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Sustainable production of fermentation-based novel proteins

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ABSTRACT

Feeding the growing global population sustainably while minimizing environmental impact is grand challenge for human society. Methane-based single-cell proteins through fermentation (bio-SCP) have emerged as a promising alternative to traditional protein sources (animal and crops), addressing the significant greenhouse gas emissions from livestock production. This study explores an innovative approach to bio-SCP production using synthetic natural gas derived from biogas. The process integrates biogas production via anaerobic digestion of food waste, biogas upgrading through a series of treatments, and SCP production via aerobic fermentation of methane. Detailed process modeling reveals that the proposed design consumes 25,000 kg/h (200 Mt/y) of food waste, producing 4,269.4 kg/h (34.2 Mt/y) of SCP and valuable by-products such as biofertilizer, elemental sulfur, low-pressure steam, and nitrogen. Notably, the proposed design achieves close to 100 % energy self-sufficiency. Techno-economic analysis indicates a capital investment of $733.5 million, annual operating costs of $43.96 million, and a minimum product selling price of $1.02/kg of bio-SCP, demonstrating promising economic viability, especially with nitrogen by-product sales. A cradle-to-gate life cycle assessment highlights the environmental benefits of bio-SCP, showing significant reductions in environmental impacts compared to fossil-driven SCP production. This study underscores the potential of bio-SCP in sustainable animal nutrition and greenhouse gas emission reduction.

1. Introduction

Feeding a burgeoning global population sustainably while mitigating the environmental impacts of agricultural practices remains a critical challenge in the 21st century. The Paris Accord of 2015 underscored the urgent need for significant reductions in carbon emissions to combat climate change, emphasizing the role of all sectors, including agriculture, in achieving these ambitious goals. Livestock production stands out as a major contributor to greenhouse gas emissions, generating approximately 7.1 gigatons of carbon dioxide equivalent annually, which accounts for about 14.5 % of human-caused greenhouse gas emissions worldwide (Benavides et al.,; Wu et al., 2014; FAO Publications Catalogue). A substantial portion of these emissions, up to 45 %, is attributed to the production and processing of livestock feed, underscoring the environmental footprint of current agricultural practices (FAO Publications Catalogue). Despite these environmental challenges, global demand for animal protein is projected to increase significantly, with estimates suggesting a 110 % growth from 2005 to 2050 (Boland et al., 2013). This demand growth poses a dilemma: how can we meet nutritional needs while reducing the environmental impact of food production?

In response to these challenges, innovative approaches to animal agriculture are being explored, with a particular emphasis on sustainable protein sources that can reduce dependence on conventional animal and plant-based proteins. Single-cell proteins (SCP), derived from microbial cultivation, have emerged as a promising alternative due to their potential to provide high-quality protein suitable for animal feed. Various microorganisms, including bacteria, fungi, and microalgae, can be cultivated for SCP production. Among the alternatives, methane-based bacterial (methanotrophic) SCP offers several advantages over conventional proteins: (1) it requires significantly less water and land for production, making it a more sustainable option; (2) it can be produced from non-human edible feedstocks such as hydrocarbons and biowastes, thereby increasing food availability without competing with traditional food sources; and (3) SCP production is highly energy-efficient and resilient, ensuring a stable protein source even during extreme food supply crises (García Martínez et al.). It is reported that food products derived from carbon building blocks found in natural gas

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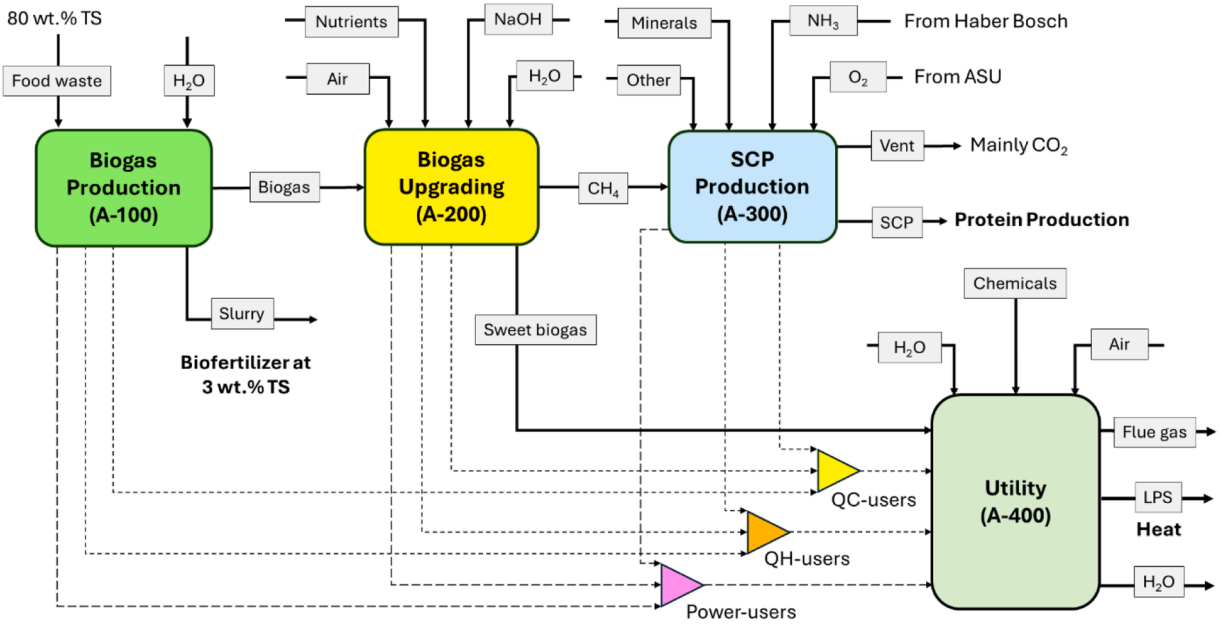

Fig. 1. Simplified block flow diagram for sustainable and energy self-sufficient bio-SCP production. Note that many process and energy streams are intentionally omitted in this hierarchical view to avoid complexity. Detailed process flow diagrams and process descriptions are summarized in Section 2.

use approximately 100 times less water and 1000 times less land per ton compared to soybeans (García Martínez et al.).

Consequently, much research has focused on methane SCP, making it one of the most advanced and accessible SCP production technologies, currently on the verge of large-scale commercialization. Key players pioneering the mass production of methane SCP include Calysta Inc., (CALYSTA) Unibio A/S, (UNIPROTEIN®) Circe Biotechnologie GmbH, (CIRCE) and String Bio Pvt Ltd. (String) These companies mainly utilize methane fermentation technology to produce SCP, aiming to address global protein demand sustainably across various sectors. Notably, methanotrophic bacteria, such as *Methylococcus capsulatus* (M. *capsulatus*), require three primary inputs for producing high-quality protein through aerobic fermentation: a carbon source, typically derived from methane found in natural gas; a nitrogen source, sourced from ammonia derived from the fertilizer industry; and an oxygen source, acquired through cryogenically separated air.

