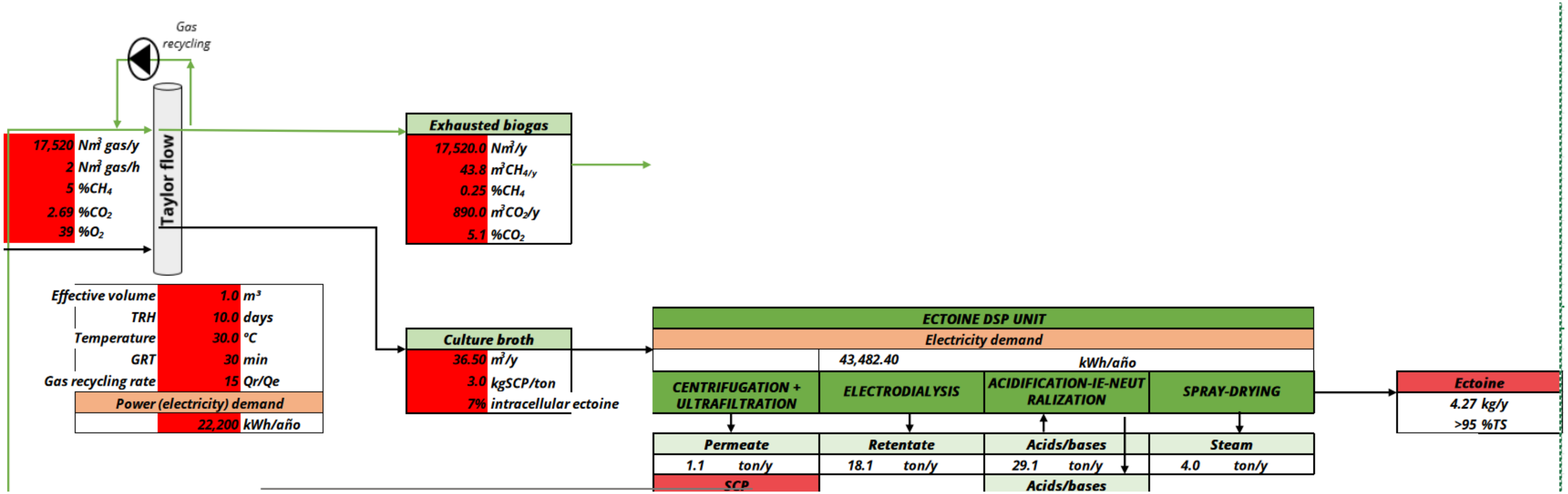


Figure 7 . Overall mass balance overview of Ectoine production value chain for the selected CHEERS demo size.

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3.4.3 Financial projection and environmental perspective

A detailed cost-benefit analysis of the CHEERS demo ectoine production plant was already included in D6.1 considering a theoretical scenario and has been updated considering the current biogas potential at Lleida case study site (303 231 Nm^3 biogas/y) is entirely valorized through the ectoine production value chain. Operational costs (OPEX) associated to power demands were calculated assuming 0.18 €/kWh. The yield observed by UVa at lab-scale, given as 0.04 kg ectoine/kgCH_4, has been considered for further calculations. Also, a scale factor for operational and investment costs was applied considering existing data at CHEERS scale (see Table 9). As observed, the financial projection does not differ so much compared to the theoretical one initially presented, with a payback of less than 3 years considering an updated ectoine price of 1000€/kg. The most significant operational costs were found to be associated to raw materials (industrial grade salts).

Table 9. Cost-benefit analysis of the ectoine production value chain (nr. 4) under full biogas valorisation.

Description	Unit	Measurement
Available biogas (65% CH_4)	m^3/y	303 325
CH_4 availability	ton/y	129 552.54
Ectoine (>95%) potential production	kg/y	4,27
Investment costs	€	349.649,13
Operational costs	€/y	37.920,70
Ectoine price	€/kg	1000
Ectoine selling revenues	€/y	4.269,72
Payback	y	3

[a]Updated considering the purity achieved along DSP trials.

Annual cash flow has been obtained from the difference between total income, depreciation and operational costs. Payback was calculated by dividing the investment costs by the annual cash flow.

*Investment costs=(200000)/0,4; Operational costs= 420000/opex (0,44) ; Revenues= Ton AGVs/y*1000*1000*

Depreciation period = 10 years.

