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FUBAF

From Urban Biowaste to Animal Feed

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Denne slutrapport er godkendt af MUDP, men det er alene rapportens forfatter/projektlederen, som er ansvarlige for indholdet.

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1. Sammenfatning (Dansk)

Med en hastigt stigende verdensbefolkning, klimaforandringer og knaphed af ressourcer opstår der stadig flere udfordringer med at følge med den globale fødevaresikkerhed og miljømæssig bæredygtighed. En høj efterspørgsel på mad fører til en forøget dyrkning af jorden, kunstvanding og brug af gødning (næringsstoffer), hvilket påvirker samfundet og økosystemerne. Især stiger efterspørgslen af proteinholdige fødevarer af høj kvalitet, såsom fisk og kødprodukter. Der er endnu ikke etableret et bæredygtigt og klimavenligt alternativ, der er i stand til at erstatte disse klimatunge produktioner. Hvis vi skal imødekomme denne stigende efterspørgsel, er vi nødt til at gøre det langt mere klimavenligt og bæredygtigt. Derfor er det helt oplagt at udforske nye alternative proteinkilder til fødevareproduktion.

FUBAF (Fra Urbant Bioaffald til Animalsk Foder) projektet præsenterer en ny innovativ tilgang til, hvorledes det fremtidige behov for højkvalitets madprodukter kan opfyldes ved at gentænke udnyttelsen af madaffald i produktionen af bakterielle proteiner (Single Cell Proteins, SCP). SCP kan direkte anvendes til dyrefoder og kan dermed erstatte de mere klimatunge alternativer, såsom fiskemel og sojabønner, som anvendes i produktionen af dyrefoder i dag. Forædlingen af urbant bioaffald, som dermed går fra affald til en ressource, som på lang sigt kan anvendes direkte til produktion af proteinholdige fødevarer af høj kvalitet, er således helt i tråd med tankerne om cirkulær økonomi. FUBAF-konceptet er et 2. generations SCP-koncept, hvor affald anvendes til SCP-produktionen, hvorimod 1. generationskoncepter anvender råmaterialer, såsom naturgas og syntetisk kvælstof. Bæredygtigheden af 2. generations produktion har et højt potentiale i sammenligning med 1. generations produktion.

Urbant bioaffald, i dette tilfælde kildesorteret organisk dagrenovation (KOD), blev udrådnet anaerobt i en pilotskala reaktor, hvorved der blev produceret biogas. Biogasreaktoren blev drevet med tilførsel af KOD i en periode på mere end 200 dage ved mesofile temperaturer og med gradvist øget TS-belastning op til 12 % TS, hvilket svarer til en organisk belastning på op til 5 g VS/L/dag. En stabil og ret robust proces blev opretholdt, og der blev opnået et metanudbytte på 0,99 L CH_4/L/d, svarende til 370 Nm^3 CH_4/t VS_{ind}.

Biogassen, som indeholdt ca. 60-65 % metan, blev derefter biologisk opgraderet til naturgaskvalitet til et metanindhold på ca. 90-95 %. Omdannelsen blev foretaget ved hjælp af mikroorganismer (blandet archaea kultur) i en reaktor, hvor brint blev tilsat. Brinten reagerer med kuldioxiden i biogassen, og metan bliver dannet. Der blev foretaget en række laboratorietests med flere forskellige reaktorkonfigurationer og typer af pakkemateriale, hvilket skulle sikre det bedst mulige design af opgraderingsreaktoren. I et pilotskala setup med en termofil rislefilter med polyurethan (PU) skum som pakkemateriale, blev der opnået rigtig gode resultater. Metanindholdet var >90 % og der blev observeret en meget hurtig genopretning af processen efter en standby periode.

Et nyt mikrobielt bioelektrokemisk system i laboratoriskala blev udviklet med det ene formål at udvinde næringsstoffer fra rejektvand fra afvanding af slam samt bioforgasset bioaffald (digestat). Reaktoren blev drevet som en mikrobiel brændselscelle (Microbial Fuel Cell, MFC). Udvindingseffektiviteten af ammoniak fra digestat var 40-42 % i en 1-trins MFC. I et 2-trins system som fik tilledt rejektvand og digestat, blev effektiviteten henholdsvis ~61% og 54 % svarende til ca. 70 % og 25 % højere effektivitet end i et 1-trins system.

Det opgraderede biogas og det udvundne kvælstof blev anvendt som hhv. kulstof- og kvælstofkilde til SCP-produktion i laboratorieskala. Den højeste SCP-produktion blev opnået i eksperimenter med disse alternative kilder til, selv i sammenligning med SCP-produktion fra naturgas og syntetisk kvælstof. Dette indikerer, at opgraderet biogas og bioelektrokemisk udvinding af kvælstof har et stort potentiale for at producere SCP og for at erstatte de traditionelle kilder. En høj proteinholdig biomasse blev produceret med et total aminosyreindhold på 49-51 % af DCW (dry cell weight) i to separate reaktorer.

Der blev udført en miljøvurdering ved anvendelse a konsekventiel livscyklusvurdering (CLCA). Et scenarie, som indeholdt samudrådning af slam og madaffald, bioelektrokemisk udvinding af kvælstof og biologisk opgraderet biogas til SCP-produktion (Sc2) overgik baseline scenariet samt andre scenarier i alle modellerede ”damage categories” (menneskelig sundhed, kvalitet af økosystem og ressourceknaphed). I både kvalitet af økosystem og ressourceknaphed, var besparelserne i Sc2 større end påvirkningerne. Resultaterne indikerede at erstatning af kemikalier som anvendes til SCP fermentering, er meget vigtig ift. at forbedre den miljømæssige ydeevne af integreret renseanlæg og SCP-produktion.

Resultatet af en livscyklus kost vurdering (LCCA) viste, at ingen af scenarierne er økonomisk rentable under projektets antagelser og omstændigheder. Der er dog en lang række potentielle forbedringer til systemet, såsom reduktion af den modificerede-fortyndede mineralsalt (dAMS) opløsning, højere udnyttelse af 1 g NH_4-N, forbedring af fermentor reaktor designet med det formål at reducere den hydrauliske opholdstid (HRT) samt forøget gas til væske overførsel. Det er værd at bemærke, da der stadig er mange udfordringer med fuldskala dyrkning, er 1. generation af bakterielle SCP sandsynligvis ikke økonomisk rentabel på den korte bane. I øjeblikket er anvendelse af SCP i animalsk foder kun rentable i nogle meget specifikke anvendelser, såsom akvakultur. Men, hvis de miljømæssige omkostninger ved integreret renseanlæg og SCP-produktion blev taget i betragtning, så ville SCP-produktion fra urbant bioaffald være en rimelig mulighed.

Der er en række lovgivningsmæssige udfordringer, som skal adresseres ved produktion af SCP til animalsk foder via FUBAF-konceptet. Den største udfordring er anvendelsen af kvælstof, som stammer fra udrådning af bioaffald. Derudover anses kravet til bakteriesammensætningen at kunne udgøre udfordringer, da bakteriesammensætningen ikke vil blive kontrolleret på samme måde som i produktionen af proteiner baseret på bakteriesammensætningen af rene kulturer.

FUBAF-projektet viste, at SCP kan succesfuldt produceres fra urbant bioaffald. Der er stadig en række tekniske, økonomiske og miljømæssige udfordringer, som skal optimeres, men konceptet viser gode potentialer til at konkurrere med 1. generationsproduktion af SCP fra naturgas og syntetisk kvælstof.

Næste skridt for FUBAF-konceptet er optimering af processerne, altså biologisk opgradering, bioelektrokemisk udvinding af kvælstof og SCP-produktion. Næste skridt ift. LCA-arbejde kunne være en sammenligning af de opnåede resultater i denne rapport med et 1. generations-koncept, såsom Unibios koncept. Fuldskala implementering af Unibios koncept er ret nyt og detaljerede data til LCA-modellering er stadig ikke tilgængelige. FUBAF-konceptet er endnu ikke økonomisk rentabelt, men dette kunne ændre sig i en nær fremtid grundet politiske ændringer. Som et eksempel kan nævnes, at Klimarådet har foreslået en general CO_2-skat, som gradvist skal øges op til 1.500 DKK/t CO_2 frem mod 2030. Dette kan fuldstændig ændre udfaldet af den økonomiske vurdering af FUBAF-konceptet. FUBAF-konceptet er også afhængig af ændringer i de relaterede sektorer, f.eks. vindmølleindustrien og udviklingen af el-, gas- og brintnettet.

2. Executive summary

With the worlds´ increasing population growth and issues related to climate change and resource scarcity, challenges arise to keep up with the boundaries of global food security and environmental sustainability. The higher demand for food is leading to an expansion of land cultivation, irrigation and use of fertilizer (nutrients) impacting the related societies and ecosystems. In particular, the manufacturing of high-quality proteinaceous food in the form of fish and meat products from both agriculture and livestock production is increasing in global demand. A sustainable and climate-friendly alternative has not yet been established that is able to replace these traditional and climate-heavy products at present time. If we are going to meet the demand for these products in the future, the basis behind production must be approached differently and to a greater extent be of a considerably more climate-friendly and sustainable nature. A need for an exploration of alternative protein sources for humans and animals is therefore prominent.

The FUBAF (From Urban Bio-waste to Animal Feed) project presents a novel approach to how the future demand of high-quality products from food production can be met by rethinking the use of urban biowaste in the production of Single Cell Proteins (SCP). SCP can be directly involved in the production of animal feed and replace the more climate-heavy alternatives such as fishmeal and soybeans used in the production of animal feed today. Thus, the valorization of urban bio-waste going from waste to a resource, which in the long run can be utilized in the production of high-quality proteinaceous food, is completely in step with the thoughts of circular economy. The FUBAF project is a 2nd generation SCP concept, where waste products are used for the SCP production, which contrasts with 1st generation concepts where raw materials, such as natural gas and synthetic nitrogen are applied. The sustainability of 2nd generation production is of course of high potential compared to 1st generation production.

Urban biowaste, in this case source-separated organic household waste, was anaerobically digested in a pilot scale reactor, thereby producing biogas. The anaerobic digestion reactor was operated with urban biowaste for more than 200 days at mesophilic conditions with gradually increasing TS content up to 12 % TS, corresponding to an Organic Loading Rate (OLR) of up to around 5 g VS/L/day. A stable and quite robust process was ensured, achieving methane yields of 0,99 L CH_4/L/d, corresponding to 370 Nm^3 CH_4/t VS_{in}.

The biogas, which consisted of appr. 60-65 % methane, was then biologically upgraded to natural gas quality with a methane content of appr. 90-95 %. The conversion was done by microorganisms (mixed archaeal culture), in a reactor where hydrogen is added. The hydrogen reacts with the carbon dioxide in the biogas and produces methane. Laboratory tests were carried out with a range of reactor configurations and types of packing materials to ensure the best possible design of the upgrading reactor. In a pilot scale setup with a thermophilic trickle bed reactor with PU foam as packing material, very good results were achieved. The methane content was >90 % and very fast recovery was observed after a standby period.

A novel microbial bioelectrochemical system in laboratory scale was developed for extracting nutrients from reject water and anaerobically digested biowaste. The reactor was operated as microbial fuel cell (MFC). The recovery efficiency of ammonia from digestate was in the range of 40-42% when using 1-stage MFC. In two-stage MFC fed with reject water and digestate, the total ammonia efficiencies in cathodic chamber were ~61% and 54%, respectively, corresponding to about 70% and 25% higher than that in one-stage operation.

The produced upgraded biogas and the extracted nutrients was utilized as carbon and nitrogen sources, respectively, for SCP production in laboratory scale. The highest SCP production was found in experiments with these alternative sources, even when compared with SCP produced from natural gas and synthetic nitrogen. This indicates that upgraded biogas and electrochemically extracted nitrogen could be very potential sources for producing SCP and even to replace the traditional sources. A highly proteinaceous biomass was produced having a total amino acids content of 49–51% of dry cell weight (DCW) in two separately operated reactors.

An environmental assessment was conducted, using consequential Life Cycle Assessment (CLCA). A scenario that included co-digestion of sludge with biopulp, bioelectrochemical nitrogen extraction and biologically upgraded biogas for the SCP production (Sc2) outperformed the baseline scenario and other scenarios in all modelled damage categories (i.e., human health, ecosystem quality, and resource scarcity). In both ecosystem quality and resource scarcity, the savings in Sc2 were larger than the impacts. The results of this study demonstrated that the substitution of chemicals used in SCP fermentation is the key in enhancing the environmental performance of integrated WWTP and SCP production.

The results of a Life Cycle Cost Assessment (LCCA) demonstrated that none of the scenarios are economically feasible under the assumptions and circumstances that were presented in this study. However, there are quite several potential improvements to the system, such as decreasing the modified-diluted mineral salt (dAMS) recipe, higher valorization of 1 g of NH4-N, and improvement of fermentor reactor design to lower the hydraulic retention time (HRT) and increased gas to liquid transfer. Since there are still many problems with large-scale cultivation, first generation of bacterial SCP are unlikely to be economically viable in the short term. Currently, the use of SCP in animal feed and only in some specific applications such as aquaculture is economically justified. However, if the environmental costs of the integration of WWTP with SCP production under the developed scenarios were considered, the SCP production from urban waste could be a reasonable option.

There are some legislative challenges that must be addressed before it can be considered possible to produce proteins for animal feed via the FUBAF concept. The primary challenge in producing proteins for animal feed via the FUBAF concept is the use of a nitrogen source which originates from the digestion of urban biowaste. In addition, it is considered that the requirement for the bacterial composition may present challenges, as the bacterial composition will not be controlled in the same way as in the production of proteins based on pure culture bacterial compositions.

The FUBAF project showed that SCP can be successfully produced from urban biowaste. There are still some technological, economic, and environmental issues that needs to be optimized, but the concept shows good potentials to compete with 1st generation SCP production from natural gas and synthetic nutrients.

When looking ahead, the next steps are improving the FUBAF concept, in relation to biological upgrading, electrochemical N extraction and the SCP production. A next step for LCA work could be to compare the FUBAF concept with 1st generation SCP production, such as the concept by Unibio. However, full-scale operation is still quite recent, so detailed data for LCA modeling is not yet available. The FUBAF concept is not yet economical feasible, but this could change in a near future, due to political changes. As an example, the Danish Council on Climate Change (Klimarådet) has proposed a general CO_2 tax, that will gradually increase up to 1,500 DKK/t CO_2 (corresponding to ~200 Euro/t CO_2) by 2030. This could completely change the LCCA outcome for the FUBAF concept. The FUBAF concept might also rely on changes in related systems, e.g. the wind power industry and the development of the electricity, gas and hydrogen grids.

3. Background

With the worlds´ increasing population growth and issues related to climate change and resource scarcity, challenges arise to keep up with the boundaries of global food security and environmental sustainability. The higher demand for food is leading to an expansion of land cultivation, irrigation and use of fertilizer (nutrients) impacting the related societies and ecosystems. In particular, the manufacturing of high-quality proteinaceous food in the form of fish and meat products from both agriculture and livestock production is increasing in global demand. A sustainable and climate-friendly alternative has not yet been established that is able to replace these traditional and climate-heavy products at present time. If we are going to meet the demand for these products in the future, the basis behind production must be approached differently and to a greater extent be of a considerably more climate-friendly and sustainable nature. A need for an exploration of alternative protein sources for humans and animals is therefore prominent.

With the increasing population growth, we also concurrently encounter accumulation of an increasing amount of urban bio-waste, including organic industrial residual products, food waste and wastewater sludge. Urban bio-waste can also be referred to as low-value waste stream, which is not sufficiently considered to be a resource today. However, the bio-waste can most noticeably be utilized as a resource in a novel method to accommodate protein production.

The FUBAF (From Urban Bio-waste to Animal Feed) project presents a novel approach to how the future demand of high-quality products from food production can be met by rethinking the use of urban biowaste in the production of Single Cell Proteins (SCP). SCP can be directly involved in the production of animal feed and replace the more climate-heavy alternatives such as fishmeal and soybeans used in the production of animal feed today. Thus, the valorization of urban bio-waste going from waste to a resource, which in the long run can be utilized in the production of high-quality proteinaceous food, is completely in step with the thoughts of circular economy. The FUBAF project is a 2nd generation SCP concept, where waste products are used for the SCP production, which contrasts with 1st generation concepts where raw materials, such as natural gas and synthetic nitrogen are applied. The sustainability of 2nd generation production is of course of high potential compared to 1st generation production.