While methane-based SCP holds promise in reducing the environmental footprint of animal agriculture by decreasing greenhouse gas emissions and land use associated with conventional feed production, using natural gas as a carbon source and fossil fuels as an energy source for synthetic protein production is not sustainable. Assuming the below stoichiometric reaction for SCP production (García Martínez et al.), approximately 1.92 mole of methane is required to produce 1 mole of SCP. Tom et al. (2016) reported carbon emissions of approximately 5.82 kg CO_2 equivalent per kilogram of SCP. Of these emissions, 46 % were attributed to waste, 35 % to fossil-driven energy and fugitive emissions, 18.7 % to raw materials, and 0.1 % to the transportation of raw materials.

$$CH_4 + 1.453O_2 + 0.104NH_3 \rightarrow 0.521CH_{1.8}O_{0.5}N_{0.2} + 1.687H_2O + 0.479CO_2$$

A more sustainable alternative is biogas obtained from the anaerobic digestion of organic matter, which can serve as a renewable source of methane for SCP production. However, food wastes and agricultural residues are often underutilized or used primarily for electricity, heat production, or transport fuel. As renewable electricity and heat can be produced more economically through other sustainable routes (solar and wind) in the near future, there is a growing need to explore more sustainable feedstocks for SCP production and develop more profitable business models for existing biogas producers. However, such investigations are limited in literature, especially from technical and sustainability perspectives.

The present study aims to address these knowledge gaps through a *process systems engineering* approach. As shown in Fig. 1, we integrate food waste processing and renewable energy sources into SCP production, eliminating the use of natural gas as a carbon source and proposing an energy self-sufficient process. Our approach involves utilizing the methane content of biogas derived from the anaerobic digestion of food waste (A-100). Since biogas primarily consists of methane, carbon dioxide, and hydrogen sulfide, we treated it through a series of operations (A-200) before cultivating SCP (A-300). Initially, hydrogen sulfide is removed from the biogas through chemical and biological treatments. We then determined the optimal amount of sweet biogas to combust to achieve a 100 % energy self-sufficient process. The remaining sweet biogas is upgraded to synthetic natural gas (SNG). Rather than simply removing and discharging carbon dioxide into the atmosphere, we valorized the carbon dioxide in the biogas via catalytic methanation to produce additional methane. The purified methane is then combined with oxygen, ammonia, and essential minerals and subsequently fermented (A-300) by M. *capsulatus* to produce high-quality SCP, referred to as bio-SCP.

The strategy depicted in Fig. 1 is rigorously modeled in a process simulator, in this case, Aspen Plus to accurately understand the interactions between various sub-systems and to obtain precise material and energy balances. Subsequently, we conducted a discounted cash flow rate of return analysis to assess the cost-effectiveness of bio-SCP production. Key economic indicators, namely the net present value (NPV) and minimum product selling price (MPSP), were used to evaluate economic viability. Various scenarios were analyzed to determine the economic feasibility of bio-SCP under different market conditions and regulatory environments, with the aim of identifying opportunities and challenges for its widespread adoption in the global animal nutrition sector. Additionally, the environmental benefits of bio-SCP production were assessed through a cradle-to-gate life cycle analysis (LCA), focusing on its potential to reduce life cycle impacts compared to SCP derived from fossil fuels. Eleven environmental indicators were evaluated to assess the complete life cycle impact of both bio-SCP and fossil-derived SCP. These environmental considerations are vital for evaluating the

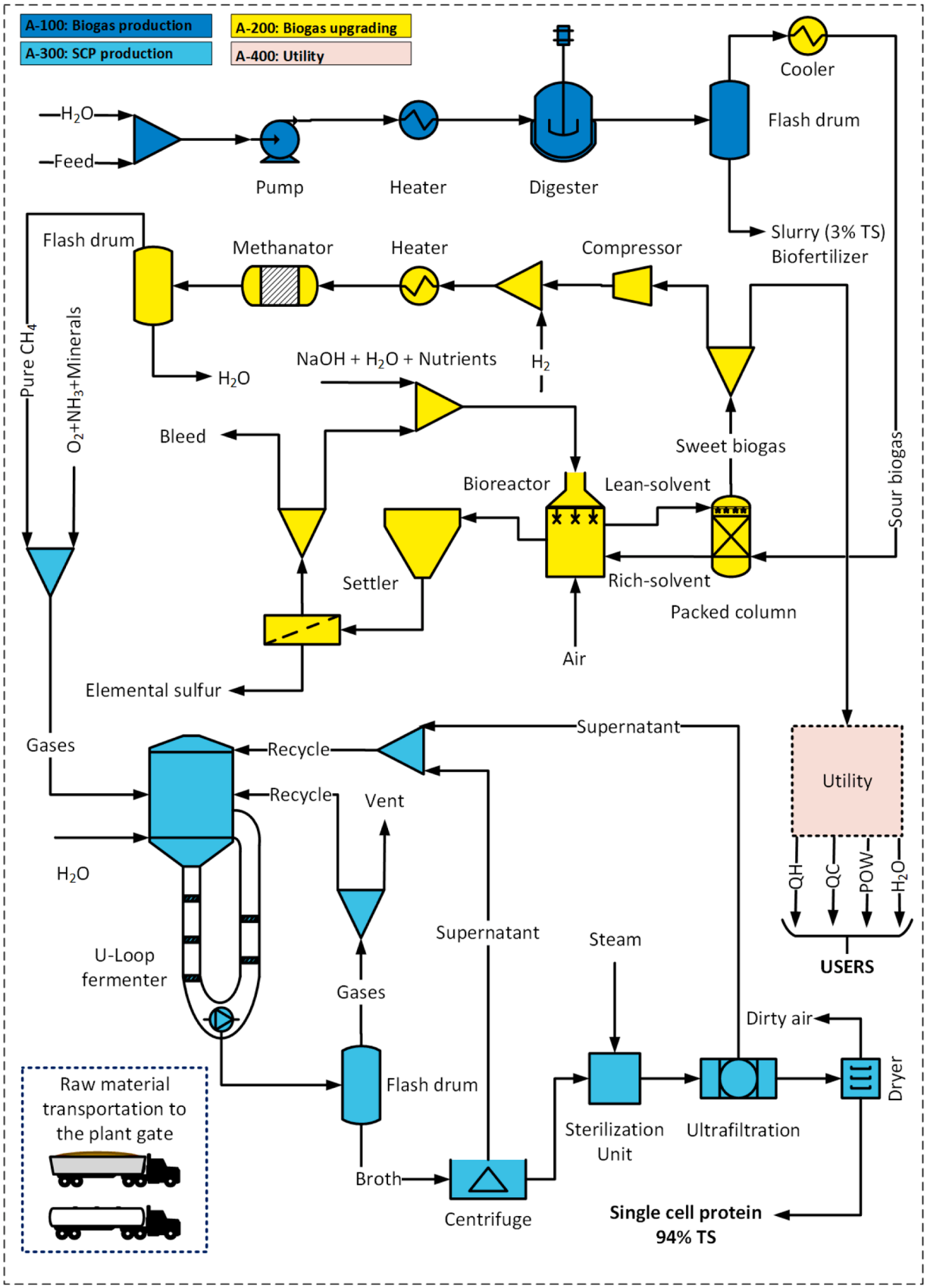

Fig. 2. Simplified process diagram of bio-SCP production. Heaters, coolers, heat integration schemes, and pumps are not shown for simplicity. For comprehensive details, refer to the Aspen Plus flow diagrams in Figures S1-S9 of the supplementary material.

Table 1
Chemical composition (wt.%) of dry food waste, reactions, and their conversions used in the process simulation to model anaerobic digestion. It is assumed that food waste received at the plant gates contains approximately 20 wt.% water. Volatile solids (VS) = Carbohydrates + Protein + Fat + Lignin.

Components	Composition	Conversion	Stoichiometric reaction
Carbohydrate	67.4 %	85 %	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$
Protein	14.6 %	85 %	$C_{13}H_{25}O_7N_3S_1 + 6H_2O \rightarrow 6.5CH_4 + 6.5CO_2 + 3NH_3 + H_2S$
Fat	4.1 %	85 %	$C_{57}H_{104}O_6 + 28H_2O \rightarrow 40CH_4 + 17CO_2$
Lignin	5.9 %	68.03 %	$C_8H_8O_3 + 4{\cdot}5H_2O \rightarrow 4.25CH_4 + 3.75CO_2$
Ash	8.0 %	–	–

overall sustainability of SCP as a protein source and its alignment with global sustainability goals, including the reduction of agricultural emissions and the conservation of natural resources.