In terms of GHG emissions, an emission factor of 0.38 kgCO_2-eq/kWh (Spanish National Markets and Competition Commission (CNMC)) was considered for further calculations*. The microbial platform described above in this section, leads to the production of 195.9 tonCO_2/y as a consequence of microbial CH_4 oxidation, according to the reaction stoichiometry.

* Tier 1 Calculation Method: GHG emission = 0.001 * Fuel Usage * High heat value *Emission factor. CO2 e = GWP*GHG emission (tons)

However, the environmental impact is strongly affected by the 5 580 tCO_2-eq/y saving resulting from the conversion of 95% CH_4 at the inlet, which exhibits an emission factor of 28 tCO_2-eq/tCH_4.

Taking into account the emissions associated to power consumption (54.9 tCO_2-eq/y), final negative 1 897.6 tCO_2-eq/y might be expected. However, further environmental assessment within WP6 will be deployed in D6.1 at different bio-based industries scale to further confirm this first approach.

3.5 Feasibility Study of Value Chain-5: Microbial Platform. Bioconversion of CH_4 to SCP

3.5.1 Introduction

Food competition is becoming more intense as the world's population continues to grow, which at current consumption levels would cause the global demand for animal-derivative protein to reach thousands of million tons per year.

On the other hand, recent evidence has shown approximately 9% of the world population is undernourished.

Single cell protein (SCP) is the first product of the fermentation process and has proven to be a good protein alternative. Soon, SCP may be able to contribute against protein deficiency. High quantities of SCP can be produced by microorganisms, such as algae, yeast and bacteria, due to their fast development rate and the significant level of protein in their chemical structure. Beside proteins, SCP contains carbohydrates, nucleic acids, lipids, minerals, vitamins and essential amino acids.

SCP has been an effective substitute for more expensive protein sources such as fish and soybean products. In conclusion, SCP can easily replace traditional protein sources in human and animal feed without side effects. (Bratosin et al., 2021).

3.5.2 Process designs

The process mechanism composed of a forced-circulation U-loop fermenter to convert CH_4 content of biogas into a protein-rich biomass called SCP.

The feedstock for this process is biogas rich in CH_4 and nutrients such as KNO_3. Methanotrophic bacteria culture grows aerobically in the U-loop fermenter using methane as its source of energy and KNO_3 as nitrogen source. Aeration may be improved further by having air supply entering the methanotrophic culture. This fermenter is followed by a purifying/drying post-treatment step composed of centrifugation and spray-drying units.

This process has been developed by AINIA and SYSPRO at TRL5 and will be upscaled in CHEERS project.

According to laboratory-scale experiments carried out by AINIA within M1-M8 (reported in deliverable D1.2) and based on the demo-size of value chain nr. 5 (SCP production) and the available substrates at Lleida, the following operational mass balances of the process at the case study site has been calculated (see Table 6).

Scale factors were applied for both upstreaming and downstreaming considering lab and demo sizes to calculate streams, dimensions and energy (thermal/electricity) demands.

As described for value chain nr. 4, a biogas-air stream of 17 520 m^3/y (2 m^3/h; 5% CH_4) is expected to be fed, after mixing and equal deviation, to the gas-recycling U-loop technology for methanotrophic growth.

The fermenter is expected to be 1 m^3 of total volume, with an HRT of 6 days according to preliminary laboratory tests. Working temperature for the cultivated microorganism/consortium (fast-growth type I methanotrophs such as *Methylococcus* sp.) has been set at 35-40ºC, which can be easily achieved with no need to heat contribution due to self-heat production derived from the liquid recycling system implemented onto the fermentation unit, which represents the highest energy (electricity) consumption at this level (about 5 times higher compared to the Taylor flow technology of value chain nr. 4).

Gas recycling at R=15x will be implemented to ensure 95% bioconversion of CH_4. Microbial diet will be complemented with a nutrient solution at 60.8 m^3/y, including industrial grade salts for cell growth and maintenance and KNO_3 being the most significant component (minimum of 1 g/L for proper performance).

The performance in terms of methane abatement is estimated to be similar to the one described for the *M.alcaliphilum* unit (value chain nr. 4), based on the GRT employed, similar mineralization rates (C-CH_4 deviated to C-CO_2) and the data provided by AINIA (preliminary CH_4 bioconversion efficiencies >80% without gas recycling optimization). Thus, exhausted biogas at 17 520 m^3/y with 0.25 %CH_4 and 5.1 %CO_2 is expected to result, susceptible to be used in value chain platforms nr. 2 and 3 to further increase overall circularity and economic feasibility at higher bio-based scales implementing CHEERS technology (Figure 8).