The FUBAF project is a spin-off of the previous/adjacent MUDP Lighthouse project, VARGA, which purpose has been to identify how a conventional wastewater treatment plant (WWTP Avedøre) can be converted into a water and resource recovery facility (WRRF) and thereby setting up the foundation for a shift of the present paradigm for wastewater treatment. This has been carried out by investigating the potentiality of a range of novel and current opportunities in the period from 2016 to 2022 (To be reported later, see www.projekt-varga.dk). The promising prospect identified in VARGA of producing animal feed from urban bio-waste derived a deeper investigation in the FUBAF project.

The purpose of the FUBAF project is specifically to develop and evaluate following topics:

1. Manufacturing of Single Cell Proteins (SCP) based on upgraded biogas produced from urban bio-waste.
2. Recovery of nutrients after anaerobic digestion of urban bio-waste for utilization in the manufacturing of proteins.
3. The sustainability of the overall production process from anaerobic digestion, upgrading of biogas, recovery of nutrients and the manufacturing of high value animal protein-feed from urban bio-waste based on circular economy principles.

4. From Urban Biowaste to Animal Feed (FUBAF)

The FUBAF (Urban Biowaste to Animal Feed) project will explore the opportunity to produce Single Cell Protein (SCP) from upgraded biogas and recovered nutrients originating from anaerobically digested organic fraction of source-separated municipal solid waste (hereafter simply called urban biowaste), in a quality that can substitute conventional protein sources for animal feed.

The FUBAF concept including affected sectors is presented in Figure 1.The overall idea is to make the best use of the waste streams from the city and convert it into something useful, in this case feed production, that can be reintroduced to the city as food. Apart from the waste generators (city, WWTPs, industry) other sectors are involved in the FUBAF concept. The SCP must be refined and used in the agricultural sector as feed. The wind power industry is involved since hydrogen is needed for the upgrading of the biogas, and this hydrogen is supplied by electrolysis of water using excess electricity from windmills.

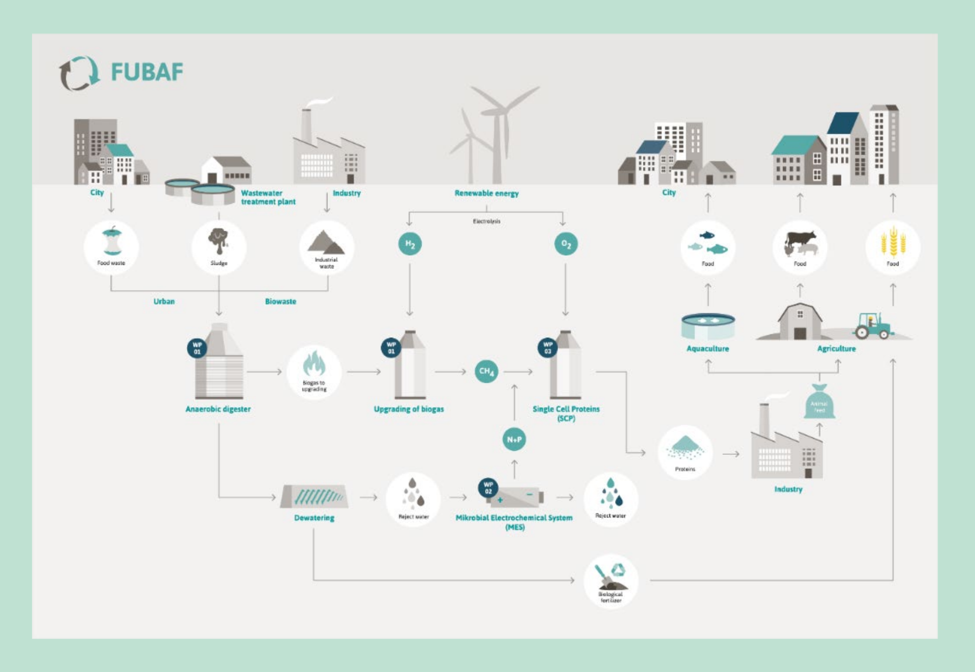

FIGURE 1. Overview of the FUBAF concept including affected sectors (project website: www.projekt-varga.dk/en/fubaf-fronteng/)

The proposed project will be realized through the expertise and knowledge of the participating partners within complementary fields; BIOFOS, VandCenter Syd and Aarhus Vand (Utility, wastewater treatment), DTU (Fermentation and microbial electrochemistry), ARC (knowledge on household waste), LiqTech (membrane technology), Unibio (production of SCP, full scale) and EnviDan (engineering, upscaling, resource recovery & environmental challenges). All partners will contribute to the assessment of circular economy aspects. FUBAF is a spin-off project of the Lighthouse project VARGA and is financed by the Danish Eco-Innovation program (MUDP). The project executed from 2019-2021.

The FUBAF project is divided into 5 Work Packages (WPs), which is described briefly below:
WP1: Anaerobic digestion and biological upgrading:
In WP1 a pilot scale operation with anerobic digestion (AD) of urban bio-waste is carried out at Avedøre WWTP. Subsequently, biological upgrading of biogas is performed in pilot scale tests in a separate process exploiting the AD digestate as a nutrient source.

WP2: Electrochemical nitrogen extraction:
In WP2 nutrients are recovered from AD digestate using a novel microbial electrochemical system in laboratory scale at DTU. Digestate from the pilot plant at Avedøre WWTP as well as reject water from the utility companies will be used as test material.

WP3: Production of bacterial protein:
In WP3 methane and nutrients are used as raw materials to produce a 2nd generation of SCP via a novel fermentation process in laboratory scale at DTU.

WP4: Circular economy:
In WP4 the proposed concept will be evaluated in the frame of circular economy using LCA and economical assessments for defining the level of sustainability and feasibility of producing the high value product, SCP based on urban waste.

WP5: Project administration:
WP5 covers administration of economy, progress according to milestones as well as coordination between work packages. Dissemination as a central and highly prioritized activity is also coordinated within WP5. The already established communication platform from the VARGA project has been used, see www.projekt-varga.dk/en/fubaf-fronteng/. The FUBAF project has contributes to six scientific papers, prepared by DTU and the Chinese Academy of Agricultural Science. These are:

1) Khoshnevisan, B., Dodds, M., Tsapekos, P., Torresi, E., Smets, B.F., Angelidaki, I., Zhang, Y., Valverde-Pérez, B., 2020. Coupling electrochemical ammonia extraction and cultivation of methane oxidizing bacteria for production of microbial protein. Journal of Environmental management. 265, 1-9, 110560.
2) Khoshnevisan, B., Tsapekos, P., Zhang, Y., Valverde-Pérez, B., Angelidaki, I., 2019. Urban biowaste valorization by coupling anaerobic digestion and single cell protein production. Bioresource Technology. 290, 1-9, 121743.
3) Tsapekos, P., Khoshnevisan, B., Zhu, X., Zha, X., Angelidaki, I., 2019. Methane oxidising bacteria to upcycle effluent streams from anaerobic digestion of municipal biowaste. Journal of Environmental management. 251, 1-7, 109590.
4) Tsapekos, P., Zhu, X., Pallis, E., Angelidaki, I., 2020. Proteinaceous methanotrophs for feed additive using biowaste as carbon and nutrients source. Bioresource Technology. 313, 1-7, 123646.
5) Zha, X., Tsapekos, P., Zhu, X., Khoshnevisan, B., Lu, X., Angelidaki, I., 2021. Bioconversion of wastewater to single cell protein by methanotrophic bacteria. Bioresource Technology. 320, 1-7, 124351.
6) Yang Z, Tsapekos P, Zhang Y, Zhang Y, Angelidaki I, Wang W. 2021. Bio-electrochemically extracted nitrogen from residual resources for microbial protein production. Bioresource Technology. 337, 125353.

Apart from the scientific papers, several presentations have been made at national and international conferences (e.g. DAKOFA, Dansk Vand, NORDIWA, REGAS, IWA conferences).

The work packages will be described in the following chapters. WP1 to WP3 deals with the experimental part of the FUBAF project and the description of these WPs are quite technical. A more holistic overview of the project is given in WP4 on the topic of circular economy. WP5 comprises project administration and is not relevant to describe further in this report.

5. WP1: Anaerobic digestion and biological upgrading

The aim of the work package is to anaerobically digest urban biowaste and to biologically upgrade the produced biogas to natural gas quality (high methane content >95 %). Anaerobic digestion includes four key phases, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. The general overall reaction equation can be exemplified by glucose digestion by anaerobic microorganisms to carbon dioxide and methane, se equation 1.

$C_6H_{12}O_6$ à 3 CO_2 + 3 CH_4 *(Equation 1)*

Anaerobic microbes

Overall, results generated during WP1 have been published in scientific paper and parts have already been presented at various conferences.

5.1 Anaerobic digestion of urban biowaste

Creating synergies with the Lighthouse project VARGA, WP1 of FUBAF efficiently demonstrated anaerobic digestion of urban biowaste in pilot scale operation at Avedøre WWTP (operated by BIOFOS). The anaerobic digestion reactor was operated with urban biowaste for more than 200 days at mesophilic conditions with gradually increasing TS content up to 12 % TS, corresponding to an Organic Loading Rate (OLR) of up to around 5 g VS/L/day. Some of the results obtained during the pilot scale test are presented in Figure 2.

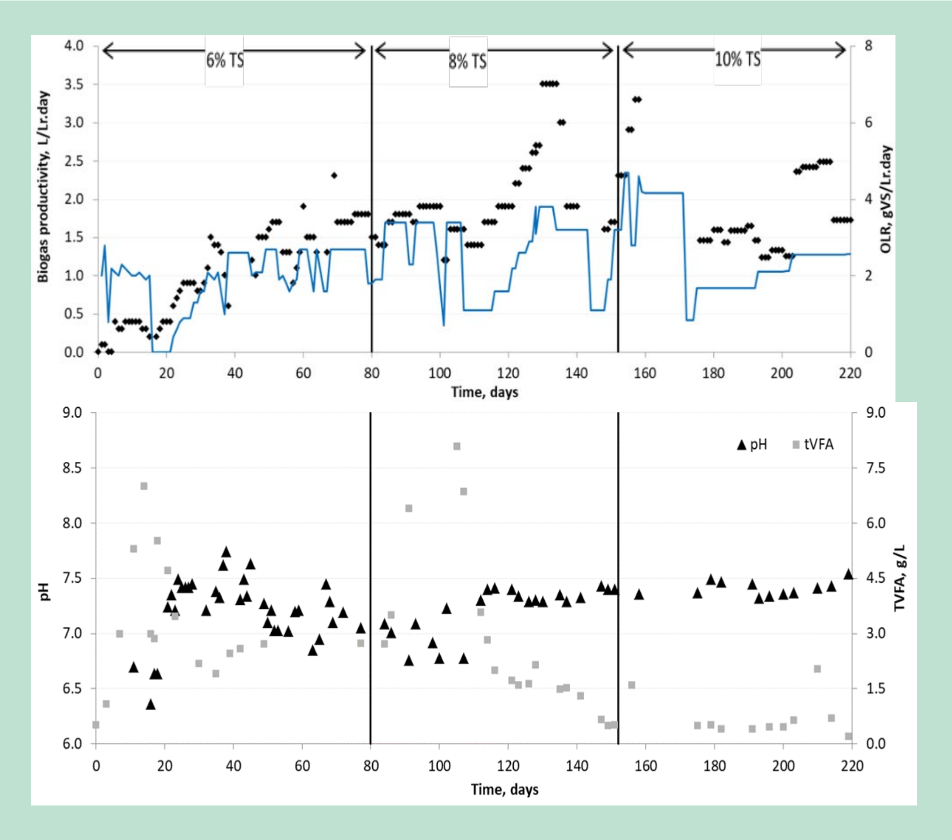

FIGURE 2. Results from ~200 days of operation of pilot scale anaerobic digestion of urban biowaste at the pilot site at Avedøre WWTP, Denmark.

The anaerobic digestion process was stable during the 200 days of operation. The biogas productivity was quite high at an average 0,99 L CH_4/L/d, corresponding to 370 Nm^3 CH_4/t VS_{in} which is in accordance with the expected level of ~340 Nm^3 CH_4/t VS_{in} (Miljøstyrelsen, 2017). The produced biogas and the effluent (digestate) were collected and exploited as carbon and nutrient source for the biological upgrading tests.

5.2 Biological upgrading of biogas

The produced biogas consisting of mainly carbon dioxide and methane is upgraded to natural gas quality by microorganisms (archaea) by adding hydrogen to the biogas, see equation 2. The CO_2 in the biogas is thereby converted to methane.

$$CO_2 + 4\,H_2 \underset{\textit{(Archaea)}}{\rightarrow} CH_4 + 2\,H_2O \qquad \textit{(Equation 2)}$$

The process of biological upgrading of biogas is being developed by many stakeholders around the world. One example is Elctrochaea GmbH, a company located in Germany and with subsidiaries located in Denmark and California. Electrochaea applies a biocatalytic process for the upgrading of biogas.

5.2.1 Laboratory scale experiments

In DTU Environment, up-flow reactors equipped with Silicon Carbide (SiC) membranes (from LiqTech, Denmark) and trickle bed reactors (TBR) equipped with porous packing material (polyurethane (PU), activated carbon foam, Rasghig rings) were exploited as potential reactor systems for biological upgrading. The experimental setup is presented in Figure 3 and the achieved methane content in the biologically upgraded biogas at different Gas Retention Times (GRTs) is presented in Figure 4.

Lab-scale results showed that the TBR had very good performance (especially with PU foam as packing material) reaching a methane contain of 90% using a retention time of 4 hours and periodically the same methane content at a reduced retention time. Based on the positive results a pilot plant with a TBR configuration was designed.

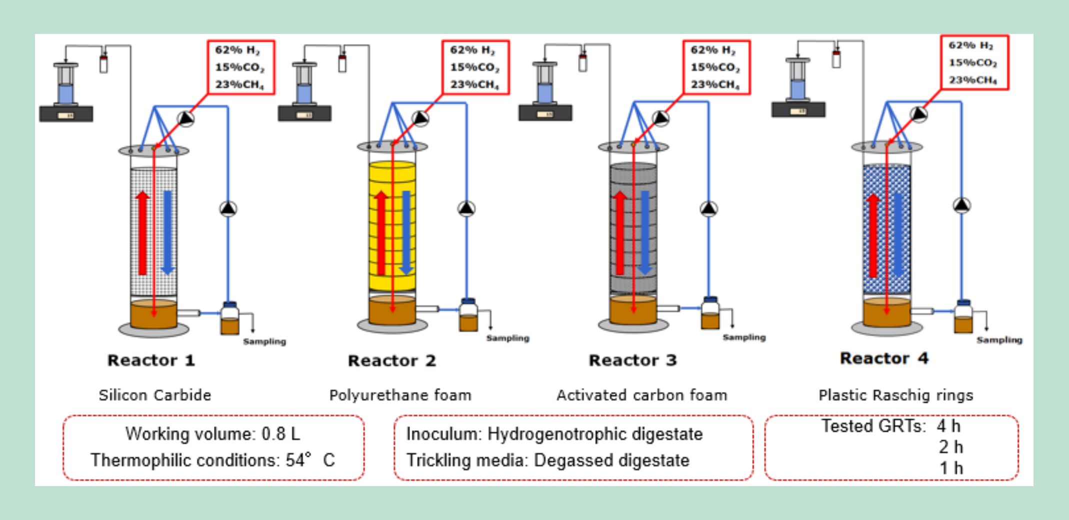

FIGURE 3. Experimental setup for the laboratory scale biological upgrading (GRT: Gas Retention Time)

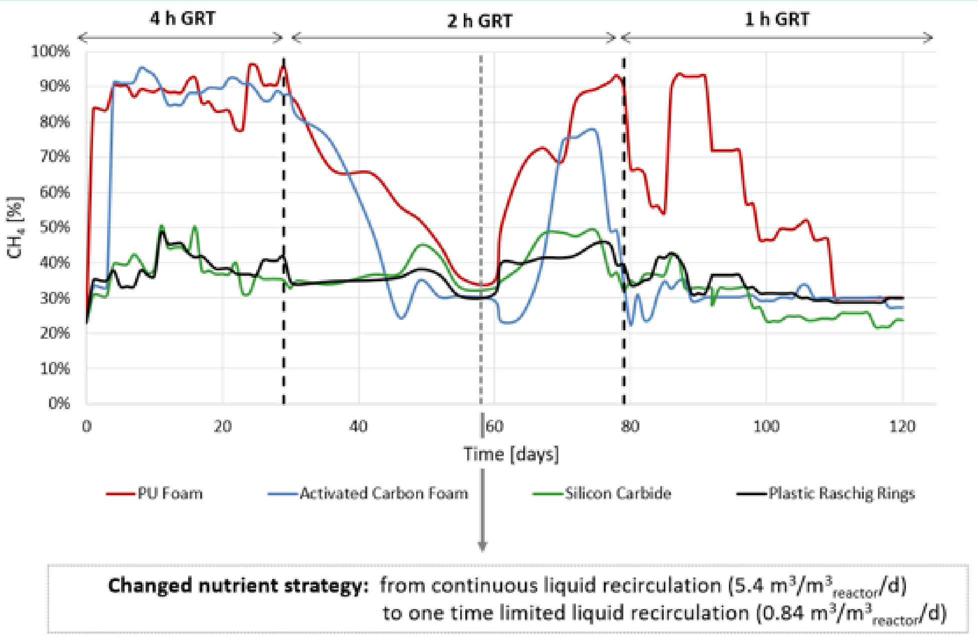

FIGURE 4. Achieved methane content in the biologically upgraded biogas for the four different reactors.