2. Methodology

We designed a bio-SCP production process that consumes 25,000 kg/h or 200 Mt/y of food waste on a dry basis. This plant capacity is equivalent to the Solrød Bioenergy process operating in Denmark (Solrød Biogas A/S: Denmark). The simplified process flow diagram of the simulated process is shown in Fig. 2, while the detailed Aspen Plus hierarchical view of the simulated process is shown in **Figure S1** of the supplementary material. These diagram reveals four main sections: biogas production (A-100), biogas upgrading (A-200), SCP production (A-300), and utility (A-400).

2.1. Biogas production (A-100)

The process starts with the anaerobic digestion of food waste. The chemical composition of the food waste, along with the stoichiometric reactions and their assumed conversions used to model anaerobic digestion and calculate biogas yield, are summarized in Table 1 (Edwiges et al., 2018; Achinas and Euverink, 2016). The fraction conversion of these reactions is adjusted to achieve a methane yield of 377.3 ml CH_4/g dry VS (Edwiges et al., 2018). Specifically, an 85 % conversion of carbohydrates, proteins, and fats was assumed. To achieve the reported experimental yield of 377.3 ml CH_4/g dry VS, a lignin conversion of 68.03 % was back calculated.

As depicted in Fig. 2, the food waste is mixed with water to achieve a total solids (TS) concentration of 10 wt.% (Edwiges et al., 2018) and then pumped to the reactor, which operates at 55 °C and 1 atm (Jiang et al., 2018; Anaya Menacho; Rajendran et al., 2014). The reaction temperature is partially achieved by utilizing the heat from the reactor's effluent, with the remaining heat provided by low-pressure steam generated in A-400. Upon completion of fermentation, flash drum separates the biogas from the fermentation broth. The recovered biogas is sent to Area-200 for upgrading, while the fermentation broth at 3 wt.% is cooled and used as biofertilizer, following current practices of biogas plants in Denmark (Solrød Biogas A/S: Denmark). A detailed Aspen Plus model of this section is shown in **Figure S2** of the supplementary material.

2.2. Biogas upgrading (A-200)

The key objectives of this section are to (1) produce sweet biogas by removing hydrogen sulfide, (2) determine the amount of sweet biogas that should be combusted to meet the energy demands of the overall facility, and (3) upgrade the remaining sweet biogas to SNG.

For the biogas sweetening, we implemented the Sulfurex®BR process due to its capability to handle high sulfur loads, achieve high hydrogen sulfide removal efficiencies (up to 98 % under optimal conditions), and suitability for upgrading sweet biogas to fuel quality (DMT Environmental Technology 2025; Campos, 2019). As shown in Fig. 2, Sulfurex®BR integrates chemical desulfurization with biological regeneration. The system comprises a packed column, a biological reactor, and a settler. The packed column captures hydrogen sulfide from the sour gas through chemical scrubbing. The biogas enters at the column's base, while the caustic solution is uniformly distributed over the packing material at the top. As the biogas rises through the packed column, hydrogen sulfide is absorbed by the solvent. Sweet gas is recovered from the column's top, while the saturated liquid accumulates in the sump at the scrubber's bottom and flows under gravity to the bioreactor. The primary objective of the bioreactor is to regenerate the caustic solution for reuse in the scrubber. This is achieved through the biological oxidation of hydrogen sulfide into elemental sulfur using *Thiobacillus* bacteria and oxygen (air). The latter is supplied by a bottom-mounted aeration system. Water, nutrients, and caustic are continuously replenished in the bioreactor to support bacterial growth and maintain operational stability. Finally, elemental sulfur is separated from the process liquid by a settler integrated within the bioreactor. The sulfur sludge, recovered from the settler's bottom, contains a high dry matter content of 5–10 % by mass and serves as a valuable fertilizer.

The sweet biogas from the packed column (absorber) is then divided into two streams: one for generating utilities and the other for upgrading to SNG via methanation. This decision-making task is performed through a design specification that adjusts the biogas flow to the utility section to meet (goal) the total electricity requirements of the entire facility.

The remaining biogas is directed to the methanation subsection, where it is first compressed to 15 atm and mixed with hydrogen gas in a 1:4 molar ratio (Chiang and Hopper, 1983). The mixture is then heated to 320 °C and sent to a plug flow reactor (methanation reactor) (Chiang and Hopper, 1983). In this reactor, the hydrogenation of CO_2 over a nickel catalyst supported on kieselguhr synthesizes SNG according to the Sabatier reaction:

$$CO_2 + 4H_2 \rightleftarrows CH_4 + 2H_2O \ \Delta H - 165.0 \ kJ/mol$$

The reaction conditions and kinetics were obtained from Chiang and Hopper, 1983) with the kinetics defined in Aspen Plus using a power law model. As the Sabatier reaction is exothermic, the heat generated from the methanation process is utilized to produce low-pressure steam in the utility section. Once the reaction is completed, the reactor effluents are cooled by heating the incoming fresh feed and utilizing cooling water. The cooled products are sent to a flash drum to separate methane from water at 50 °C and 1 atm. The recovered SNG is then sent to A-300 for cultivating SCP.

A detailed Aspen Plus models of this section are shown in **Figures S3** and **S4** of the supplementary material.

2.3. SCP production (A-300)

In this study, a process developed by Unibio was used for bio-SCP synthesis (United States Patent 2002). As shown in Fig. 2, the process initiates in the aerobic fermenter known as the U-Loop, where M. *capsulatus* bacteria is utilized as methanotrophic microorganism for utilization of supplied gases at 45 °C and 1 atm (United States Patent 2002). M. *Capsulatus* metabolizes methane into biomass and carbon dioxide. Minerals such as NH_4Cl, $MgSO_4$, and $CaCl_2$ are also supplied to nourish the methane-consuming microorganism. The oxygen required for fermentation is produced onsite through cryogenic air separation. Notably, the air separation unit (ASU) itself is not explicitly simulated in this study; instead, the material and energy balance data are sourced from Morgan's work (Morgan, 2013). The following stoichiometric reaction, with an 85 % fraction conversion of methane, was retrieved from literature to calculate material balance for protein synthesis (García Martínez et al.)

$$CH_4 + 1.453O_2 + 0.104NH_3 \rightarrow 0.521CH_{1.8}O_{0.5}N_{0.2} + 1.687H_2O + 0.479CO_2$$

After fermentation, the gaseous mixture of carbon dioxide, unreacted methane, and oxygen is separated from the protein-rich broth in a flash drum. To minimize methane losses, 85 % of the recovered gases are recycled, while the remaining vented to prevent carbon dioxide accumulation. The protein broth is then concentrated to 15 wt.% TS using a centrifuge (United States Patent 2002). Following this, the concentrated broth undergoes rapid heating in a sterilization unit to approximately 140 °C, followed by quenching to around 70 °C (United States Patent 2002). During sterilization, superheated steam is injected, which reduces the TS to 14.7 % (United States Patent 2002). This process (sterilization and quenching) effectively deactivates the biomass, induces cell lysis, and enhances protein accessibility. The sterilized biomass is further concentrated to 22 wt.% TS through an ultrafiltration unit and finally dried in a spray dryer to achieve a non-dusty agglomerated product at 94 wt.% TS, with a particle size of 150–200 um (United States Patent 2002). This product contains 70.6 % crude protein, 9.8 % crude fat, 7.1 % ash, 0.7 % crude fiber, and 11.8 % nitrogen-free extract (United States Patent 2002). To minimize water consumption and wastewater generation, process water (supernatant) separated by the centrifuge and ultrafiltration unit is recycled, along with makeup water continuously added to maintain the TS of 2–3 % in the fermentation broth (United States Patent 2002).