Table 10. Main waste and by-products mass flows of the CHEERS case study site to be used in the SCP production value chain (nr. 5).

Description	Unit	Measurement
Biogas inlet (65% CH_4)	m^3/y	1 401.6
Air inlet (21% O_2)	m^3/y	16 118.5
Nutrient solution inlet (including 0.1 %KNO_3)	m^3/y	60.8
Culture broth produced (7 g/L, 50-70% protein content)	m^3/y	60.83
Liquid fraction produced (susceptible for upstream nutrient recycling)	m^3/y	57.39
Type I methanotroph-based SCP produced (70% protein content)	ton/y	0.4

A total of 60.8 m^3/y culture broth is minimally expected considering the HRT indicated, hence containing about 7 g/L of type I methanotrophic biomass to further be purified. Such downstreaming involves a two-step process consists of a centrifugation and a spray-drying, the first representing the highest electricity consumption at this level (90%) considering the operation hours required throughout the year.

A nutrient-rich liquid fraction, mainly produced at centrifugation level, will be produced (57,4 m^3/y) and could be recycled upwards to minimize nutrient consumption and demo plant OPEX. As a result of downstreaming, a minimum of 0.4 ton/y of SCP with proper humidity (less than 5%) will be produced, which is in line with the associated KPI and can be even exceeded if the *M.alcaliphilum*-based SCP produced within value chain nr.4 is considered too.

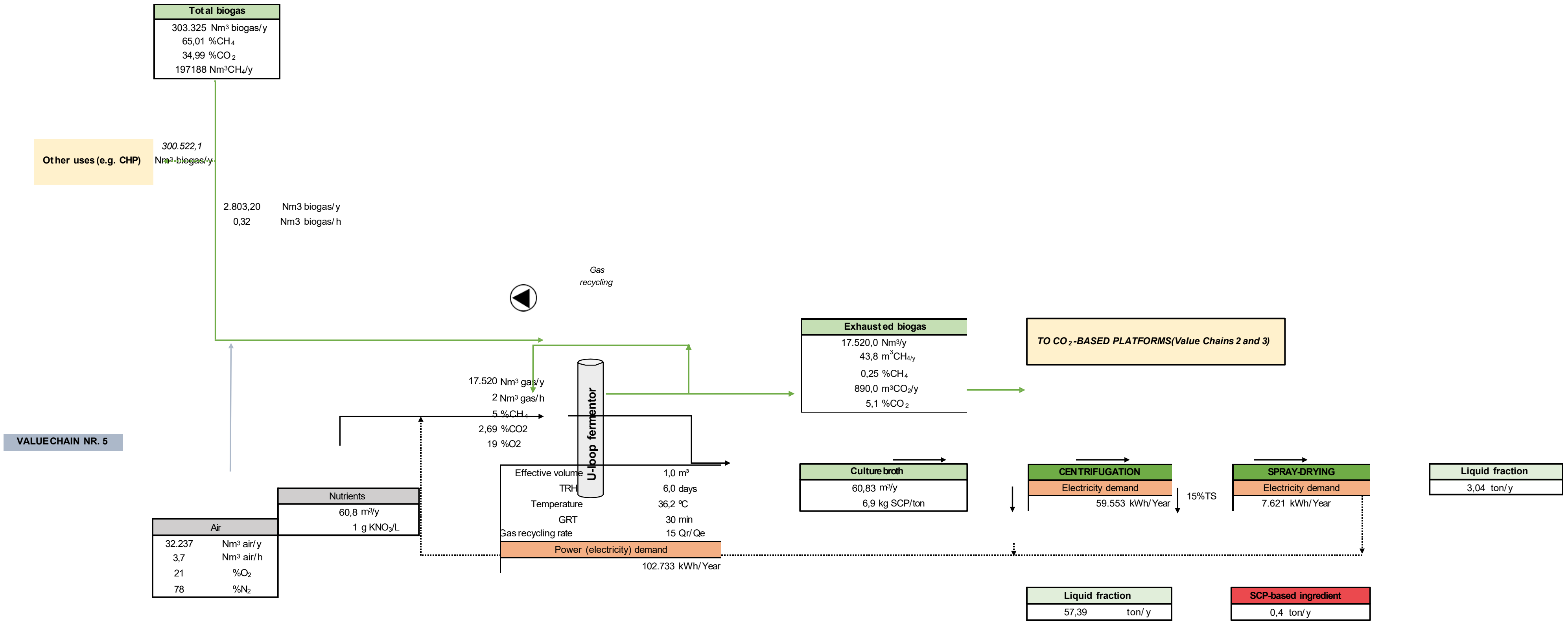

Figure 8. Overall mass balance overview of SCP production value chain for the selected CHEERS demo size.