5.2.2 Pilot scale experiments

The pilot plant main part was the TBR having a total volume of 68 liters. The TBR was equipped with a heating cable to ensure stable operating temperature of ~55°C (thermophilic conditions). A technical drawing is presented in Figure 5.

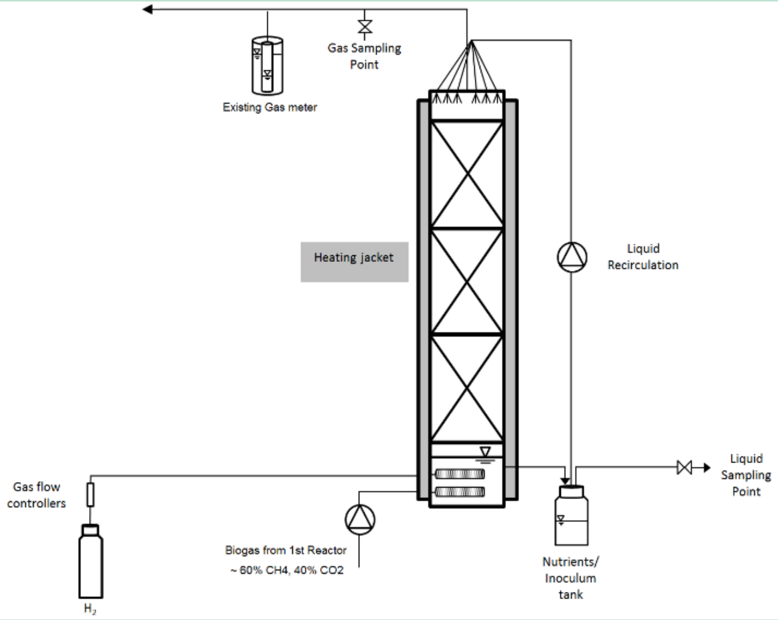

FIGURE 5. Technical drawing of the pilot TBR for biological upgrading of biogas.

The pilot TBR was filled with PU foam as packing material and equipped with SiC membrane to improve the hydrogen gas diffusion. The pilot plant was integrated in the container containing the anaerobic digestion pilot reactor (Figure 6). The set-up poses the ability to couple biogas derived CO_2 with exogenous H_2. Knowing the volumetric biogas production from the VARGA project, the daily CO_2 production was also known and thus, the needed amounts of CO_2 and H_2 to be injected to the TBR were calculated. Pressurized H_2 cylinders provided the exogenous gas using an automated flow controller.

Thermophilic hydrogenotrophic inoculum (Archaea) was collected from the DTU laboratory, while the effluent of the anaerobic digestion pilot reactor was used as source of nutrients for the upgrading microbiome.

FIGURE 6. Pilot-scale operation at BIOFOS WWTP in Avedøre. Left: biological upgrading TBR. Right: Anaerobic digestion reactor.

High biomethanation efficiency was achieved since the first week of operation reaching more than 90% methane in the output (Figure 7). After feed regime adjustment at the stoichiometrically optimum at day 8, a slight drop of upgraded methane was detected from day 10 to18. However, prior to the "standby" period no residual H_2 was detected in the output and the CH_4 content was equal to 90%.

In the period after the “standby” period, the highest CH_4 concentration (98%) was detected at the gas output since the beginning of the experiment. A deviation to the H_2 flow was faced from day 72 to 75 reducing the upgrading efficiency. Once the H_2 flow was adjusted to the correct value, the biomethane content reached again more than 95%. Doubling the feeding load to reach a gas retention time of 5 h at the following period, the biomethanation performance was negatively affected. Specifically, the CH_4 was decreased to 58% after 4 days of operation and stabilized to 76% at day 110. To overcome limited biomethanation, perforated stainless steel diffusers were replaced with SiC membranes at day 110 as means to improve the gas-liquid contact. Indeed, the positive impact was quickly revealed and biomethane content reached a value of 98% at the end of the experiment.

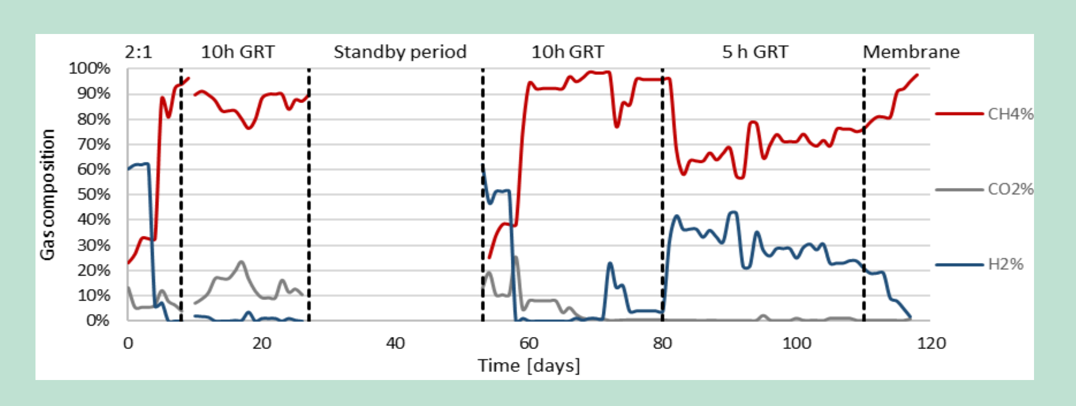

FIGURE 7. Monitoring of biological upgrading of biogas at pilot scale (TBR) at Avedøre WWTP.

The pilot scale TBR experiments showed Proof of concept for biological upgrading of biogas to natural gas quality (in terms of methane content). The focus from this point should be optimization of the process and increase of the amount of gas treated (decrease of GRT).

5.2.3 Effect of biogas composition

On top of biogas originated from digested biowaste, the composition of different biogas sources was also evaluated. Specifically, biogas content at Marselisborg (Figure 8a) and Egå WWTPs (both operated by Aarhus Vand) (Figure 8b) showed that the CH_4 content was ~60% at both WWTPs for a 100 days monitoring period. Furthermore, biogas produced in Avedøre WWTP (operated by BIOFOS) consisted of ~62% CH_4, ~38% CO_2 and 90 ppm H_2S. Similarly, biogas produced in Ejby Mølle WWTP (operated by Vandcenter Syd) consisted of ~65% CH_4, ~35% CO_2 and 630 ppm H_2S. Hence, the biogas composition at four full-scale WWTPs and pilot biogas reactor had adequate CH_4 content to be valorized for biological upgrading and subsequently, microbial protein production.

The H_2S content can inhibit the subsequent SCP production, so it is important that the upgraded biogas is cleaned before use in SCP production. This would be in the same way as cleaning of biogas, e.g. scrubbers or filters.

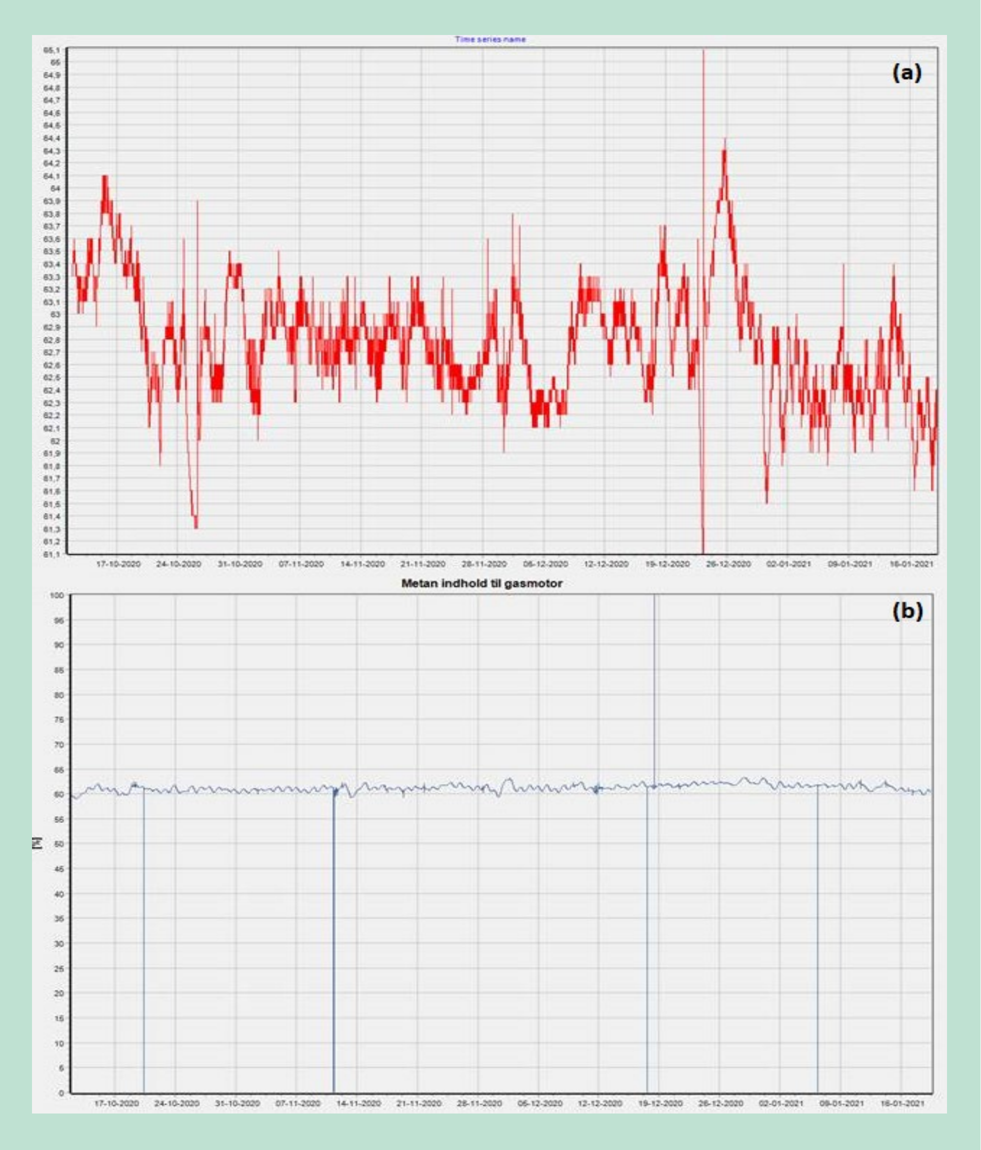

FIGURE 8. Historic CH_4% at Marselisborg (a) and Egå WWTP (b)

5.2.4 Sub-conclusion

WP 1 in the FUBAF project showed stable and successful anaerobic digestion of urban bio-waste in pilot scale with biogas productivity at an average of 0,99 L CH_4/L/d, corresponding to 370 Nm^3 CH_4/t VS_{in}. Biological upgrading was tested in laboratory scale and the results were used to design a pilot TBR with PU foam as packing material. The pilot TBR had good performance with produced methane contents of >90 %. This biogas is used for the SCP production in WP3.

6. WP2: Electrochemical nitrogen extraction

The overall aim of this work package was to investigate new ways of extracting nitrogen from nitrogen sources that are available on site, such as nitrogen in digestate from the anaerobically digestated biowaste or reject water from dewatering of sludge. The N sources cannot be utilized directly, since the methane oxidizing bacteria (MOB) for the SCP production needs a certain quality, in terms of concentration and purity. Utilizing nitrogen sources that are available on site, has a potential of being much more sustainable than using virgin resources such as natural gas and synthetic nitrogen.

Recently, bio-electrochemical systems, mainly including microbial fuel cell (MFC) and microbial electrochemical cell (MEC), have been developed for ammonia recovery. These methods have advantages of relatively low energy consumption (even energy production) and no need of additional chemicals. MFC can operate without external energy input by using microorganisms as biocatalyst for oxidation of organic matter to gain electrons. MFC, can convert chemical energy contained in wastewaters into electricity and thereby can be considered as a low-cost and green approach to recover ammonia from waste streams.

All experiments were carried out at DTU Environment during 2019-2020. The obtained results generated during WP2 have been published in scientific papers and have already been presented in conferences.

6.1 One-stage nitrogen recovery

A novel microbial electrochemical system was developed for extracting nutrients from reject water and anaerobically digested biowaste. The reactor was operated as a MFC and consisted of two chambers (working volume of 250 mL per chamber), cation exchange membrane (CEM; CMI 7000, Membrane international, NJ) and a resistance (1 kΩ). Stainless steel woven mesh coated with 0.5 mg/cm^2 Pt was used as cathode electrode. The anode electrode was carbon fiber brush (4 cm diameter, 4 cm length), pretreated at 450 ºC for 30 min before usage. After pretreatment, the brush was pre-acclimated in wastewater for 16 days, operating as two-chamber MFC to create biofilm. The experimental setup is presented in Figure 9.

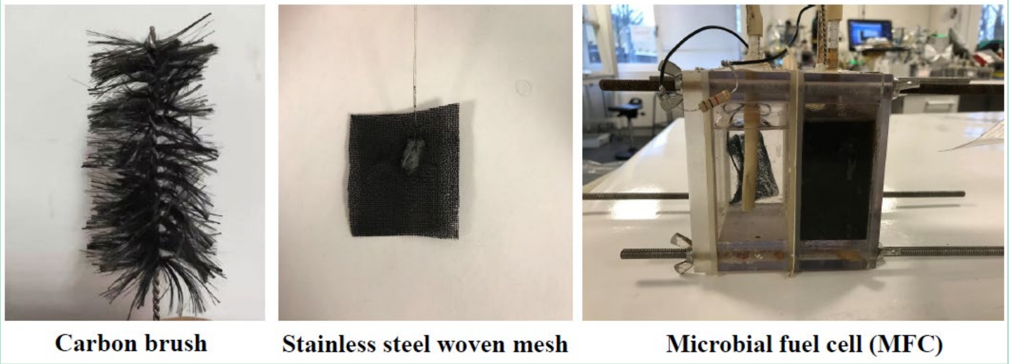

FIGURE 9. Microbial electrochemical system for nitrogen extraction from digested sludge and digestate

After 16 days, the voltage could be kept over 0.6 V for about 5 days, and thus, the system was ready for testing (Figure 10). The tests for ammonia recovery from each waste was carried out in three runs, to detect the stability and reproducibility of system. Liquid in both anodic and cathodic chamber was refilled in each run to keep the same initial conditions. Reject water from Ejby Mølle WWTP (Vandcenter Syd) and digested biopulp from pilot reactor were used for ammonia recovery by MFC. When reject water was used as waste stream, from batch 1 to batch 3, voltage was increased gradually, with a 2-fold increment on electricity energy production (from 14.4 J to 38.5 J). This phenomenon was attributed to the adaptation of microorganisms to the reject water in the anode. After 30 h, no significant changes of ammonia concentration in two chambers were appeared (Figure 11Figure a), with a downward tendency on voltage.