A detailed Aspen Plus model of this section is shown in **Figure S5** of the supplementary material.

Table 2
Parameters for economic analysis of bio-SCP production and methodology to determine total capital investment and total operating cost. F_i is the flowrate of raw material i estimated from Aspen Plus simulation; and C_i the is cost of raw material i. Note utilities costs are not calculated as the designed process is energy self-sufficient.

Economic parameters (Humbird et al., 2002; Dickson and Liu; Dickson et al., 2018)	**Value**
a. **Assumptions in techno-economic model**	
Analysis year	2024
Plant life (y)	30
Plant operating (h/y)	8000
Rate of interest	10 %
Tax rate per year	35 %
Depreciation method (recovery period)	200 % declining balance (7 years)
Salvage value	0
b. **Methodology for calculating TCI** (Davis et al., 2018)	
x_1, Total installation cost (TIC)	100 %
x_2, Warehouse	4 % of Inside battery limits (ISBL)
x_3, Site development	9 % of ISBL
x_4, Additional piping	4.5 % of ISBL
Total direct costs (TDC)	$\sum_{i=1}^{4} x_i$
y_1, Prorateable costs	10 % of TDC
y_2, Field expenses	10 % of TDC
y_3, Home office & construction fee	20 % of TDC
y_4, Project contingency	10 % of TDC
y_5, Other costs (start-up, permits, etc.)	10 % of TDC
Total indirect costs (TIDC)	$\sum_{i=1}^{5} y_i$
Fixed capital investment (FCI)	TDC + TIDC
Land costs (L)	6 % of installation costs
Working capital (W)	5 % of FCI
TCI	FCI + L + W
Methodology for calculating total operating cost (Turton et al., 2009)	
Operating cost (O_C)	$O_C = V_C + F_c$
Variable costs (V_C)	$\sum_i F_i \times C_i$
Fixed operating costs (F_c)	$L_C + M_c + I_c$
Labour cost (L_C)	1.6 % of TCI
Maintenance cost (M_c)	3.0 % of TCI
Insurance cost (I_C)	0.7 % of FCI

2.4. Utility (A-400)

This section tracks the plant's utility demands, including power, heating, cooling, and process water. A detailed Aspen Plus models of this section are shown in **Figure S6–S9** of the supplementary material.

2.4.1. Power

For power generation, we employed a three-stage stationary gas/combustion turbine specialized for biogas fuel in combined heat and power plants (U. S. Environmental Protection Agency (EPA) 2007). The selected pressure ratio was 20:1. The technical specifications regarding fuel distribution across the stages or the inlet temperature at each stage of the gas turbine are often not available in the literature. However, such information is crucial to ensure the turbine operates at peak efficiency by converting the maximum possible fuel energy into mechanical work. To optimize performance of the gas turbine's performance, the optimization problem is formulated in Aspen Plus as follows:

Objective function:

$$\max P^{NET}, \tag{1}$$

Constraints:

$$T_i^{IN} \leq 1200, \ \forall \ i \ \in I, \tag{2}$$

$$T^{FLUE} \leq 550 \tag{3}$$

$$O_2^{FLUE} \geq 0.15 \tag{4}$$

Variables:

$$0 \leq x_i^{IN} \leq 1, \ \forall \ i \ \in I, \tag{5}$$

$$0 \leq F^{Air} \leq 100, \tag{6}$$

where P^{NET} represents the net power, defined as $P^{Gross} - P^{Turbine}$. T_i^{IN} denotes the inlet temperature at each stage (i) of turbine. T^{FLUE} and O_2^{FLUE} are the exhaust gas temperature and oxygen mass fraction at the outlet of the third stage of the turbine, respectively. x_i^{IN} and F^{Air} are the main decision variables corresponding to fuel distribution split across the stages (i) and air flow rate, respectively. Notable, F^{Air} will be optimized to satisfy Eq. (4). Considering material limitations, efficiency, and operational safety, an upper bound of 1200 °C is set for the inlet temperature at each stage (i) of the turbine (Eq. (2)).

The optimal fuel distribution was determined to be 55.22 %, 20.92 %, and 23.86 %, resulting in turbine inlet temperatures of 877.3 °C, 854.0 °C, and 851.3 °C, respectively, and an exhaust temperature of 524 °C with a flow rate of 58.8 kg/s. The calculated gross electrical power generation is 44 MW, with 60 % consumed by the gas turbine (compressor) itself. This results in a net efficiency of 35.8 % and a heat rate of 10,000 kJ/kWh, aligning well with the technical specifications for such turbines.

2.4.2. Heating

The exhaust gas from the gas turbine is cooled from 524 °C to 120 °C, with the recovered heat initially used to satisfy the heating requirements of high-pressure steam users. The remaining heat is then blended with that from the methanation reactor, which operates at 320 °C. After accounting for a 10 % loss, this combined heat produces low-pressure steam at 130 °C and 1.62 atm. Of this steam, 33.2 % is utilized within the process, and the rest is sold.

2.4.3. Cooling

The cooling tower system is designed to meet the process's cooling requirements by providing water at an inlet temperature of 28 °C and an outlet temperature of 37 °C (Humbird et al., 2002). The evaporation rate is calculated for a temperature decrease from 37 °C to 28 °C. Water

losses in the cooling towers occur through blowdown and windage, with assumed values of 0.006 % of the total flow to the tower and 0.15 % of the water leaving the tower basin, respectively. The power requirement for cryogenic refrigeration in the ASU is obtained from Morgan (2013).

Additionally, this subsection provides water at a constant pressure to the users (A-100 to A-400).

2.5. Process simulation

The process described in Section 2 was simulated using Aspen Plus V.14. Detailed Aspen Plus process flow diagrams, along with material and energy balances, are provided in **Figures S1–S9** and the accompanying Excel file. The simulation incorporated 26 chemical components, 189 material streams, 54 heat streams, and 53 work streams to model bio-SCP production. The activity coefficient models are particularly effective for characterizing liquid-phase behavior in systems involving both polar and non-polar components, such as methane, carbon dioxide, and water. The non-random two-liquid (NRTL) thermodynamic model was used to simulate the non-ideal behavior of the liquid phase, while the Peng-Robinson and STEAM-TA (steam tables) property packages were used to model the power generation and boiler units, respectively (Humbird et al., 2002; Fasahati et al.). Non-condensable gases were treated as Henry components to determine their solubility in water (Fasahati et al., 2017). Design specifications were utilized to ensure the process met all design requirements.

2.6. Techno-economic analysis

We developed a discounted cash flow rate of return analysis model to determine economic viability of bio-SCP production. The MPSP of bio-SCP ($/kg) that results in a NPV of zero was used as the primary economic measure. All techno-economic assumptions, methodology to estimate total capital investment (TCI), and total operating costs are summarized in Table 2.

The total installation cost (TIC) was estimated as follows:

$$TIC = \sum_j C_{j,o} \times \left(\frac{Q_j}{Q_{j,o}}\right)^{a_j} \times I_j \times \left(\frac{CEPCI_{2024}}{CEPCI_{ref}}\right),$$

where $C_{j,o}$ is the cost of the baseline equipment j, a_j is the scaling variable of the equipment j, Q_j and $Q_{j,o}$ are the adjusted and baseline capacities of equipment j, respectively, and $I_{j,o}$ is the installation factor of equipment j. All baseline equipment cost, capacities, scaling variables, and installation factors used to calculate TIC are given in **Table S1** of the supplementary material. Once the TIC of the plant was determined, the factor approach given in Table 2 was used to calculate the TCI.