3.5.3 Financial projection and environmental perspective

The preliminary cost-benefit analysis of the CHEERS demo for SCP production plant included in D6.1 was correspondingly updated, considering current biogas potential at Lleida case study site (303 231 m^3 biogas/y) is entirely valorized through the SCP production value chain.

The yield observed by AINIA at lab-scale, given as 0.5 kgSCP/kgCH_4, has been considered for further calculations.

Operational costs (OPEX) associated to power demands were calculated assuming 0.18€/kWh. Also, a scale factor for operational and investment costs was applied for lab-scale to demo-scale data conversion (see Table 11). As observed, the financial projection does not differ so much compared to the theoretical one initially presented, with a payback of less than 4 years considering an SCP ingredient price of 4.5€/kg. The most significant operational costs were found to be associated to energy demands and raw materials (industrial grade salts).

Table 11. Cost-benefit analysis of the SCP production value chain (nr. 5) under full biogas valorisation.

Description	Unit	Measurement
Available biogas (65% CH_4)	m^3/y	303 325
CH_4 availability	ton/y	129.5
SCP (>95%) potential production	ton/y	64.8
Investment costs	€/y	12 465 424.6
Operational costs	€/y	1 454 299.5
SCP price	€/kg	4,5
SCP selling revenues	€/y	291 453.9
Payback	y	3

Annual cash flow has been obtained from the difference between total income, depreciation and operational costs. Payback was calculated by dividing the investment costs by the annual cash flow.
Operational costs considered include raw materials, electrical power, personnel and maintenance costs.
Depreciation period = 10 years..

In terms of GHG emissions, an emission factor of 0.38 kgCO_2-eq/kWh (Spanish National Markets and Competition Commission (CNMC)) was considered for further calculations*. Also, the microbial platform depicted leads to the production of 195.92 tCO_2/y as a consequence of microbial CH_4 oxidation, considering the metabolic path involved corresponds to the one described for ectoine production (see value chain nr. 4).

* Tier 1 Calculation Method: GHG emission = 0.001 * Fuel Usage * High heat value *Emission factor. CO2 e = GWP*GHG emission (tons)

Again, the environmental impact is strongly affected by the 5580 tCO_2-eq/y saving resulting from the conversion of 95% CH_4 at the inlet, which exhibits an emission factor of 28 tCO_2-eq/tCH_4. Taking into account the emissions associated to power consumption (145.3 tCO_2-eq/y), final negative 1 807.7 tCO_2-eq/y might be expected. However, further environmental assessment within WP6 will be deployed in D6.4 at different bio-based industries scale to further confirm this first approach.

4 Conclusions

The current deliverable helps defining all value chains involved in CHEERS project and providing novel solutions at demo-scale for the production of cosmetic, feed and chemical bulk bioproducts of commercial interest.

The feasibility of the technologies was also assessed based on i) their technical viability through lab- scale experiments as well as theoretical assumptions carried out by the corresponding partners, ii) their lower environmental impact (mainly expressed as CO_2-eq emission reductions) and iii) their economical profit (based on investment, operational costs and revenues).

The preliminary cost-benefit analysis of the CHEERS demo plants included in D6.1 was updated considering the maximum valorisation of substrates (waste/by-products) available on-site given the current potential at the different pilot sites and the main limiting substrate due to its lower availability. For this reason, most of the data regarding investment costs, operational costs and revenues related to the production obtained on a real scale in each pilot and in an estimated manner have been updated based on the total valuation and based on the latest tests carried out on a laboratory scale and the consequent updated mass and energy balances.

The outcomes of the present deliverable will serve as basis for the final feasibility study carried out in WP6, led by ZHAW and the retrofitting of the experimental data obtained in the demo plant at Lleida.

The following process flow diagram (Figure 9) is a helpful tool for visualizing the mentioned five complex processes, process equipment, inputs, by-products and main outputs, their compositions, etc. It serves as a roadmap to understand how the two CHEERS platforms and the five bio-processes are interconnected, techniques through which the streams and the efficiencies of bio-conversions could be optimized, in the end, control and decision points.