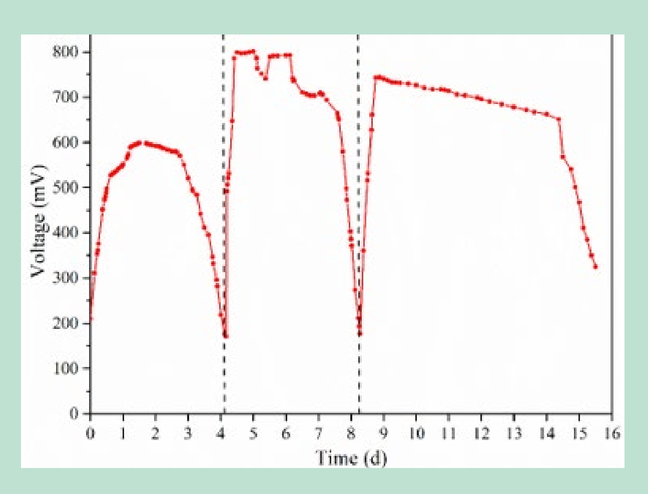

FIGURE 10. Voltage of MFC at pre-acclimated period

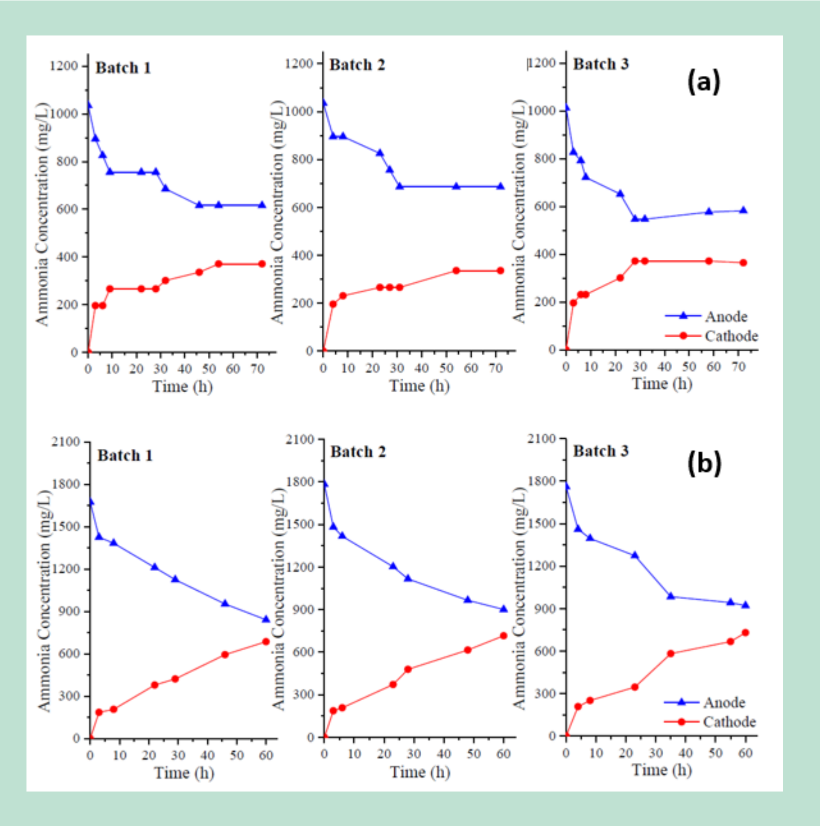

FIGURE 21. Evolution of ammonia concentration using reject water (a) and digested biowaste (b)

NH_4^+ migration and NH_3 diffusion, which was mainly depended on current intensity and environmental factors (such as concentration gradient, pH, temperature), respectively, were considered as two dominant ways of ammonia transportation through cation exchange membrane. Stable recovery efficiency and recovery rate of ammonia could be obtained in MFC system in the three batches. To detect the stability and efficiency of MFC from ammonia-rich waste, digestate (about 1,750 mg NH_4^+-N/L) was tested as substrate (Figure b).

The recovery efficiency of ammonia from digestate was in the range of 40-42% in three batches, which was higher than that in MFC feeding reject water. Recovery rate of ammonia was about 12 mg/L/h, which was about 2.5 times higher than that from reject water (about 5 mg/L/h). The results revealed that higher initial ammonia concentration could boost the ammonia transfer in MFC, resulting in a higher recovery efficiency as well as a higher recovery rate. Based on above discussion, the ammonia recovery efficiency was lower than 42%.

6.2 Two-stage nitrogen recovery

For system optimization, a two-stage recovery experiment was followed in which the waste stream in anodic chamber was remained, and the liquid in cathodic chamber was refilled when the average recovery rate of ammonia was lower than 10 mg/L/h. The cut-off point of two stages in MFC fed with reject water and digestate was at 48 and 40 h, respectively. The recovery rate of ammonia in cathodic chamber was lower than 10 mg/L/h after the cut-off point in both reactors. Ammonia balances were higher than 93%, indicating the feasibility and stability of two-stage ammonia recovery.

In two-stage MFC fed with reject water and digestate, the total ammonia efficiencies in cathodic chamber were ~61% and 54% (Figure 12a and b), respectively, corresponding to about 70% and 25% higher than that in one-stage operation. High ammonia recovery rates (8.50 and 15.93 mg/L/h) were obtained in both reactors.

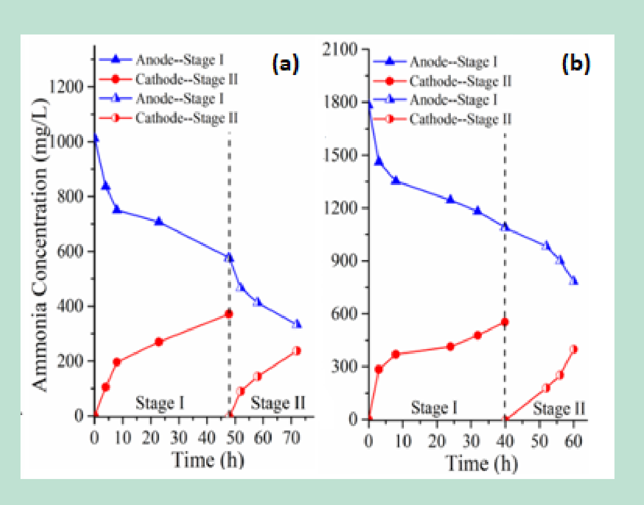

FIGURE 32. Evolution of ammonia concentration using reject water (a) and digested biowaste (b) in two-stage recovery operation

6.3 Sub-conclusion

Reject water from a WWTP and digested biopulp from the anaerobic digestion pilot reactor were used for ammonia recovery by MFC. The recovery efficiency of ammonia from digestate was in the range of 40-42% when using 1-stage MFC. In two-stage MFC fed with reject water and digestate, the total ammonia efficiencies in cathodic chamber were ~61% and 54%, respectively, corresponding to about 70% and 25% higher than that in one-stage operation.

7. WP3: Production of bacterial proteins

The overall aim of this work package is to produce SCPs from the sources that were obtained in WP1 and WP2, the carbon source being biogas or upgraded biogas and the nitrogen sources being electrochemically extracted nitrogen from either reject water or digestate.

A very simple reaction equation of the SCP production, with a general formula for the amino acids in protein, is shown in Equation 3.

$$CH_4 + O_2 + \text{minerals} \xrightarrow[\textit{Methane oxidizing bacteria (MOB)}]{} RCH(NH_2)COOH \text{ (protein)} \qquad \textit{(Equation 3)}$$

By utilizing waste products that are already available on site, the FUBAF concept (as a 2nd generation concept) has a potential of being more sustainable than 1st generation SCP concepts where raw materials, such as natural gas and synthetic nitrogen are utilized. The Technology Readiness Level (TRL) of 2nd generation concepts are, however, low compared to 1st generation concepts. 1st generation concepts have already been developed for full-scale implementations, e.g. by the Danish company Unibio A/S, who is a partner in the FUBAF project. Unibio has, together with their partner and licensee Protelux, built a full-scale SCP plant in Ivangorod, Russia.

All experiments in WP3 were carried out at laboratory scale at DTU Environment during 2019-2020. Similarly, with previous WPs, results obtained during WP3 were published in scientific papers and conferences.

7.1 Selection of methane oxidizing bacteria

Community enriched in the bacteria genera *Methylomonas* and *Methylophilus* spp. was mainly used in the FUBAF project. *Methylomonas* is widely examined for single cell protein (SCP) production, reaching 69% $w_{\text{-protein}}/w_{\text{-dry weight}}$ when fed with natural gas. *Methylomonas* is one of the typical type I methane oxidizing bacteria (MOB) that have higher growth rates and higher methane affinity compared to other MOB types. Moreover, the ability of *Methylomonas* strains to fixate CO_2 is well-known and this characteristic can help the mixed culture to grow on biogas. On the other hand, *Methylophilus* strains can grow on methanol to produce high content protein. *Methylomonas* and *Methylophilus* spp. can be enriched together to grow syntrophically providing bidirectional advantages. For instance, the role of *Methylomonas* can be to oxidize methane to methanol while the role of *Methylophilus* can be to assimilate the accumulated metabolites (i.e. methanol) to avoid the inhibition. The species were found as the dominant ones in articles produced during FUBAF. Hence, the co-cultivation of *Methylomonas* and *Methylophilus* strains on biogas and residual nitrogen can lead to the establishment of a specialized community to produce SCP.

7.2 Effect of different carbon sources

The performance of the mixed culture to produce SCP was initially explored using synthetic cultivation media and gases. At batch bottles of 0.1 L working volume, more than 1.0 $g_{\text{-SCP}}/L$ was produced at a growth rate higher than 0.6^{-d}, showing the potential of the mixed culture to oxidize CH_4 for biomass assimilation (Figure 13a).

The replacement of synthetic gas with raw biogas, led to a slightly lower biomass production (~0.6 g-$_{SCP}$/L). The used biogas consisted of 61% CH_4, 39% CO_2 and 913 ppm H_2S. Besides CH_4, some methanotrophs can tolerate and in some cases assimilate CO_2 for biomass generation. On the contrary, H_2S can have negative impact on MOB growth. To elucidate the effect of H_2S in the used culture, a subsequent batch experiment was prepared at different concentrations (i.e. 0, 1,000, 5,000, 10,000 ppm) keeping the headspace at 40:60 CH_4:O_2. Results revealed a clear inhibition at 1,000 ppm, as the biomass production was 44% lower than the bottles fed with synthetic CH_4 in the absence of H_2S (Figure 13b). Furthermore, the toxic effect of H_2S was significantly more intense at 5,000 and 10,000 ppm in which the growth was hindered by 83 and 93%, respectively. Based on Levenberg-Marquardt algorithm, the IC_{50} was calculated at 1,376 ppm. Consequently, the computational method predicted that 913 ppm of H_2S –amount that was available in the raw biogas – could lead to ~38% inhibition. The calculated percentage is insignificantly different ($p > 0.05$) compared to the experimentally observed decrease and thus, adequate prediction accuracy was achieved. Removal of H_2S from biogas is a rather established technology and thus, biogas cleaning should be applied before MOB cultivation.

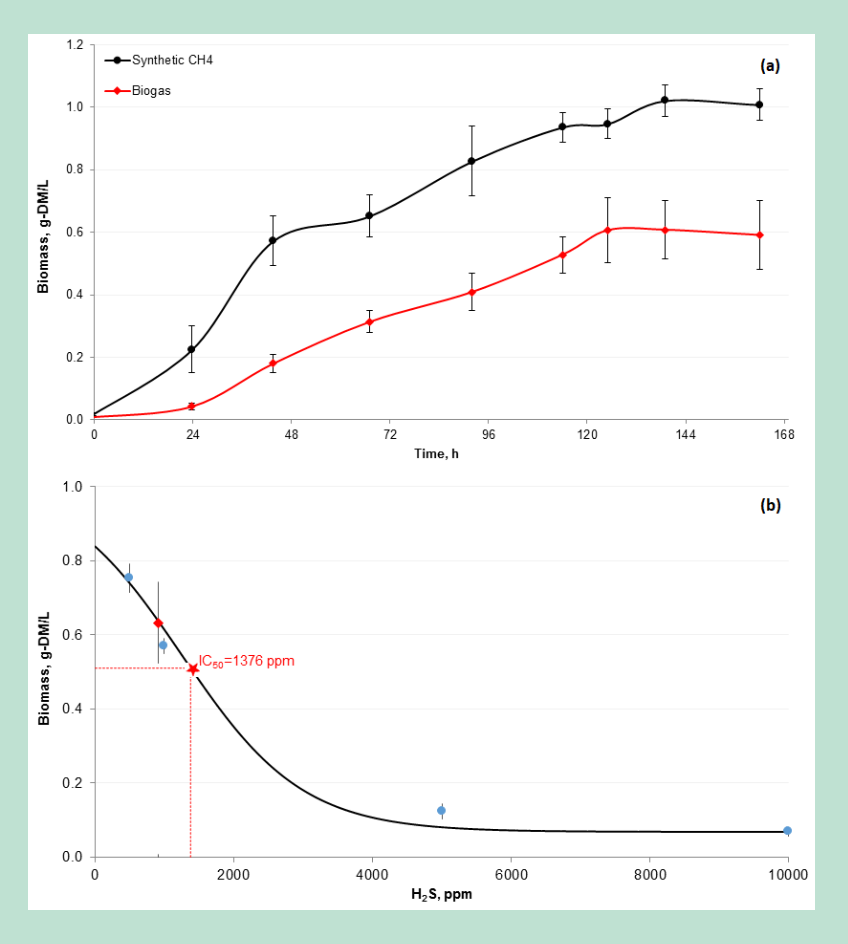

FIGURE 43. MOB growth on different (a) C source and (b) H_2S levels (Tsapekos et al., 2019)

A picture of the SCP that was produced in the laboratory is shown in Figure 14.

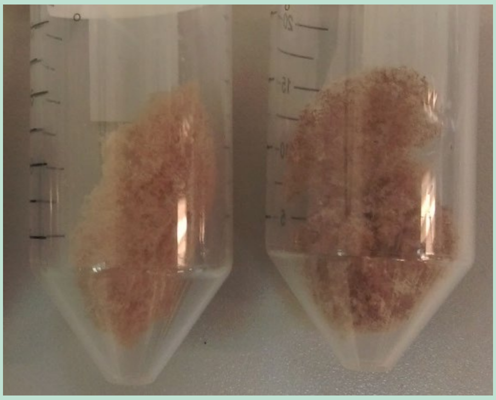

FIGURE 54. Picture of the produced SCP in the laboratory at DTU.

7.3 Effect of different nitrogen sources

The extracted nitrogen from pilot reactor digestate was supplemented with required trace elements and nutrients according to diluted mineral salt (dAMS) recipe. Then, it was used as growing medium for SCP cultivation. The bacterial growth curves can be seen in Figure 15Figure . MOB could grow well in both standard medium and extracted nitrogen supplemented with trace elements without the risk of transferring pathogens and contaminants through membrane. Insignificant difference on optical density (OD), total dry weight and growth rates were achieved between treatments showing that MOB could grow at the same performance, marking extracted nitrogen a proper source for MOB cultivation. Also, having grown the mixed-culture in dAMS and extracted nitrogen, no significant difference was observed in terms of nitrogen fixation rate. The fixation rate was estimated at ~0.37 mg N/L for both treatments. Hence, efficient replacement of synthetic media with extracted nitrogen was achieved.