Table 3
Area-wise breakdown for material and utility utilization/production.

	A-100	A-200	A-300	A-400
Raw materials (kg/h)				
Food waste	25,000.0	–	–	–
Nutrient	–	7.4	–	–
Sodium hydroxide	–	144.8	–	–
Hydrogen	–	1249.4	–	–
Ammonia	–	–	566.2	–
Minerals	–	–	32.0	–
Oxygen	–	–	11,763.4	–
Makeup water	218,702.1	329.2	4435.5	171,083.9
Cooling tower chemicals	–	–	–	1.3
Boiler chemicals	–	–	–	0.1
Waste streams (kg/h)				
Wastewater	–	–	6157.3	1563.2
Dirty air	–	–	455.6	313,961.0
Flue gas	–	–	–	203,210.1
Products (kg/h)				
Slurry	225,169.3	–	–	–
Sulfur	–	1952.3	–	–
Nitrogen	–	–	49,550.7	–
Bio-SCP	–	–	4269.4	–
Low-pressure steam	–	–	–	30,382.6
Utilities (MW)				
Power	1.23	0.99	14.05	1.38
Heating	1.59	0.38	8.99	–
Cooling	6.96	2.64	58.56	–

The total operating cost of the process was calculated as sum of the variable and fixed operating costs. These costs are estimated based on the methodology summarized in Table 2. The unit prices of raw materials and products considered in the analysis are summarized in **Table S2** of the supplementary material.

The non-discounted cash flow, NCF_n for year n, is given by

$$NCF_n = -r_n TCI + a_n W_C + (R - TCOM)(1 - t) + D{\cdot}t,$$

where r_n is the ratio of the TCI consumed during year n, D is the depreciation, and W_C is the working capital. a_n is a parameter equal to -1 during year 3, 1 during the last year of the project, and zero for all other years. Finally, the NPV is defined as

$$NPV = \sum_{n=0}^{20} \frac{NCF_n}{(1+r)^n}.$$

2.7. Life cycle analysis

A cradle-to-gate LCA was conducted to assess the environmental impact of bio-SCP production. The objective was to compare the life cycle impacts of bio-SCP production and fossil-driven SCP production by assessing eleven environmental indicators. The functional unit chosen was one kilogram of SCP. The system boundary, which includes raw material extraction, transportation, conversion at the chemical plant, and infrastructure is depicted in Fig. 2. Life cycle inventory data were derived from the process simulation and scaled according to the functional unit. This inventory (**Table S3 and Table S4**) was then implemented in SimaPro software for analysis. Characterization data were sourced from three databases (**Table S5**) and used for the life cycle impact assessment utilizing the CML-IA baseline V3.03 method.

3. Results and discussion

3.1. Technical results

Overall, the process consumes 25,000 kg/h of food waste by consuming 17.65 MW of electricity, 10.96 MW of heat, and 68.16 MW of cooling. In return, the process generates 225,169.3 kg/h of biofertilizer (3 wt.% TS), 1952.3 kg/h of elemental sulfur (10 wt.% TS), 49,550.7 kg/h of nitrogen, 4269.4 kg/h of bio-SCP, and 30,382.6 kg/h of low-pressure steam.

Results showed that in A-100, about 24,782.8 kg/h or 18,925 Nm^3/h of biogas and 225,169.3 kg/h of slurry with 3 wt.% TS are produced by utilizing 25,000 kg/h of food waste. The slurry is assumed to be transported to farmers (users) as is, without any further processing (Solrød Biogas A/S: Denmark; Fredenslund et al., 2014). The dry molar composition of biogas is calculated to 51.2 % methane, 47.5 % carbon dioxide, 0.8 %, hydrogen sulfide, and 0.5 % ammonia. As shown in, the utility demand of this section is calculated to be 1.23 MW of electricity, 7.90 MW of heating, and 6.96 MW of cooling. About 96.6 % of the required electricity in this section is utilized by agitators. Heating utility required in this section is used for heating the digester feed. About 75 % of the required heating is provided by recovering heat from slurry (biofertilizer) via heat integration. This decreases the temperature of the digester's effluent (slurry) from 55 °C to 30 °C and increases the temperature of the fresh feed entering the digester by preheating it from 25 °C to 49 °C, enabling the recovery of about 6.31 MW of heat duty. Therefore, the net heating requirement of this section is 1.59 MW. The cooling utility required in this section is used to keep the digester at the

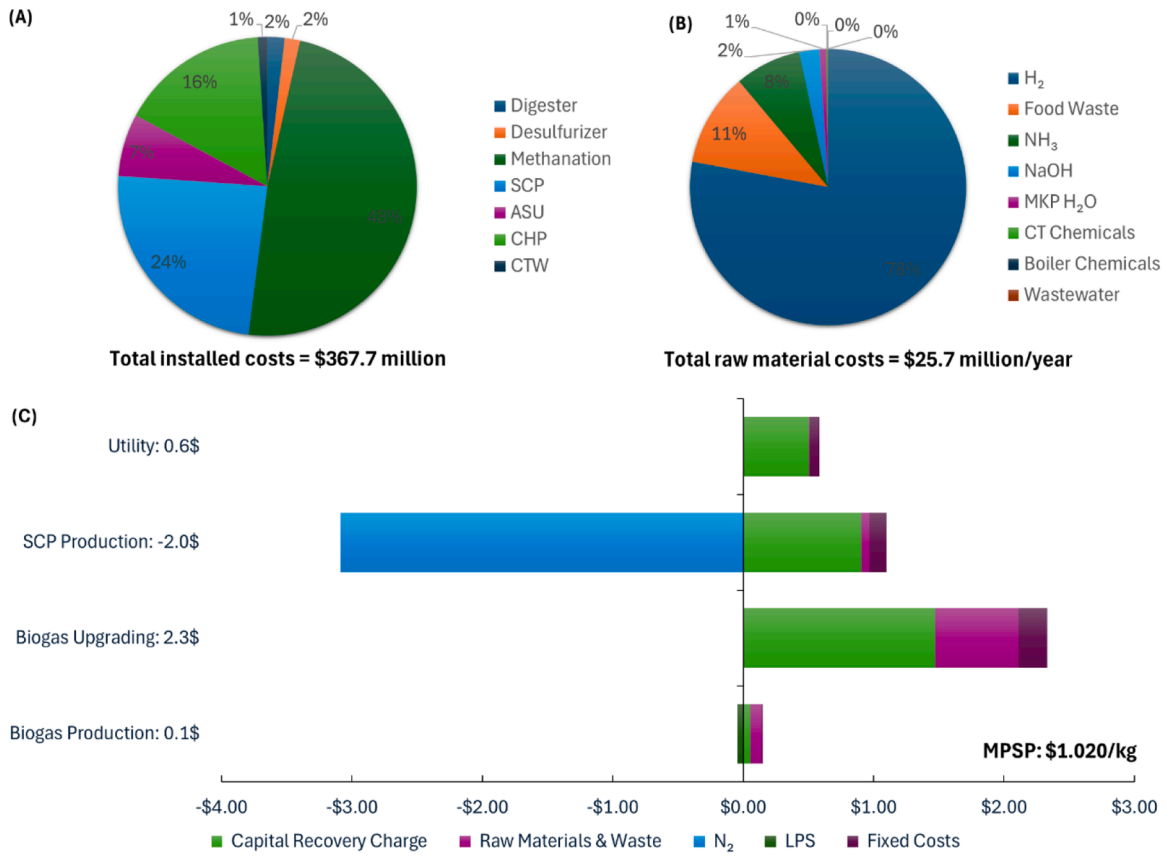

Fig. 3. Summary of the key techno-economic indicators: (A) Total installed cost breakdown; (B) Total raw material cost breakdown; and (C) Minimum product selling price breakdown. Abbreviations used are as follows: SCP (Single-Cell Protein), ASU (Air Separation Unit), CHP (Combined Heat and Power Plant), CTW (Cooling Tower Unit), and CT (Cooling Tower).

desired temperature.