The overall preliminary TEA (Technical, Economical, Environmental) feasibility is resulted optimistic, as described throughout this report, for each process at demo-scale, expecting an enhancement through CHEERS implementation stage.

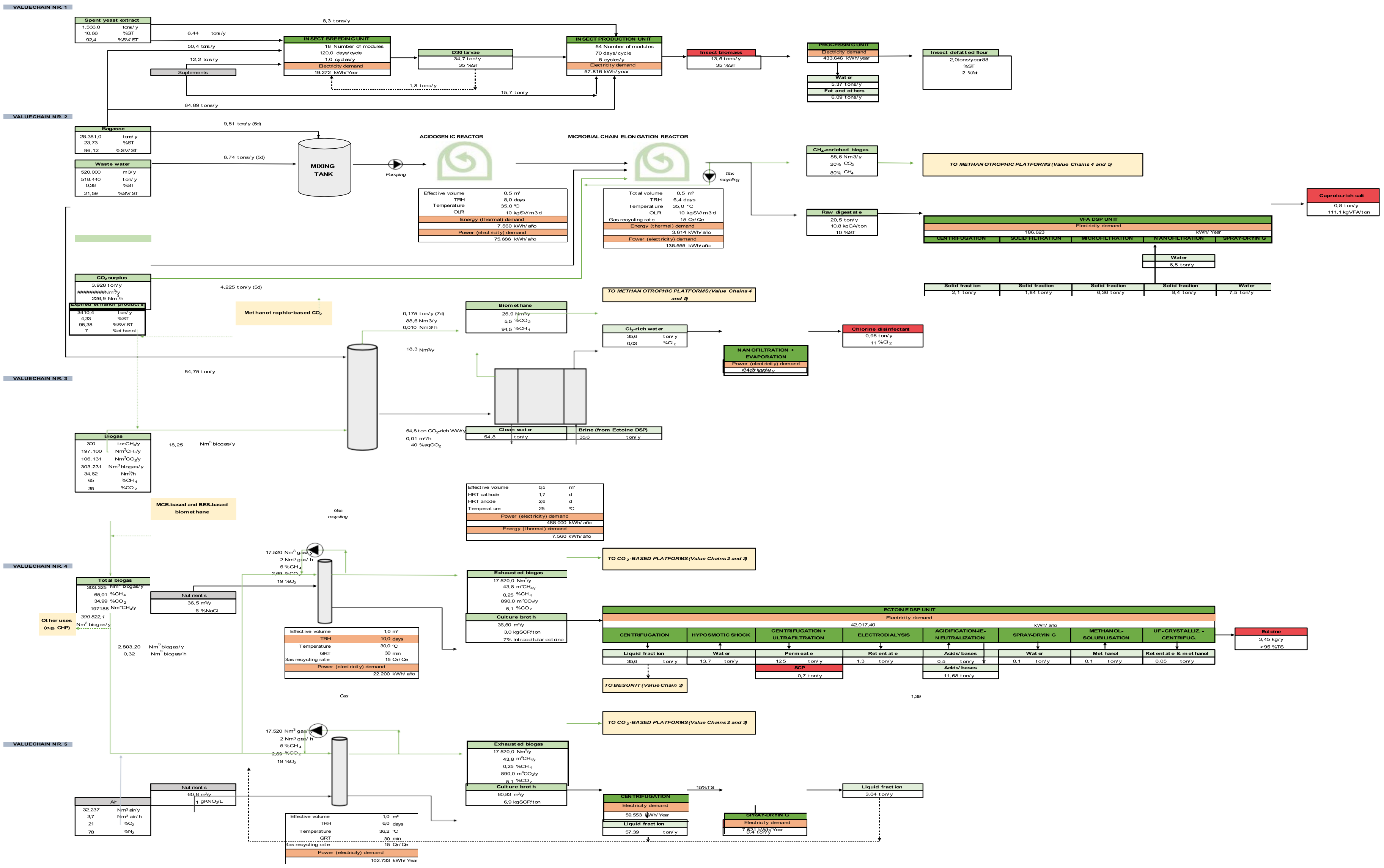

Figure 9. Overall mass balance overview of CHEERS concept at demo scale.

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CHEERS

Producing novel non-plant biomass feedstocks and bio-based products through upcycling and the cascading use of brewery side-streams

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