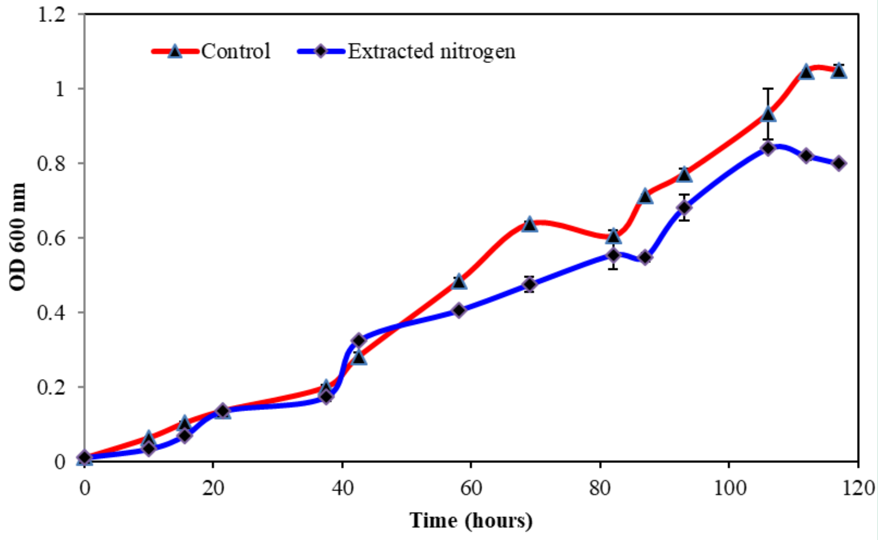

FIGURE 65. MOB growth on different N source

7.4 Upscaling

Subsequently, the process was scaled-up from 0.1 to 1.8 L working volume. The operational conditions of the semi-continuous tests are presented in Table 1. The dry cell weight (DCW) production was monitored throughout the experiments. Two individual reactors (R1 and R2) were used and they were inoculated with the same mixed bacterial seed as the batch tests, fed with high-purity CH_4 and supplied with dAMS medium and then gradually, replaced with electrochemically extracted NH_4 and upgraded biogas (bio-CH_4). MOB tend to create biofilms for carbon uptake and so, their rapid establishment at the gas feeding source led to clogging and malfunction of feeding pump. Back-flushing with air was occasionally applied to decrease diffuser biofouling. Moreover, biofouling was also observed using the novel SiC membranes (LiqTech, Denmark). For further optimization and avoiding operational problems in the future, the ceramic membranes is suggested to be cleaned by soaking, filtering, and backflushing for 30 min with NaOH 2 wt% (30 °C), followed by 30 min backflushing air at 1.5 bar.

TABLE 1. Operational conditions of semi-continuous test (Tsapekos et al., 2020).

	Dilution rate (d^{-1})	dAMS	EC-NH_4^+	Pure CH_4	Bio-CH_4
P-1 (0-5 days)	R1: -	X		X	
	R2: -	X		X	
P-2 (6-20 days)	R1: 0.200	X		X	
	R2: 0.200	X		X	
P-3 (21-35 days)	R1: 0.200		X	X	
	R2: 0.200	X			X
P-4 (36-50 days)	R1: 0.200		X		X
	R2: 0.200		X		X
P-5 (51-60 days)	R1: 0.100		X		X
	R2: 0.100		X		X
P-6 (61-80 days)	R1: 0.050		X		X
	R2: 0.050		X		X
P-5 (81-120 days)	R1: 0.025		X		X
	R2: 0.025		X		X

Remarkably, the highest MOB production (2.32 g_{DCW}/L) since the beginning of the experiment was observed in R2 at the end of P-4 (see Table 1) using alternative carbon and nitrogen sources (Figure 16a). Despite the performance of R1 slightly dropped at the end of P-4, MOB production was insignificantly lower ($p > 0.05$) than the control period (i.e. P-2). Both alternative sources of carbon and nitrogen had the potential to replace the traditional supply of substrate and nutrients.

7.5 Amino acid profile

The produced proteins were further validated determining the amino acids profile (Figure 16b). In the initial periods, relatively low content of amino acids (26–30% of DCW) was obtained at bacterial seed and control operation (i.e. P-2). The low protein contents can be attributed to the fact that the initial seed was taken at the stationary phase, during which the depletion of nutrients (e.g. N or P) can lead to the production of polyhydroxyalkanoates, lipids and extracellular polysaccharides.

At the following periods, higher amino acid contents were detected. The highest protein content (53% of DCW) was detected at the end of P-3 in R1 which was grown on electrochemically extracted NH_4 and fed with pure CH_4. Subsequently, both fermenters were stabilized to insignificantly different ($p > 0.05$) protein contents (49–51% of DCW).

All individual amino acids were augmented; nevertheless, tyrosine, serine and lysine had more distinct boosts equal to 1.7-, 1.4-, and 1.3-fold, respectively. Lysine increase is of high importance because it is found at low levels in all major cereal species and legumes. Likewise, methionine and threonine were both increased by 1.0-fold at the end of the experiment. Methionine and threonine are potentially limiting in legumes and cereals, respectively; thus, the high content in MOB is of high importance.

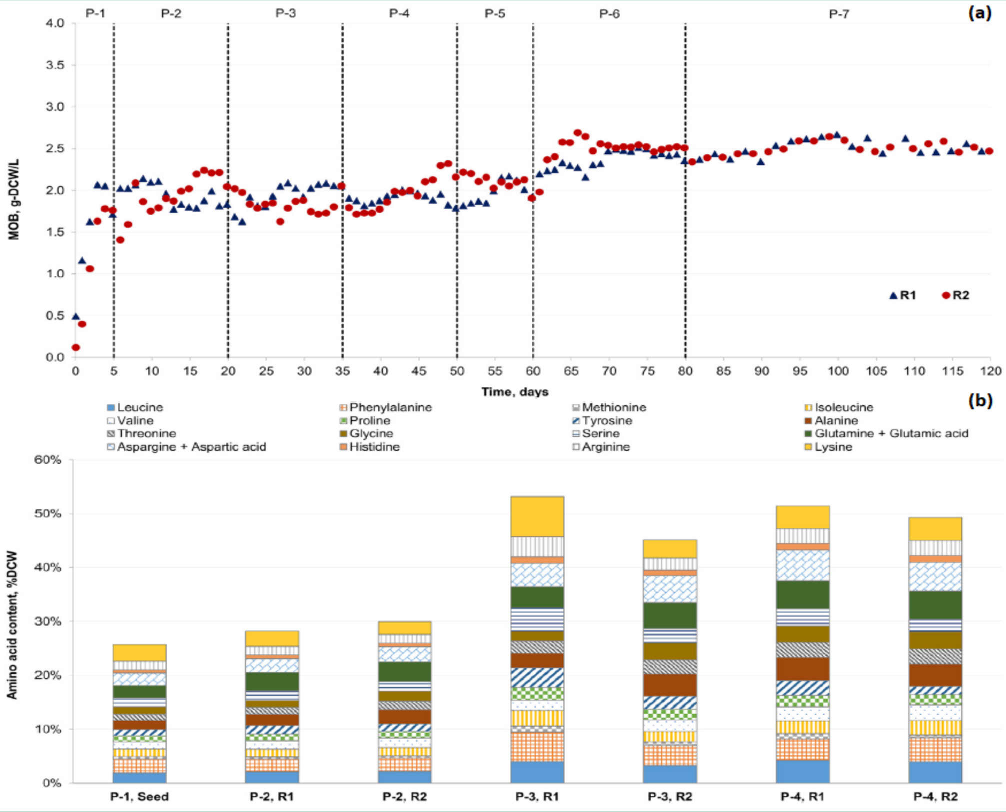

FIGURE 76. Scale-up of MOB cultivation (a) and amino acid contents (b) (Tsapekos et al., 2020).

7.6 Sub-conclusion

Alternative sources of carbon (upgraded biogas) and nitrogen (electrochemically extracted) was successfully used to produce SCP. The highest SCP production was found in experiments with these alternative sources, even when compared with SCP produced from natural gas and synthetic nitrogen. This indicates that upgraded biogas and electrochemically extracted nitrogen could be very potential sources for producing SCP and even replace the traditional sources.

A protein content of 49–51% of DCW was obtained in two separately operated reactors.

8. WP4: Circular economy, Life cycle assessment

This work package (WP4) covering circular economy in the FUBAF concept, contains a description of the performed life cycle assessment (LCA) and Life cycle cost assessment (LCCA) of the FUBAF concept. This chapter describes the LCA and chapter 9 describes the LCCA. Data collected in WP1 to WP3 has been used as inputs to the LCA and LCCA together with data from literature.

To correctly implement the LCA, the guidelines set by the ISO standards (Finkbeiner et al., 2006) as well as the instructions detailed in the ILCD handbook (Wolf et al., 2011) were followed. Accordingly, the following sections describes four mandatory steps of LCA studies: 1) goal and scope definition, 2) life cycle inventory (LCI), 3) impact assessment, and 4) interpretation.

8.1 Goal and scope definition

The goal of this study was to identify the most sustainable pathway for the valorization of biogas and reject water (or digestate) into SCP production by integrating WWTP and SCP production facilities. Since the results from this study would have consequences out of the system boundary adopted, consequential life cycle assessment (CLCA) was chosen herein. Accordingly, system expansion was used to deal with multi functionality problems instead of allocation method and marginal data was used as to model the developed scenarios.

The geographical scope of this study was Denmark, and the temporal scope was 2030. The development of the frameworks suggested herein for the valorization of reject water from WWTP into SCP (as animal feed) and the subsequent competition of the final product with commercial proteinaceous feeds (e.g., soybean meal) need a minimum time frame of 10 years. Furthermore, the biological biogas upgrading as an up-stream process for SCP facility (i.e., replacing biogas with biomethane as a carbon source for SCP cultivation) has not been commercialized yet as the dominant upgrading technology, although it has been established as a pilot operation. Accordingly, a 10-year timeframe would be the minimum time required to commercialize SCP production, subject to process optimization, approval of product safety, and establishment of biological upgrading in Denmark. This study was considered as a medium-term study due to changes in WWTP capacity and its associated demand for chemicals and energy. Therefore, this study is part of a medium-term CLCA.

The Functional Unit (FU) is the reference to which all system inputs and outputs are measured and has so far been used as mass or volume, distance, or area of land (Khoshnevisan et al., 2020). Based on the objectives set for the current study, FU was ‘‘Total amount of raw sewage entering the Avedøre WWTP in 2030”. The amount of wastewater was projected to be ~27 mio.m^3/year. The reason for choosing such FU is that we aimed at understanding if constructing a SCP facility downstream of the WWTP can improve the environmental and economic performance of a conventional WWTP.

8.2 Scenarios

Avedøre WWTP was selected as the baseline scenario (business as usual model) to which different approaches for integrating WWTP and SCP production facility were compared. Six scenarios have been created differing in terms of source of carbon (i.e, biogas or biomethane), sewage sludge mono- or co-digestion with biopulp (i.e. the organic fraction of municipal solid waste undergo to mechanical pretreatment), and the pretreatment method applied on the reject water (or digestate) to extract nutrients (i.e., centrifugation + filtration + pasteurization, electrochemical extraction, bio-electrochemical extraction). For the sake of simplicity, two scenarios were chosen for presentation in this report.

According to our preliminary mass and energy flow analyses, the biogas produced in the Avedøre WWTP cannot be sufficient to simultaneously supply internal heat and power demand as well as carbon demand of SCP production facility. Accordingly, two alternatives were considered: 1) All the plant’s heat and power demand is satisfied from the network (which is expected to be 100% renewable based on the time frame of this study) and the biogas produced in the plant is valorized into SCP, and 2) The produced biogas from anaerobic digestion of sludge is used to supply internal heat and power demand while the required biogas/biomethane is bought from biogas plants in the vicinity. The following scenarios are presented in this report and an overview of the scenarios are presented in Table 2:

0) Baseline scenario
   Business as usual scenario: Normal operation at Avedøre WWTP without production of SCP, all data projected for 2030. The WWTP consists of the following main processes: primary sedimentation, secondary treatment (nitrogen removal), secondary sedimentation / clarifiers, digestors for primary and secondary sludge, CHP, oven, and boiler for biogas valorization.

1) Scenario 1 (Sc1)
   In this scenario the reject water from the Avedøre WWTP is considered as the nitrogen source. Before being used as culture medium for SCP production, reject water is centrifuged (to decrease the content of total solids), microfiltered (to remove all the impurities), and pasteurized (at 70 ˚C for 1h to eliminate potential pathogens). The carbon source to produce SCP was supplied via biologically upgraded biogas from the biogas produced internally at Avedøre WWTP. Surplus upgraded biogas (15-20%) was used to generate heat and power to meet the internal energy demand. Therefore, the required electricity and heat of the system was supplied from the CHP unit and the rest was purchased from the grids.

2) Scenario 2 (Sc2)
   In this scenario the nitrogen was extracted from reject water via bio-electrochemical systems. A 2-stage process was employed for the ammonia recovery from the reject water. The upgraded biomethane in the biological upgrading unit was used as the carbon source for culture medium. The electricity used in the Electrolyzers to supply H_2 for upgrading unit was supplied from the surplus electricity generated from renewable wind sources, i.e., wind turbines. The biodegradability of sewage sludge is quite low, which means that the amount of biogas produced within the Avedøre WWTP would not be sufficient to supply both internal energy demand of the plant and carbon source for SCP facility. Accordingly, the co-digestion of sewage sludge and biopulp was opted as a strategy, on the one hand to produce more biogas within the WWTP, and on the other hand to enhance the biomethane potential of sewage sludge caused by the synergetic effects of sewage sludge and biopulp co-digestion. This co-digestion would increase not only the concentration of ammonium nitrogen in the reject water, but also the phosphorous content coming from biopulp. Accordingly, in this scenario both ammonium nitrogen and phosphorous were recovered using bio-electrochemical systems.

TABLE 2. Summary of the developed scenarios (year 2030).

Scenarios	N source	Treatment technology	C source
Baseline	Normal operation of the WWTP without production of SCP		
Sc1	Reject water	Centrifugation + filtration + pasteurization	Biomethane produced in Avedøre WWTP
Sc2	Reject water + biopulp	Bio-electrochemical extraction	Biomethane produced in Avedøre WWTP

The system boundary and process flow diagram are shown with an example for Sc2 in Figure 17. Dashed lines represent the substitution of marketable products/bioenergy produced within the plant by their marginal counterparts.

All the processes that were included in the LCA modeling are shown in Appendix 1.