In Area-200, the Sulfurex®BR process effectively reduces the hydrogen sulfide concentration in sour gas from 11,251.2 mg/Nm^3 to 221.1 mg/Nm^3 in the biogas. Approximately 52.1 % of the resulting sweet gas is designated for generating plant utilities, while the remainder undergoes methanation. This process consumes 1249.4 kg/h of hydrogen to produce 5510.5 kg/h of SNG, with a lower heating value of 33.74 MJ/kg. Notably, the Sulfurex®BR process generates 1952.3 kg/h of elemental sulfur at 10 wt.% TS as a byproduct. The electricity demand for this section is 0.99 MW, with 99 % of it utilized to compress biogas from 1 atm to 15 atm for methanation. The total heat demand is 2.28 MW, with 83.3 % met through heat recovery from reactor effluents via heat integration, and the remainder supplied by high-pressure steam generated in the utility section. The total cooling demand is 2.64 MW, allocated as follows: 52 % for cooling biogas, 32 % for inter-stage cooling of the multistage compressor, and the rest for other equipment.

In Area-300, a reaction involving 5510.5 kg/h of methane, 11,763.4 kg/h of oxygen, and 566.2 kg/h of ammonia yields 4269.4 kg/h of bio-SCP at 94 wt.% TS. Additionally, 49,550.7 kg/h of nitrogen with a purity exceeding 99 mol% is produced as a valuable byproduct from the ASU. As shown in Table 3, this section is the most energy-intensive, consuming 14.05 MW of electricity, distributed as follows: 45.7 % for fermenter agitators, 38.2 % for the ASU, and 12.9 % for the dryer's air blower. The cooling demand is calculated at 58.56 MW, with 98.7 % utilized to maintain the digester at the optimal temperature. The heating demand stands at 8.99 MW, with 94.6 % required to heat air to 150 °C for drying the bio-SCP to 94 wt.% TS.

Finally, Area-400 fulfills the utility demands as follows: 17.65 MW of electricity, 10.96 MW of heating, and 68.16 MW of cooling. These needs are met through (1) the combustion of 52.1 % of sweet biogas, (2) heat recovery from turbine exhaust gases and methanation reactor, and (3) the use of cooling water. Additionally, this section supplies 391,200 kg/h of freshwater, of which 55.9 % is used for the anaerobic digestion unit, 31.8 % for the cooling tower, 11.1 % for boiler makeup water, and 1.2 % for other purposes.

3.2. *Techno-economic results*

A techno-economic analysis reveals that a TCI of $733.5 million is required for the erection, commissioning, and start-up of the proposed plant design. This expenditure is distributed as follows: 50.1 % is allocated to total installed costs, 7.6 % to warehousing, site development, and additional piping, 34.6 % to indirect costs (including portable expenses, field expenses, home office and construction fees, project contingencies, and other miscellaneous costs), 3.0 % to land acquisition, and 4.6 % to working capital. As illustrated in Fig. 3**A**, the methanation unit is the most expensive, consuming 48 % of the total installed costs. Following this, the SCP unit, CHP unit, and ASU unit contribute 24 %, 16 %, and 7 %, respectively.

The estimated total annual operating costs for the plant amount to $43.96 million. Of this, 58.3 % is allocated to variable costs (raw materials), and 41.7 % to fixed costs (including labor, maintenance, and property insurance & taxes). As depicted in Fig. 3**B**, the total annual raw material costs are $25.7 million, with hydrogen accounting for 78 %, food waste for 11 %, and ammonia for 8 %.

MPSP is calculated to be $1.02/kg of bio-SCP. Fig. 3**C** provides a detailed breakdown of the MPSP, where positive values indicate economic burdens due to various costs, and negative values represent economic savings from the sale of by-products. The results reveal that biogas upgrading ($2.30) and utility ($0.60) sections are the primary

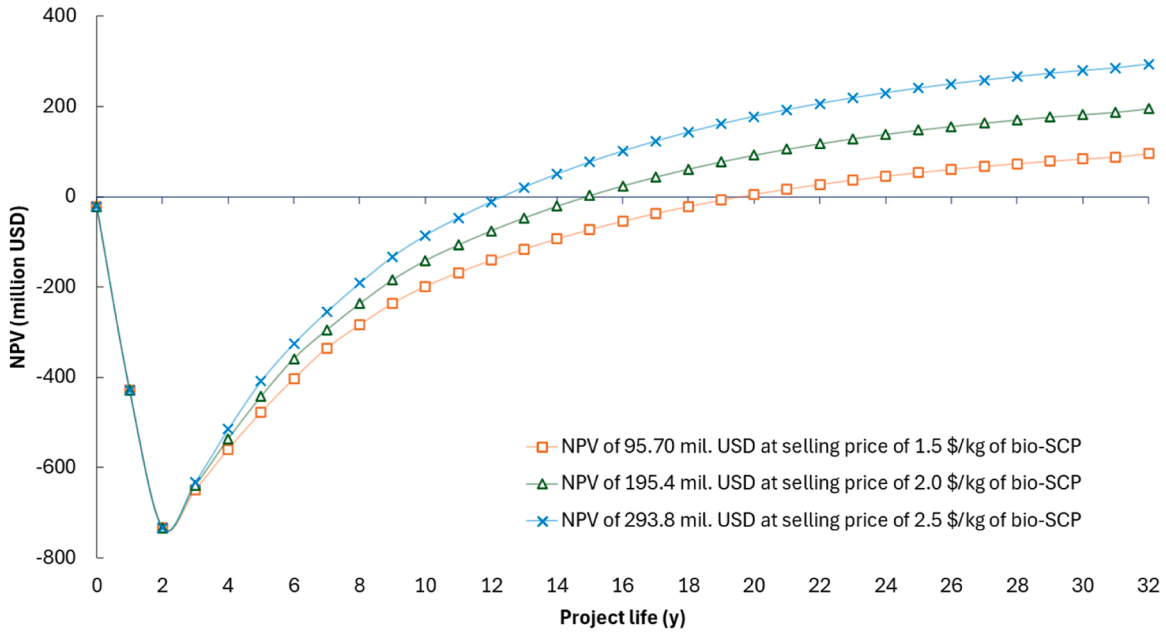

Fig. 4. Net present value as a function of bio-SCP selling price.

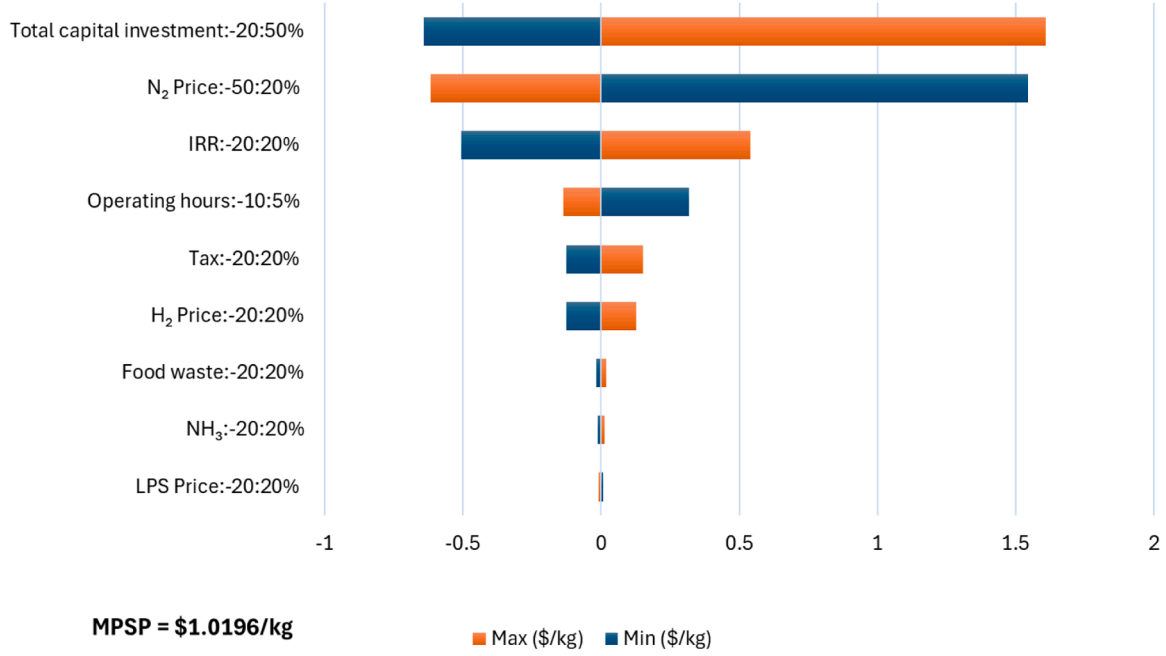

Fig. 5. Sensitivity analysis of process parameters.

contributors to the MPSP. Across all areas, capital recovery charges are the main contributors to the MPSP. The cost of raw materials predominantly impacts the biogas upgrading section compared to other areas.

Significant economic savings (-$3.09) are achieved through the sale of nitrogen, largely due to its high production rate of 49,550.7 kg/h and a market price of $0.25 per kilogram (Nitrogen Pricing Report 2024). The impact of nitrogen's market price on the MPSP is quantified through a sensitivity analysis. Additionally, marginal benefits (-$0.04) are obtained from the sale of low-pressure steam. It is noteworthy that the sales of biofertilizer and elemental sulfur are not included in the economic evaluation, as these products are distributed to farmers (users) free of charge. However, the specific cost of transporting biofertilizer to end users is included in the feedstock purchase price for a more realistic cost estimation. More details are summarized in the footnotes of Table S2.

Fig. 4 illustrates the NPV as a function of bio-SCP selling prices, revealing that the proposed design is highly profitable when bio-SCP is sold at or above $1.50/kg. Specifically, NPVs are calculated to be $98.9 million, $198.5 million, and $296.9 million at bio-SCP prices of $1.50/kg, $2.00/kg, and $2.50/kg, respectively, over a 30-year project lifespan. Given that the market price of SCP typically ranges from $1.00/kg to $3.00/kg, (Voutilainen et al.; Jean and Brown, 2024) these results underscore the exceptional economic viability of the process. This favorable outcome is largely due to the additional revenue generated from selling nitrogen as a value-added product. However, we also explore how a decrease in nitrogen market value could adversely affect the process's economic performance.

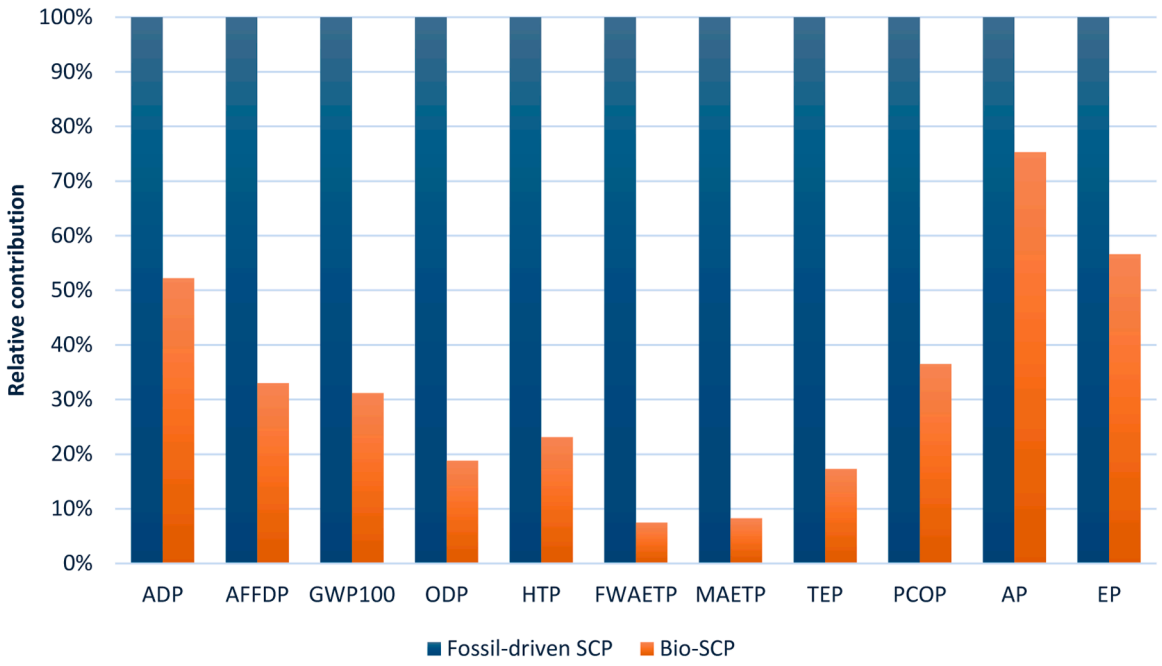

Fig. 6. Characterization results for comparison of life cycle impact assessment for fossil-driven SCP and bio-SCP.

Table 4
Life cycle indicator results of fossil-driven SCP and bio-SCP.

Impact category	Unit	Fossil-driven SCP	Bio-SCP
Abiotic depletion	kg Sb eq	4.50E-06	2.35E-06
Abiotic depletion (fossil fuels)	MJ	96.80	31.93
Global warming	kg CO2 eq	6.47	2.02
Ozone layer depletion	kg CFC-11 eq	7.01E-07	1.32E-07
Human toxicity	kg 1,4-DB eq	1.64	0.38
Fresh water aquatic ecotox.	kg 1,4-DB eq	1.58	0.12
Marine aquatic ecotoxicity	kg 1,4-DB eq	4915.89	405.73
Terrestrial ecotoxicity	kg 1,4-DB eq	0.01	0.001
Photochemical oxidation	kg C2H4 eq	0.0014	0.0005
Acidification	kg SO2 eq	0.03	0.03
Eutrophication	kg PO4— eq	0.01	0.01

Sensitivity analysis was performed on 9 process indicators shown in Fig. 5 along with their variations from the base case value. Results indicate that TCI and nitrogen selling price are the two main factors in determining economic viability of the process. The -20 % to 50 % variation in TCI can vary MPSP between $0.39/kg to $2.64/kg. Likewise, -50 % to 20 % variation in selling price of nitrogen can vary MPSP between $2.58/kg to $0.42/kg. Therefore, strategies to reduce TCI through process intensification and competitive market for nitrogen selling are crucial for economic viability.

3.3. Life cycle assessment

The characterization scores and indicators results for fossil-driven SCP production and bio-SCP production are presented in Fig. 6 and Table 4. Environmental burdens are assessed across the following stages: raw materials extraction, transportation, and plant conversion processes (including biogas production, biogas upgrading, SCP production, and utilities), as well as infrastructure impacts. Notably, this analysis does not account for the environmental savings realized by displacing an equivalent amount of fossil-driven products.