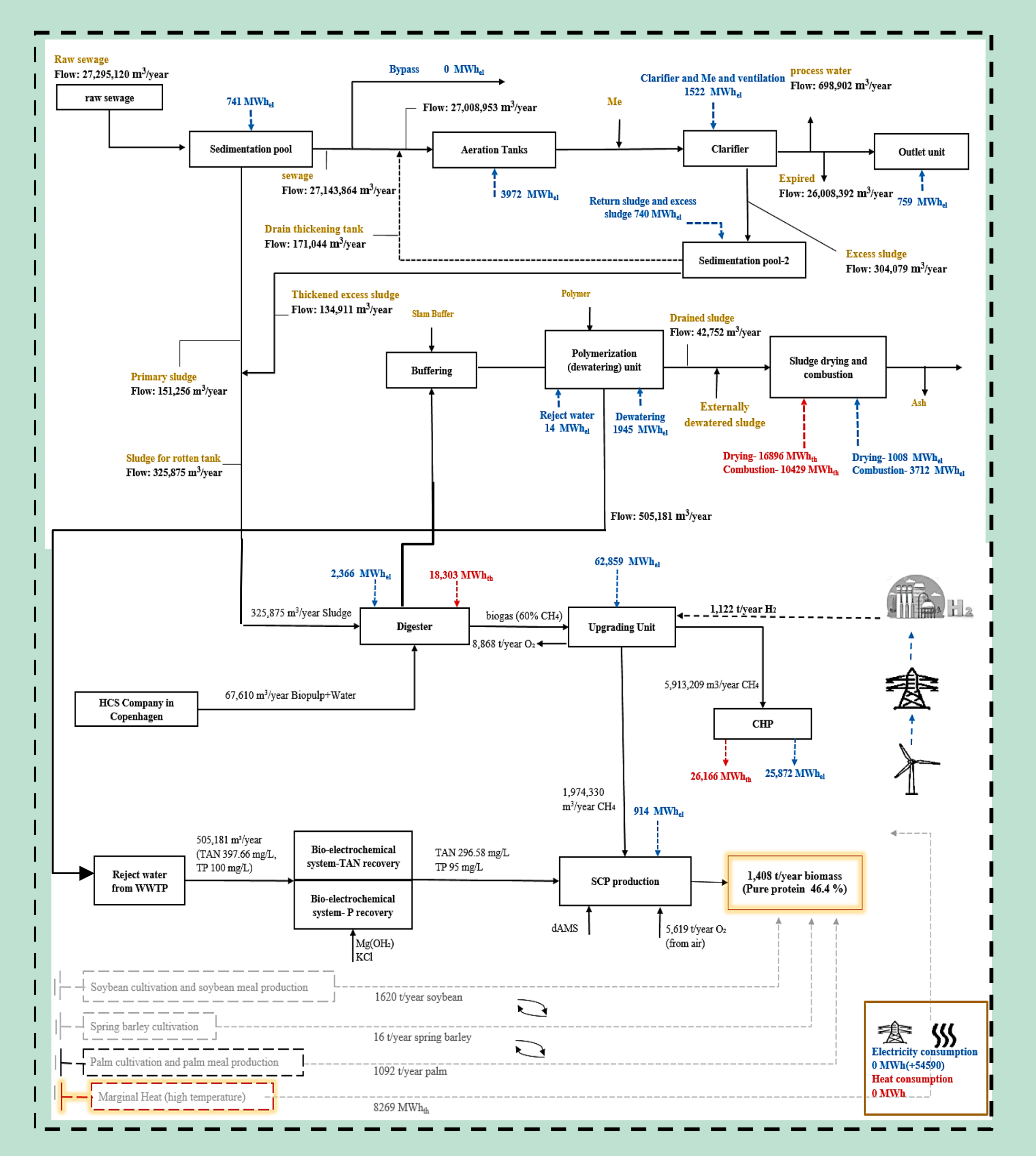

FIGURE 87. Process flow, mass flow and energy flow through the WWTP (including SCP production) in Sc2.

8.3 Life cycle inventory (LCI)

All processes that are included in the modeling are presented in Appendix 1. The full life cycle Inventory (LCI) data for all scenarios are not published yet and will be available by request only until publication.

8.4 Life cycle impact assessment (LCIA)

Interpretation of LCA studies is performed using life cycle impact assessment (LCIA) characterization factors. Midpoint and endpoint are two mainstream ways to show characterization factors (Huijbregts et al., 2017). ReCiPe2016, comprising of 18 impact categories and 3 areas of protection was used for LCIA. The three areas of protection are human health, ecosystem quality and resource scarcity. The unit for human heath damage is DALYs (disability adjusted life years), represents the years that are lost or that a person is disabled due to a disease or accident. The unit for ecosystem quality is local relative species loss in terrestrial, freshwater, and marine ecosystems, respectively, integrated over space and time (potentially disappeared fraction of species x m^2 x year or potentially disappeared fraction of species x m^3 x year). The impacts of terrestrial, freshwater, and marine ecosystems are aggregated into one single unit (species x year). The unit for resource scarcity is dollars ($), which represents the extra costs involved for future mineral and fossil resource extraction.

List of midpoint impact categories that affect ecosystem quality, human health, and resource scarcity are shown in Figure 18 (Huijbregts et al., 2017; Huijbregts et al., 2016).

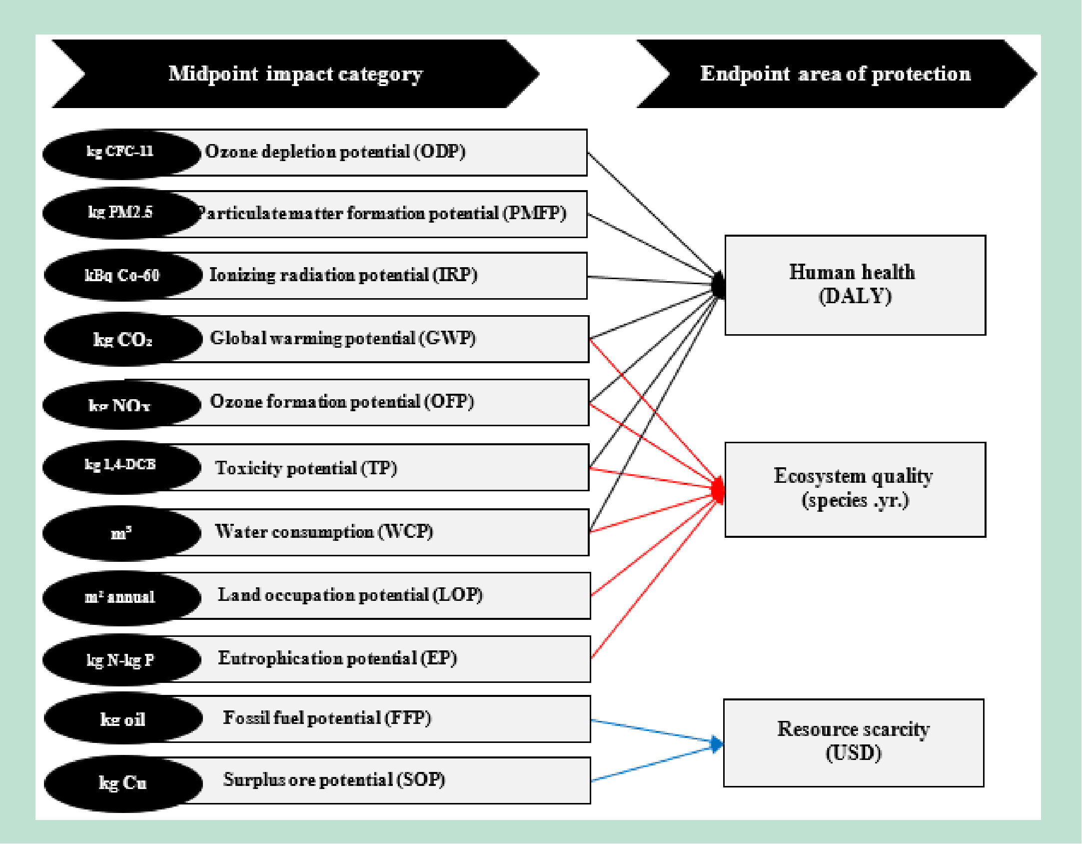

FIGURE 98. Overview of the midpoint impact categories and their relation to the endpoint impact categories. Modified from Huijbregts et al. (2017).

In this study SimaPro version 9.1 was used to model all the created scenarios and evaluate their impact assessment.

8.5 LCA results

In all impact/damage categories, there are two segments for each scenario: the positive values which will be referred to as impact and the negative values which will be referred to as saving. The net balance is calculated by subtracting the saving values by impact values. In WWTP, as can be observed the net balance in three damage categories is positive meaning that WWTP under the current condition could not be considered environmentally friendly. Nevertheless, the results achieved herein demonstrated that the integration of SCP facility into WWTP could help to decrease the environmental impacts of baseline scenario, i.e., WWTP without SCP facility, in all categories.

As depicted in Figure 19, Sc2 with co-digestion of sludge with biopulp, bioelectrochemical nitrogen extraction and biologically upgraded biogas as carbon source for the SCP production outperformed the baseline scenario in all damage categories (i.e., human health, ecosystem quality, and resource scarcity) and in both ecosystem quality and resource scarcity, the savings are larger than the impacts. Sc1 with centrifugation, filtration, and pasteurization pretreatment from reject water and biologically upgraded biogas as carbon source for the SCP production showed better performance than the baseline scenario in the ecosystem quality damage category.

In the following sections, scenarios are evaluated in detail to find out the hotspots and figure out the future possibilities to optimize the process and improve the environmental impacts of SCP production from waste effluents.

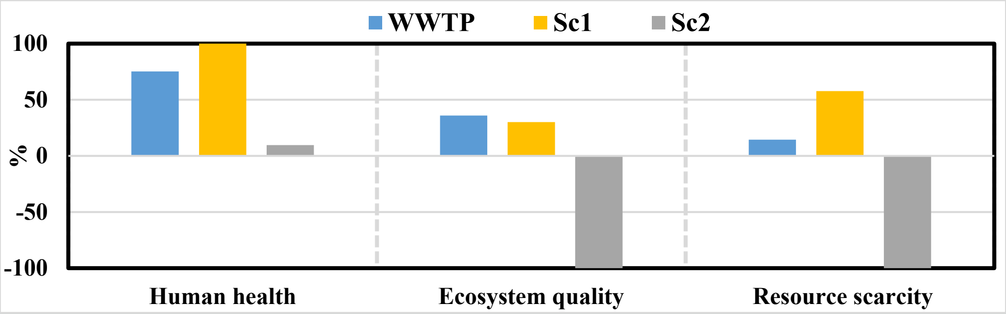

FIGURE 109. Comparison of developed scenarios in ecosystem quality, human health, and resource scarcity. The unit is % relative to the worst performing scenario, which is 100 %.

8.5.1 Human health

As can be observed in Figure 20, in Sc1, the consumption of heat for pasteurization of cultivation media imposed the highest environmental impacts to the human health damage category. The reject water used for SCP production does not contain sufficient nutrients for methanotrophs; hence, the cultivation media needed to be supplemented with modified dAMS. Modified-dAMS is composed of many chemicals. The detail analysis showed that the use of H_3PO_4 and KH_2PO_4 that provide the buffering capacity and P source in the cultivation media had the highest contribution to the fermentation unit process and consequently to the overall results.

It should be highlighted that, in both scenarios, the water electrolyzer unit process imposed high environmental impacts to the human health damage category, due to the consumption of wind electricity and water. It is noteworthy that environmental impact of wind electricity arises from their construction and maintenance.

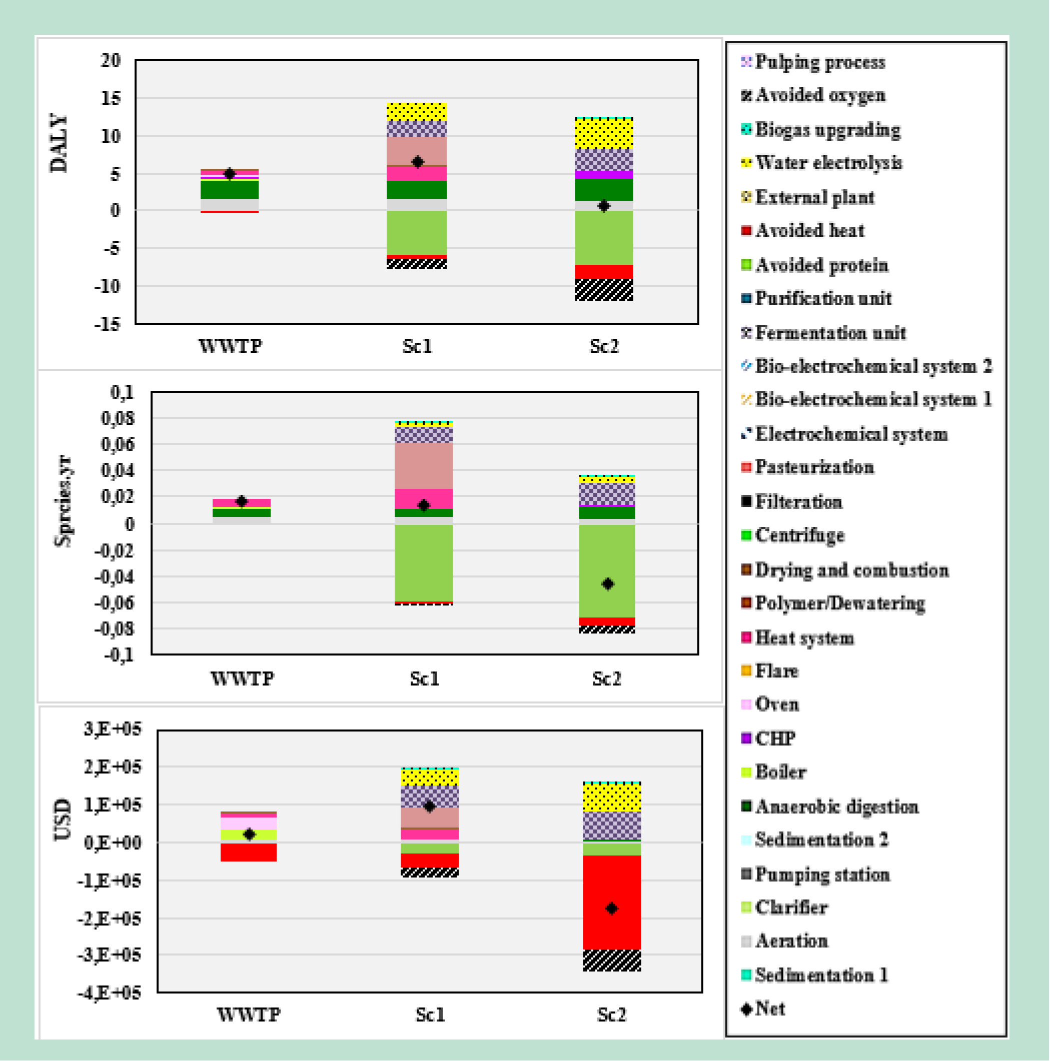

FIGURE 20. Contribution of the developed scenarios in human health, ecosystem quality, and resource scarcity area of protection.

Sc2 had the lowest net impacts on the human health damage category. This scenario did not have electricity demand in the nitrogen recovery unit and did not have a heat demand for pasteurization. In addition, Sc2 led to higher environmental net savings, due to higher SCP production. Hence, Sc2 is more competitive with the baseline scenario compared with Sc1. All results were investigated further by using sensitivity analysis; however, this is not published yet.

8.5.2 Ecosystem quality

In this damage category, Sc2 ranked first in terms of environmental performance. In Sc1, pasteurization and fermentation imposed the highest impact. The impacts of pasteurization originated from the background emissions caused by heat production and the impacts from fermentation process attributed to the background emissions from chemical industry to produce chemicals used in dAMS solution. Correspondingly, Sc2 was affected by the environmental impacts caused by the fermentation unit process; however, the total impact of Sc2 was considerably lower than Sc1.

Related to TAN recovery, the bio-electrochemical system in Sc2 had an efficiency of ~61%. In other words, the conversion of nitrogen to protein and the amount of TAN recovered for SCP fermentation were less than for Sc1. Therefore, any improvements in nitrogen recovery efficiency and conversion of nitrogen to protein can help increase the saving from the substitution of soybean meals in Sc2. All results were investigated further by using sensitivity analysis; however, this is not published yet.

8.5.3 Resource scarcity

Like the two previous damage categories, Sc2 outperformed other scenarios in the resources damage category. Sc2 had the highest rate of heat substitution compared to other scenarios. Avoided heat constituted about 79% of the total saving in Sc2. The results achieved herein implied that the substitution of soybean meal had a tiny contribution to the total impacts; in contrast, management practices within the WWTP and the use of resources would have a considerable impact on the overall results. The higher avoided heat in Sc2 was achieved by the CHP unit due to having the highest amount of biomethane produced from the biological biogas upgrade process and its combustion by CHP.

In this damage category, the impacts caused by the water electrolyzer, pasteurization, and fermentation unit process were dominant in all scenarios. All results were investigated further by using sensitivity analysis; however, this is not published yet.

8.6 Sub-conclusion

Sc2 that included co-digestion of sludge with biopulp, bioelectrochemical nitrogen extraction and biologically upgraded biogas as carbon source for the SCP production outperformed the baseline scenario and Sc1 in all damage categories (i.e., human health, ecosystem quality, and resource scarcity) and in both ecosystem quality and resource scarcity, the savings in Sc2 was are larger than the impacts.

The results of this study demonstrated that the substitution of chemicals used in SCP fermentation is the key in enhancing the environmental performance of integrated WWTP and SCP production and producing new protein sources with lower impacts than soybean meal as conventional animal feed.

The results of the LCA study showed a potential for savings in human health, ecosystem quality and resource scarcity when introducing SCP production based on carbon from biogas and nitrogen from reject water to a WWTP. The savings consisted mainly of avoided protein production (conventional) and avoided heat.

9. WP4: Circular economy, Life cycle cost assessment

The cost and revenue corresponded to each scenario is calculated, in the order shown in Figure 21. The expenditures of all scenarios including the capital expenditures (CAPEX), fixed operation expenditure (FOPEX), and variable operational expenditure (VOPEX) are calculated.

All details of the economical assessments are not published yet and will only be available upon request until publication.

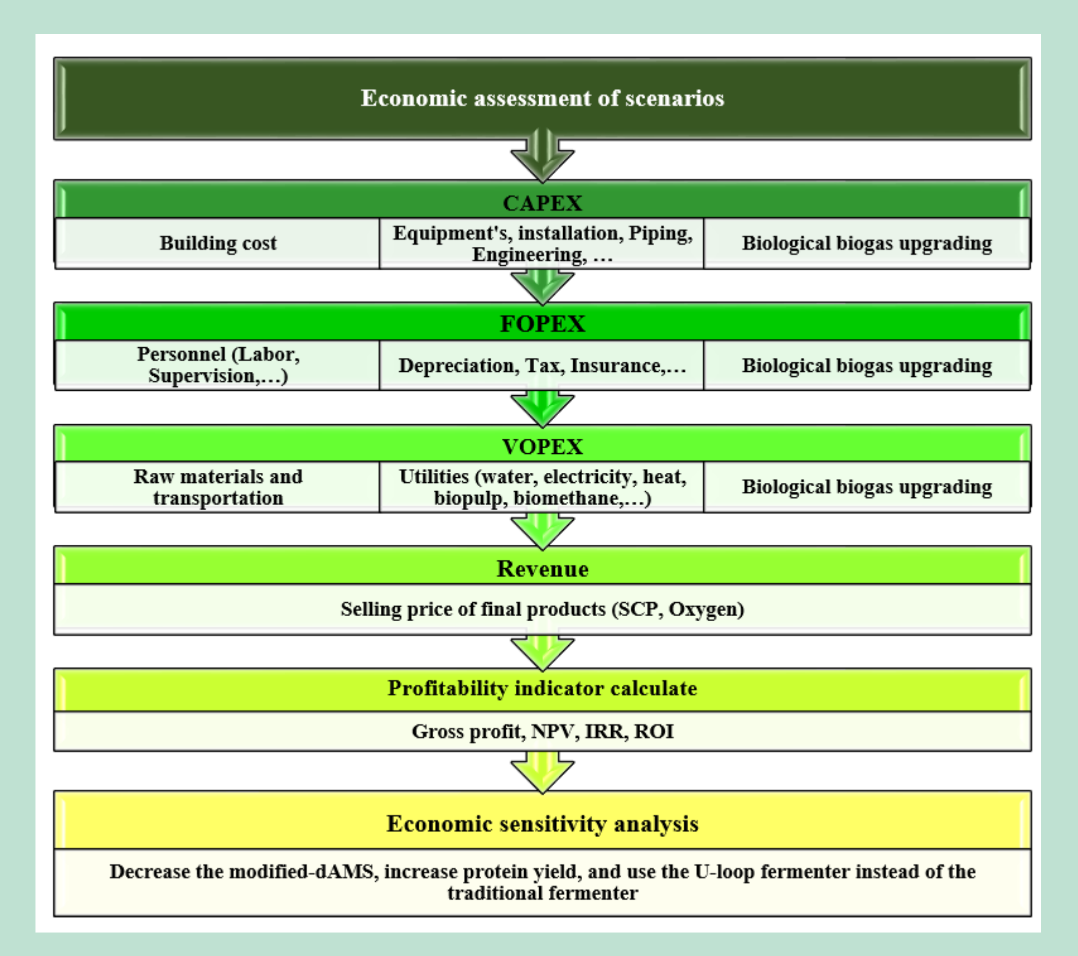

FIGURE 21. Economical assessment steps in the extended scenarios

9.1 Life cycle cost assessment results

The life cycle cost assessment consists of evaluation of CAPEX, OPEX, revenue and profitability indicators.

9.1.1 CAPEX

The CAPEX included the cost of equipment in scenarios and additional expenditure for building, equipment installation, piping, electrical systems, engineering, etc. (projected for 2030). The new installations for Sc1 included centrifuge, filters, pasteurizer, and fermenters and for Sc2 fermenters, and bioelectrochemical system.

The price of the fermentor for SCP production was by far the biggest contribution of CAPEX in the developed scenarios. The high price is due to use of traditional fermenters which need high volumes to increase the SCP production due to their limited mass transfer rates. Sc2 has the highest CAPEX due to co-digestion of sludge and biowaste and subsequently increase of fermentor capacity. The summarized CAPEX is shown in Table 3.

9.1.2 OPEX

The OPEX included cost of raw materials / chemicals, personnel, depreciation/tax, utilities, energy, water etc. for the processes related to the SCP production. Fermentation and bio-electrochemical system are the units that consume raw materials. Raw material costs in Sc1 and Sc2 constitute about 21 % and 35 % of the total OPEX for each scenario, respectively, mainly due to modified dAMS, pH adjustment, struvite precipitation, and the feeding of living microorganisms in bioelectrochemical system. The heat for pasteurization of cultivation media constituted 37 % of the total OPEX for Sc1. The biopulp that was supplied from external plants constituted 11 % of the total OPEX for Sc2. Overall, the total OPEX of Sc1 and Sc2 was found to be 1.41 and 1.15 M€/year, respectively. The summarized OPEX is shown in Table 3.

9.1.3 Revenue

The profit from the scenarios are products that can be sold to the market (SCP and oxygen). Considering optimistic, base case, and pessimistic conditions, revenue from sales of final products was calculated for the scenarios. Sc1 had the highest annual income from sale of oxygen, due to the higher upgraded biomethane consumption in SCP facility. The summarized income is shown in Table 3 (optimistic case).

9.1.4 Profitability

The profitability of the scenarios was assessed by calculating the gross profit, Return of investments (ROI), Net Present Value (NPV), and Internal Rate of Return (IRR). The gross profit for all scenarios was calculated considering the FOPEX, VOPEX, and revenue.

The gross profit for Sc1 was higher than Sc2, due to both lower OPEX and larger revenue. The CAPEX had the largest contribution in the result related to NPV, ROI, and IRR. For the least amount of profitability, it should be NPV>0 and IRR=discount rate (i.e., 8%). Evaluating the IRR and NPV indicated that none of scenarios were profitable, mainly due to high CAPEX. The summarized profitability indicators are shown in Table 3 (optimistic case).

TABLE 3. Summarized economic indicators (optimistic case for revenue).

Item	Unit	Sc1	Sc2
CAPEX	Mio. Euro	18.8	23.1
OPEX	Mio. Euro/yr.	1.41	1.07
Revenue	Mio. Euro/yr.	1.30	1.18
Gross Profit*	Mio. Euro/yr.	0.09	0.29
ROI	%	6.9	5.1
NPV	Mio. Euro	-18.0	-20.2
IRR	%	-16.9	-11.0

The gross profit does not take the property tax and depreciation into account.
ROI, return of investment; NPV, net present value; IRR, internal rate of return

9.1.5 Sensitivity analysis

The most sensitive parameters and the ones that contribute the most to the economical assessments are the modified-dAMS, valorization of 1 g of NH4-N into 5.25 g of protein and replacing conventional fermenters with U-Loop reactors (developed by Unibio). These parameters were exposed to a sensitivity analysis. The result demonstrated that although none of the scenarios could be economically feasible, economic sensitivity analysis replacing conventional fermenters with U-Loop reactors had the highest contribution to the reduction of economic costs compare to other sensitivity analysis parameters.

Considering all the possible improvements (valorization of 1 g of NH4-N into 5.25 g of protein, 20% reduction in the consumption of modified-dAMS, use of U-loop fermenter instead of the traditional fermenters), under optimistic conditions, significantly affect the final profitability, reducing CAPEX and increasing revenue simultaneously, however SCP production still are not favorable. In this case Sc2 had the greatest gross profit, ROI, NPV, and IRR i.e., 0.59 M€, 15.1%, 7.13 M€, and -3.23%, respectively.

9.2 Sub-conclusion

The result demonstrated that none of the scenarios are economically feasible under the assumptions and circumstances the were presented in this study. However, there are several potential improvements to the system, such as decreasing the modified-dAMS, higher valorization of 1 g of NH4-N, and improvement of fermentor reactor design to lower HRT.

It would be worth noting that because there are still many problems with large-scale cultivation, first generation of bacterial SCP are unlikely to be economically viable in the short term. Currently, the use of SCP in animal feed and only in some specific applications such as aquaculture is economically justified. However, if the environmental costs of the integration of WWTP with SCP production under the developed scenarios were considered, the SCP production from urban waste could be a reasonable option.

10. Feed and food legislation (DK and EU)

The FUBAF project demonstrates the production of bacterial proteins in a quality that can replace traditional protein sources for animal feed from e.g. soybeans and fishmeal. The production of bacterial proteins takes place from biogas which originates from the decomposition of Source-separated organic fraction of municipal solid waste (SS-OFMSW). However, the biogas could just as well have originated from the digestion of wastewater sludge or another organic residual fraction of wastes. The biogas produced subsequently undergoes biological upgrading by the addition of hydrogen. To produce bacterial proteins, a nitrogen source must be used for this purpose. In the FUBAF project the nitrogen source originates from the digested SS-OFMSW – specifically the reject water. In the FUBAF project the ammonium-rich reject water undergoes electrochemical treatment before being applied to the bacterial protein production.

A similar method to produce bacterial proteins has already been approved for the Danish manufacturer Unibio A/S. Therefore, the method is not foreign to either Danish legislation or EU legislation. However, the bacterial protein production in the FUBAF project, differs from the method used by Unibio A/S, in the sense that the FUBAF project uses other input materials / raw materials in the production of the bacterial proteins. Unibio A/S use natural gas in their production. Other raw materials, as the nitrogen source, are of food quality and are therefore guaranteed free of toxins, dioxins and heavy metals due to the highly controlled production process. In addition, it must be emphasized that Unibio A/S in their protein production uses "pure" bacterial cultures in the formation of the bacterial proteins, hence the exact bacterial composition of the protein product is known. In the following, comments are made in accordance to the legislation with a focus on the differences in the production methods between the already approved product Uniprotein® from Unibio A/S and the production of bacterial proteins in the FUBAF project.

10.1 The biogas source

The biogas used in the FUBAF project differs from the biogas used in the production of bacterial proteins by Unibio A/S. The biogas in the FUBAF project is not of natural gas quality, but instead biogas with a high content of methane (after upgrading). The question is whether the current legislation only approves the use of biogas of natural gas quality for production of bacterial proteins or whether the upgraded biogas can be used without further approvals, if the quality meets the quality requirements to natural gas? The Danish Veterinary and Food Administration (DVFA) states that it legal to use the upgraded biogas for production of bacterial proteins, if the upgraded biogas contains methane in a similar concentration as that of the natural gas and otherwise meets the same quality as natural gas. In this regard, the upgraded biogas can be used to produce bacterial protein under entry 12.1.2 in the feed list (Fodermiddelfortegnelsen).

10.2 The nitrogen source

The source of nitrogen used in the FUBAF project differs from the nitrogen source used in the production of bacterial proteins by Unibio A/S. The nitrogen source used in the Unibio process is of food quality. In the FUBAF project the nitrogen source originates from the reject water from the digested wastewater sludge and is hence not of food quality. The question is whether it follows the feed and food legislation to use a nitrogen source originating from the decomposition of organic residues (SS-OFMSW, wastewater sludge, organic industrial waste, etc.). The nitrogen source in the FUBAF project has been recovered from the reject water through electrochemical treatment before being applied to the bacterial protein production. The Danish Veterinary and Food Administration (DVFA) states that the reject water is considered a fraction of the digested biomass and can hence not be used in the bacterial protein production without further pre-treatment.

If the nitrogen source originates from the digestion of SS-OFMSW, the nitrogen source is considered a residual product, most likely containing animal by-products and must be handled in accordance with the Regulation on animal by-products (no. 1069/2009) and its implementing Regulation (no. 142/2011). This means, that all steps in the chain from the digestion of SS-OFMSW to recycled nitrogen must be approved or registered by DVFA in accordance with the regulation on animal by-products. It is possible to read more about the regulations mentioned here . The same legislative conditions are expected to apply if the reject water originates from the digestion of wastewater sludge or other organic waste fractions.
Degassed biomass (regardless of origin; SS-OFMSW, wastewater sludge, other organic wastes, etc.), or fractions thereof (e.g. reject water), must not be used in animal feed products. It is therefore important that there are no residues of reject water in the feed protein produced by the bacteria. If the bacterial protein contains residues of reject water, it will not be permitted to use it for animal feed-purpose.

DVFA can thus only allow the use of recycled nutrients from the digestion of SS-OFMSW, wastewater sludge or organic wastes etc., provided that there is documentation on a treatment which ensures that there is no transfer of infectious agents (bacteria/viruses/parasites, etc.), toxic and otherwise harmful substances from the reject water to the nutrients which will be used for the production of bacterial proteins. It is the responsibility of the producer to provide this documentation.

The organic material used, regardless of whether it is SS-OFMSW, wastewater sludge or other organic wastes, is assumed to contain toxic and infectious substances that is unwanted in animal feed. Therefore, it is essential to the FUBAF concept and method that the recycled nutrient from these fractions are separated from the unwanted substances in the reject water before addition to the bacterial protein production. DVFA does not consider a pasteurization of the reject water to be sufficient, because a pasteurization not necessarily remove all infectious organisms/particles or toxins from the reject water. DVFA also states that it is the responsibility of the protein producer to prepare a risk assessment on the use of reject water in the production of proteins and to demonstrate that the production methods complies with the current requirements of the legislation. Based on this, DVFA can then assess the provided documentation.

10.3 Bacterial composition

In the FUBAF project, the dominant bacterium responsible to produce bacterial proteins belonged to *Methylomonas* genus, while Unibio A/S culture is enriched in *Methylococcus capsulatus* in the Uniprotein®. Both *Methylomonas* and *Methylococcus* genera belong to the family of *Methylococcaceae*. *Methylococcus capsulatus* is highlighted under entry 12.1.2 in the feed list (Fodermiddelfortegnelsen). In addition, smaller populations of other microorganisms are expected to be present in the bacterial composition, responsible for the transformation of the residual fractions of gases in the fermentation process that are not methane. The question is whether there are legislative requirements for the bacterial composition in reference to the entry 12.1.2 in the feed list (Fodermiddelfortegnelsen). Does the exact bacterial composition have to be known? Does it have to be a pure culture? In this regard, DVFA states that it is important to know (among other things for safety reasons) which microorganisms produce the bacterial proteins. If the product is to match entry 12.1.2 in the feed list, it must be bacteria of the same type that are responsible for the production of bacterial proteins.

10.4 Primary challenge

There are some legislative challenges that must be addressed before it can be considered possible to produce proteins for animal feed via the FUBAF concept. The primary challenge in producing proteins for animal feed via the FUBAF concept is the use of a nitrogen source which originates from the digestion of SS-OFMSW, wastewater sludge, other organic wastes etc. In addition, it is considered that the requirement for the bacterial composition in relation to entry 12.1.2 in the feed list (Fodermiddelfortegnelsen) may present challenges, as the bacterial composition will not be controlled in the same way as in the production of Uniprotein® using controlled mixed culture bacterial composition in the process.

There is no actual procedure of approval (performed on the basis of risk assessments) when feed materials are recorded and notified in the feed list (Fodermiddelfortegnelsen) and the feed material register (fodermiddelregistret), respectively. Feed materials are also not recorded nor notified specifically for certain animal species. To be in compliance with the feed and food legislation the feed materials need to be known, i.e. they must either be found in the feed list or in the feed register.

It is possible to find more about the procedure for including new feed materials in the feed list as well as about the procedure for notifying feed materials to the feed register in the digital feed guide (feed guide) in the feed list (from page 60) and about the feed register (from page 67).