Fig. 6 demonstrates that bio-SCP production surpasses fossil-driven SCP production in all environmental indicators. Table 4 provides detailed comparisons of various environmental potentials between fossil-driven SCP production and bio-SCP production. Specifically, the potentials for abiotic depletion (ADP), abiotic fossil fuels depletion (AFFDP), global warming (GWP100), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FWAETP), marine aquatic ecotoxicity (MAETP), terrestrial ecotoxicity (TEP), photochemical oxidation (PCOP), acidification (AP), and eutrophication (EP) for bio-SCP production are lower by 1.91, 3.03, 3.20, 5.31, 4.32, 13.38, 12.12, 5.78, 2.74, 1.33, and 1.77 times, respectively, compared to fossil-driven SCP production. Therefore, bio-SCP production is the most environmentally friendly option.

Fig. 7 illustrates the lifecycle profiles of bio-SCP production. The results indicate that all environmental indicators, except AFFDP, AP, and EP, are significantly impacted by the SCP production stage. Further analysis reveals that the use of ammonia in SCP production and fugitive methane emissions are the primary contributors to these environmental burdens. Specifically, 66.4 % of the GWP of this stage is attributed to fugitive emissions resulting from SPC production.

In the biogas production stage, the transportation of biofertilizer (3 wt.% TS) is the main source of emissions.

Hydrogen for methanation and sodium hydroxide for the chemical treatment of sour gas are the main environmental burdens in the biogas upgrading stage. Hydrogen accounts for 86.1 % of the GWP of this stage, while the rest is mainly due to fugitive emissions. The ODP of this stage is 58.7 % affected by sodium hydroxide use and 35.4 % due to transportation.

Emissions from the utility and infrastructure stages are found to be minimal.

Overall, the results suggest that reducing methane fugitive emissions, capturing carbon dioxide, replacing chemical treatment of sour biogas with more environmentally friendly technology, and using renewable-driven hydrogen can further improve the lifecycle profile of bio-SCP production. To address these challenges, several potential future research and development directions include:

Integration with Additional Processes: Exploring opportunities to integrate fermentation with other processes to utilize off-gases as substrates for further bioconversion, thereby minimizing waste.

Research on Alternative Fermentation Strains: Investigating alternative microbial strains that may produce higher yields and lower off-gas emissions during fermentation.

Optimized Gas Separation: Implementing advanced gas separation

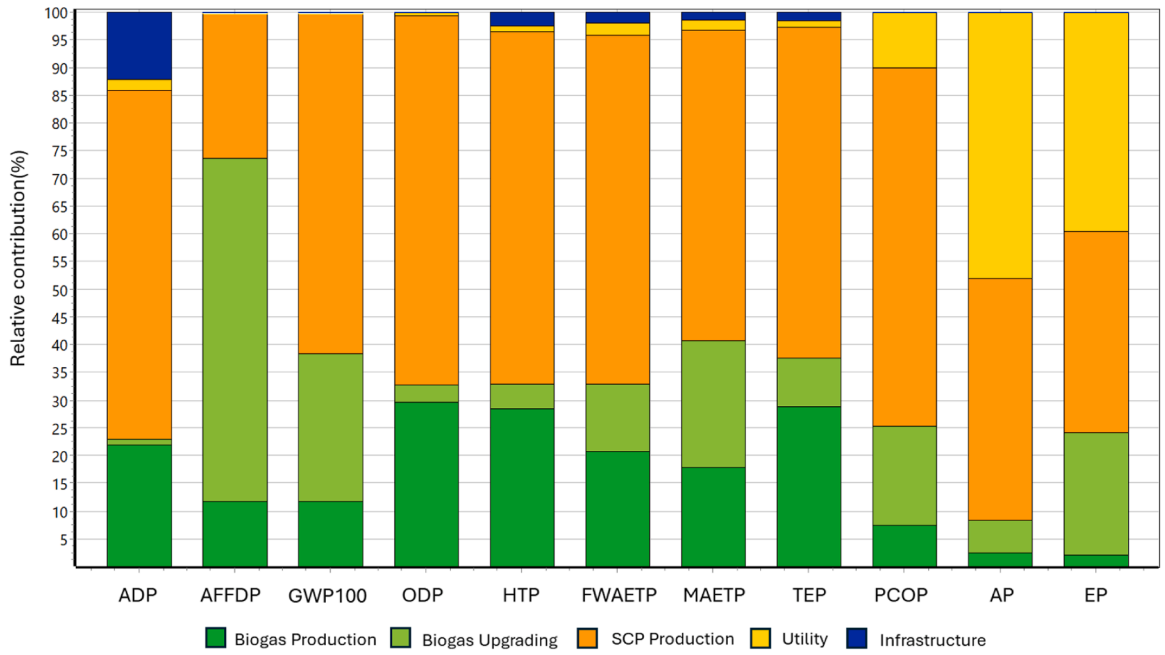

Fig. 7. Lifecycle profiles of bio-SCP production.

technologies to more effectively capture unreacted methane and other valuable components from the off-gases before venting.

Increased Recirculation Rates: Developing systems to increase the recirculation rates of the captured gases, ensuring that a higher percentage is reused in the fermentation process.

Process Monitoring and Control: Employing real-time monitoring and control systems to dynamically adjust operational parameters, allowing for better management of gas flows and reducing emissions.

4. Conclusion

The sustainable production of single-cell proteins (SCP) from food waste offers a transformative solution to the dual challenges of meeting global protein demand and reducing the environmental impact of livestock production. This study demonstrates the feasibility and benefits of using biogas-derived methane as a carbon source for SCP production, referred to as bio-SCP. The comprehensive process design, modeled in Aspen Plus, showcases the integration of food waste processing for biogas production, biogas upgrading, and SCP production through aerobic methane fermentation, resulting in an energy self-sufficient system. The production of 4269.4 kg/h of bio-SCP, along with valuable by-products such as biofertilizer, elemental sulfur, and high-purity nitrogen, underscores the multifunctional advantages of this approach.

The techno-economic analysis underscores the economic viability of the proposed process, with a total capital investment of $733.5 million, annual operating costs of $43.96 million, and a minimum product selling price of $1.02/kg of bio-SCP. The sensitivity analysis reveals that capital investment and nitrogen market price are critical factors influencing economic outcomes. The sale of nitrogen, due to its high production rate, provides substantial economic savings, enhancing the profitability of the process. The life cycle assessment further validates the environmental superiority of bio-SCP production, showing significant reductions in various environmental impact categories compared to fossil-driven SCP production. Key environmental benefits include lower global warming potential, reduced resource depletion, and minimized ecotoxicity impacts.

However, the study also identifies areas for further improvement, such as reducing fugitive methane emissions, enhancing carbon dioxide capture, and adopting renewable-driven hydrogen for methanation. Addressing these challenges can further optimize the environmental performance and economic sustainability of the bio-SCP production.

In conclusion, bio-SCP production offers a promising and sustainable alternative to conventional protein sources. By converting food waste into high-quality protein and valuable by-products, this approach not only mitigates environmental impacts but also supports circular economy principles. The successful commercialization of this technology can significantly contribute to global efforts in achieving sustainable food production and reducing greenhouse gas emissions, aligning with international climate goals and sustainable development objectives.

Data availability

The data supporting this article are included in the Supplementary Information.

CRediT authorship contribution statement

Rofice Dickson: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Seyed Soheil Mansouri:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Seyed Soheil Mansouri reports financial support was provided by Technical University of Denmark. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.compchemeng.2025.109033.

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