If the regulatory challenges of producing proteins for animal feed via the FUBAF concept are addressed, and the FUBAF concept ultimately meets all legislation, the product can eventually be marketed. In that case, there are two possible way of addressing this:

1) If the product matches already existing products in the feed inventory, it is covered and can be marketed, and in this case a review of the product is not required. The product of the FUBAF concept is best suited with entry 12.1.2 in the feed list (where Unibios Uniprotein® is also located). If the final product from FUBAF is in accordance with the criteria described for entry 12.1.2, it will be a viable path.
2) If the protein derived from the FUBAF concept does not sufficiently match the entry of the feed material list 12.1.2, it is a possible alternative to notify product one to the feed material register, after which it can be marketed.

In the Danish Veterinary and Food Administration's digital feed guide, it is possible to obtain further knowledge about the feed list (from page 60) and about the feed register (from page 67). Regardless of the marketing of the protein product is caried out under 1 or 2 it is the responsibility of the producer to guarantee the safety of the product.

11. Conclusion

The FUBAF 2nd generation SCP concept was tested in laboratory and pilot scale experiments. The overall idea was to convert urban biowaste to bacterial proteins for animal feed, through some innovative processes. 1st generation concepts, in which virgin materials such as natural gas and synthetic nutrients were utilized, has already been proven in large scale. The FUBAF concept aims at optimizing the system in terms of sustainability and better environmental performance.

Urban biowaste, in this case source-separated organic household waste, was anaerobically digested in a pilot scale reactor, thereby producing biogas. The anaerobic digestion reactor was operated with urban biowaste for more than 200 days at mesophilic conditions with gradually increasing TS content up to 12 % TS, corresponding to an Organic Loading Rate (OLR) of up to around 5 g VS/L/day. A stable and quite robust process was ensured, achieving methane yields of 0,99 L CH_4/L/d, corresponding to 370 Nm^3 CH_4/t VS_{in}.

The biogas, which consisted of appr. 60-65 % methane, was then biologically upgraded to natural gas quality with a methane content of appr. 90-95 %. The conversion was done by microorganisms (mixed archaeal culture), in a reactor where hydrogen is added. The hydrogen reacts with the carbon dioxide in the biogas and produces methane. Laboratory tests were carried out with a range of reactor configurations and types of packing materials to ensure the best possible design of the upgrading reactor. In a pilot scale setup with a thermophilic trickle bed reactor with PU foam as packing material, very good results were achieved. The methane content was >90 % and very fast recovery was observed after a standby period.

A novel microbial bioelectrochemical system in laboratory scale was developed for extracting nutrients from reject water and anaerobically digested biowaste. The reactor was operated as microbial fuel cell (MFC). The recovery efficiency of ammonia from digestate was in the range of 40-42% when using 1-stage MFC. In two-stage MFC fed with reject water and digestate, the total ammonia efficiencies in cathodic chamber were ~61% and 54%, respectively, corresponding to about 70% and 25% higher than that in one-stage operation.

The produced upgraded biogas and the extracted nutrients was utilized as carbon and nitrogen sources, respectively, for SCP production in laboratory scale. The highest SCP production was found in experiments with these alternative sources, even when compared with SCP produced from natural gas and synthetic nitrogen. This indicates that upgraded biogas and electrochemically extracted nitrogen could be very potential sources for producing SCP and even replace the traditional sources. A highly proteinaceous biomass was produced having a total amino acids content of 49–51% of DCW in two separately operated reactors.

An environmental assessment was conducted, using consequential Life Cycle Assessment (CLCA). A scenario that included co-digestion of sludge with biopulp, bioelectrochemical nitrogen extraction and biologically upgraded biogas for the SCP production (Sc2) outperformed the baseline scenario and other scenarios in all modelled damage categories (i.e., human health, ecosystem quality, and resource scarcity). In both ecosystem quality and resource scarcity, the savings in Sc2 were larger than the impacts. The results of this study demonstrated that the substitution of chemicals used in SCP fermentation is the key in enhancing the environmental performance of integrated WWTP and SCP production and producing new protein sources with lower impacts than soybean meal as conventional animal feed.
The results of a Life Cycle Cost Assessment (LCCA) demonstrated that none of the scenarios are economically feasible under the assumptions and circumstances that were presented in

this study. However, there are quite several potential improvements to the system, such as decreasing the modified-dAMS, higher valorization of 1 g of NH4-N, and improvement of fermentor reactor design to lower HRT and increased gas to liquid transfer. It would worth note that because there are still many problems with large-scale cultivation, first generation of bacterial SCP are unlikely to be economically viable in the short term. Currently, the use of SCP in animal feed and only in some specific applications such as aquaculture is economically justified. However, if the environmental costs of the integration of WWTP with SCP production under the developed scenarios were considered, the SCP production from urban waste could be a reasonable option.

There are some legislative challenges that must be addressed before it can be considered possible to produce proteins for animal feed via the FUBAF concept. The primary challenge in producing proteins for animal feed via the FUBAF concept is the use of a nitrogen source which originates from the digestion of urban biowaste. In addition, it is considered that the requirement for the bacterial composition may present challenges, as the bacterial composition will not be controlled in the same way as in the production of proteins based on pure culture bacterial compositions.

The FUBAF project showed that SCP can be successfully produced from urban biowaste. There are still some technological, economic and environmental issues that needs to be optimized, but the concept shows good potentials to compete with 1st generation SCP production from natural gas and synthetic nutrients.

12. Future perspectives

The FUBAF concept has been successfully proven in this project in laboratory / pilot scale. In the next phase, the system needs to be scaled up to get more realistic experiences and numbers for future full-scale applications.

Some technical suggestions for the experimental work have been proposed:

1) Biological upgrading of biogas:
    a. New and more efficient packing materials to enhance biofilm formation and improve trickling. The polyurethane foam (that was applied in the pilot) does not favor proper trickling and thus, biofilm is not homogenously formed via TBR bed).
    b. Add more raw biogas to reduce the retention time as a means to capture more CO_2 in a certain time frame
    c. Collect the off-gases from SCP production, which should be rich in CO_2, and feed them in TBR to be coupled with H_2 and produce CH_4
    d. Define inhibitors for the biomethanation when the off-gases of MOB cultivation are used (residual O_2 after MOB cultivation?)

2) Electrochemical nitrogen extraction
    a. New electrodes, ratio of electrode to volume and reactor configuration (e.g. multiple chambers)
    b. Extraction of phosphorus using electrochemical and microbial electrochemical system

3) Production of SCP
    a. When using digestate, closely monitor contaminants (pathogens, heavy metals, antibiotics, pesticides) at the final product to ensure suitability for feed applications
    b. Up-scale the process to collect and assess the biogas-based SCP as poultry feed, evaluating digestibility and absorption
    c. Unveil the metabolism of pure or mixed cultures via meta-omics approaches to reveal activity and metabolism shifts at different operational conditions. Reveal how the desired amino acid profile is formed.

The LCA results showed potentials for savings in all damage categories by implementing the FUBAF concept. Next step for LCA work could be to compare the FUBAF concept with 1st generation SCP production, such as the concept by Unibio. However, full-scale operation is still quite recent, so detailed data for LCA modeling is not yet available.

The results from the LCCA showed that none of the developed FUBAF scenarios were economically feasible. However, the processes are also still relatively low in TRL and there are many potential improvements to the system, as mentioned above. One thing that has not been evaluated during the LCCA, is the political circumstances, that might be changed soon. This could potentially change the assumptions done in this study. As an example, the Danish Council on Climate Change (Klimarådet) has proposed a general CO_2 tax, that will gradually increase up to 1,500 DKK/t CO_2 (corresponding to ~200 Euro/t CO_2) by 2030. This could completely change the LCCA outcome for the FUBAF concept.

In general, what is needed, is a political will to change the existing system, to be able to implement new and more sustainable technologies and to drive the green transition. New technologies might be more costly in the short run, but with optimization of the processes as well as political intervention, new technologies might be even cheaper in the long run. The FUBAF concept might also rely on changes in related systems, e.g. the wind power industry and the development of the electricity, gas and hydrogen grids.

The FUBAF concept will not be able to supply proteins for the entire growing population, but at least it can contribute to more sustainable and maybe as important, local production of proteins. In a Danish context, that means, that we can rely less on import of proteins in the form of soya from South America, for the large scale pig production in Denmark.

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Appendix 1. Modules in SimaPro

Unit/unit	Module on SimaPro v9.0
Sedimentation unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Aeration unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Clarifier unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Outlet unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Sedimentation unit 2	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Digester unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Heat[1]	1- Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, wood chips, 6,667 kW, state-of-the-art 2014 | Conseq, U. 2- Heat, central or small-scale, other than natural gas {RoW}| heat production, wood pellet, at furnace 300kW | Conseq, U. 3- Heat, central or small-scale, other than natural gas {Europe without Switzerland}| heat production, wood pellet, at furnace 15kW | Conseq, U. 4- Heat, district or industrial, other than natural gas {GLO}| treatment of straw, organic, in furnace 300kW | Cut-off, U.
Water * (in sc6: pulping process)	Water, cooling, unspecified natural origin, DK
Transportation * (in sc. 6: pulping process)	Transport, freight, lorry 7.5-16 metric ton, euro3 {RoW}| market for transport, freight, lorry 7.5-16 metric ton, EURO3 | Conseq, U
Boiler unit	
Oil	Diesel {GLO}| market group for | Conseq, U
Oven unit	
Oil	Diesel {GLO}| market group for | Conseq, U
Polymer/dewatering unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Drying and combustion unit	

Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Heat *(marginal heat)*	1- Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, wood chips, 6,667 kW, state-of-the-art 2014 | Conseq, U. 2- Heat, central or small-scale, other than natural gas {RoW}| heat production, wood pellet, at furnace 300kW | Conseq, U. 3- Heat, central or small-scale, other than natural gas (Europe without Switzerland)| heat production, wood pellet, at furnace 15kW | Conseq, U. 4- Heat, district or industrial, other than natural gas {GLO}| treatment of straw, organic, in furnace 300kW | Cut-off, U.
Transportation	Transport, freight, lorry 7.5-16 metric ton, euro3 {RoW}| market for transport, freight, lorry 7.5-16 metric ton, EURO3 | Conseq, U
Electrolyze unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U.
Water	Water, unspecified natural origin, DK.
Centrifuge unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Filter unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Pasteurization unit	
Heat *(marginal heat)*	1- Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, wood chips, 6667 kW, state-of-the-art 2014 | Conseq, U. 2- Heat, central or small-scale, other than natural gas {RoW}| heat production, wood pellet, at furnace 300kW | Conseq, U. 3- Heat, central or small-scale, other than natural gas {Europe without Switzerland}| heat production, wood pellet, at furnace 15kW | Conseq, U. 4- Heat, district or industrial, other than natural gas {GLO}| treatment of straw, organic, in furnace 300kW | Cut-off, U.
Fermentation unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Purification unit	
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U
Bio-electrochemical system (TAN recovery)	
Chemical materials	• Ammonium chloride {GLO}| market for | Conseq, U. • Potassium chloride, industrial grade {GLO}| market for potassium chloride, industrial grade | Conseq, U.

	• Calcium chloride {GLO}| market for | Conseq, U. • $MgCL_2$ (This module is defined) • KH_2PO_4 (This module is defined) • Na_2HPO_4 (This module is defined) • NaH_2PO_4 (This module is defined)
Transportation	Transport, freight, lorry 7.5-16 metric ton, euro3 {RoW}| market for transport, freight, lorry 7.5-16 metric ton, EURO3 | Conseq, U
Bio-electrochemical system (P recovery)	
Chemical materials	• Na_2HPO_4 • $Mg(OH_2)$
Transportation	Transport, freight, lorry 7.5-16 metric ton, euro3 {RoW}| market for transport, freight, lorry 7.5-16 metric ton, EURO3 | Conseq, U. This module is defined.
Avoided protein	
Material	• Marginal soybean production-Average {GLO} • Marginal oil palm production-Average {GLO} • Barley grain {GLO}| market for | Conseq, U
Avoided heat	
Heat *(marginal heat)*	1- Heat, district or industrial, other than natural gas {DK}| heat and power co-generation, wood chips, 6,667 kW, state-of-the-art 2014 | Conseq, U. 2- Heat, central or small-scale, other than natural gas {RoW}| heat production, wood pellet, at furnace 300kW | Conseq, U. 3- Heat, central or small-scale, other than natural gas {Europe without Switzerland}| heat production, wood pellet, at furnace 15kW | Conseq, U. 4- Heat, district or industrial, other than natural gas {GLO}| treatment of straw, organic, in furnace 300kW | Cut-off, U
Avoided oxygen	
Oxygen	Oxygen, via cryogenic air separation, production mix, at plant, gaseous EU-27 S
Pulping process	
Water	Water, cooling, unspecified natural origin, DK
Electricity	Electricity, high voltage {DK}| electricity production, wind, 1-3MW turbine, offshore | Conseq, U

FUBAF: From Urban Biowaste to Animal Feed

FUBAF-projektet præsenterer en ny innovativ tilgang til anvendelse af organiske restprodukter til produktion af proteiner (Single Cell Proteins, SCP), som kan anvendes til dyrefoder og erstatte de nuværende klimatunge alternativer (soya bønner og fiskemel). FUBAF-konceptet er et 2. generations SCP-koncept med en øget bæredygtighed og et højt potentiale sammenlignet med 1. generation, hvor råmaterialerne naturgas og syntetisk kvælstof anvendes.

I FUBAF-projektet blev en biopulp af kildesorteret organisk dagrenovation (KOD) udrådnet under anaerobe og mesofile betingelser for at producere biogas (middelproduktion på 370 Nm3 CH_4/t VSin med ca. 60% CH_4 og 40% CO_2). I næste trin blev biogassen opgraderet biologisk (blandet archaea kultur) til naturgaskvalitet (90-95 % metan) vha. tilsætning af brint. Både biogasproduktionen og opgraderingen af biogassen foregik i pilotskala. Et nyt mikrobielt bioelektrokemisk system til udvinding af kvælstof fra bioforgasset bioaffald blev udviklet i laboratorieskala. Den opgraderede biogas og det udvundne kvælstof blev anvendt som hhv. kulstof- og kvælstofkilde til SCP-produktion i laboratorieskala, med lovende resultater som følge heraf.

Miljøvurdering af FUBAF-konceptet ved anvendelse af konsekventiel livscyklusvurdering (CLCA) viste en favorabel miljømæssig performance for 2. generationsproduktion af SCP. Resultatet af en livscyklus kost vurdering (LCCA) viste, at ingen af de udviklede scenarier er økonomisk rentable under projektets antagelser og omstændigheder. Der er peget på forhold til forbedringer til FUBAF-konceptet og samtidig er udpeget en række tekniske, økonomiske, miljømæssige udfordringer samt politisk tiltag, som skal sikre at FUBAF-konceptet kan implementeres i fuldskala i fremtiden.

The FUBAF-project presents an innovative approach to utilization of organic residues for production of Single Cell Proteins (SCP), which can be used as animal feed to replace more climate-heavy alternatives (fishmeal and soybeans). The FUBAF-concept is a 2nd generation SCP-concept with improved sustainability and a higher potential than 1st generation, where raw materials (natural gas and synthetic nitrogen) are used.

In the FUBAF-project a biopulp from source-separated organic household waste was anaerobically digested under mesophilic conditions to produce biogas (average production of 370 Nm3 CH_4/t VS in with ~60% CH_4 and 40% CO_2). In the next step the biogas was upgraded biologically (mixed archaea culture) to natural gas quality (90-95% CH_4) by adding hydrogen. These two processes were tested in pilot scale. A novel microbial bioelectrochemical system for extraction of nitrogen from digested biopulp was developed in laboratory scale. The upgraded biogas and the extracted nitrogen were then used for SCP production the laboratory, with promising results.

A consequential life cycle assessment (CLCA) of the FUBAF-concept showed a favorable environmental performance for 2nd generation production of SCP. The result of a life cycle cost assessment (LCCA) showed that none of the developed scenarios are economically feasible under the assumptions and circumstances that were presented in the study. A range of possible improvements to the FUBAF-concept were identified. In addition, several technical, economic, and environmental challenges as well as political initiatives that can ensure future full-scale implantation of the FUBAF-concept were designated.

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