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ABSTRACT

The pace at which the human population is growing raises serious concerns related to food security while at the same time conventional agriculture-based food production is becoming a major cause of environmental pollution and greenhouse gas emissions. Numerous solutions have been proposed to boost food production among which edible microbial biomass is considered a promising alternative to conventional sources of food and feed with lower environmental footprint. This work introduces the Neo-Carbon Food concept that is a pilot-scale hybrid biological–inorganic process suitable for the production of microbial biomass. The concept includes integrated hydrogen production by water electrolysis, direct air capture (DAC) of carbon dioxide, and its subsequent assimilation by autotrophic hydrogen-oxidizing bacteria (HOB). The hydrogen production with in situ electrolysis achieved specific energy consumption just below 100 kWh/kgH_2 while the specific energy consumption of DAC was around 20 kWh/kg$_{CO_2}$.

1. Introduction

To achieve the climate mitigation goal of the Paris Agreement, 4–5 Gigatonnes of CO_2 must be stored annually (Dowell et al., 2017). According to IPCC, 23% of total anthropogenic greenhouse gas emissions (2007–2016) derive from Agriculture, Forestry and Other Land Use (AFOLU) (IPCC, 2019). Further, maintaining food security is becoming more challenging due to the growing population, lack of additional arable land and freshwater resources, overfishing, and climate change (FAO, 2017). Additionally, unwanted environmental impacts of the conventional agricultural practices such as loss of biodiversity, eutrophication, and salinization of groundwater resulting from fertilizer runoff, inefficient irrigation, and overuse of pesticides harm the sustainability of food production (Mateo-Sagasta et al., 2017). Because of agriculture, six out of nine planetary boundaries have exceeded safe operational spaces (Campbell et al., 2017). Therefore, net-zero carbon emission society requires net CO_2-free food production, which is less dependent on arable land, weather conditions, etc.

1.1. Microbial protein as an emerging technology

One emerging strategy that might significantly contribute to the decoupling of food production from the conventional agricultural production routes is microbial assimilation of carbon. Diverse microbes, such as bacteria, yeast, algae, and fungi, have been known for years for their ability to produce a microbial protein (MP) also known as single-cell protein (Pikaar et al., 2018). The idea of generating an essential protein for human and animal nutrition using microbes, anywhere renewable energy is available, is astonishing.

Indeed, the production of MP as a meat substitute is regaining attention in recent years (Pikaar et al., 2018; Sillman et al., 2019). An attractive feature of microbial protein-based systems is that the production can be performed in fully controlled, enclosed, and automated fermentation bioreactors showing minimal environmental impact (Sillman et al., 2020). Reactor-based MP production is not dependent on using organic substrates, such as starch or cellulose, does not require the utilization of toxic pesticides to control pests and weeds, emits no phosphorous, and requires a

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limited amount of freshwater inputs. Moreover, the main nutrient ammonia, which is conventionally produced by Haber–Bosch method using fossil fuels, is fully utilized in the closed system (Pikaar et al., 2017). Taking into account that globally the major part of the arable land is currently dedicated to agricultural production of food and feed, the transition towards reactor-based protein production might considerably decrease the environmental pressure. As a consequence, a net positive greenhouse gas emissions from agricultural land use can be diminished drastically while at the same time problems related to deforestation, biodiversity loss, and land-use change would be tackled.

The main carbon source in the process is captured CO_2 which is accessible from anywhere in the world while the main energy source for bacterial growth is H_2 which can be produced through water electrolysis powered by renewable energy. These unique features of the reactor-based process open up the opportunity to launch the production of MP in any geographical location making it almost independent of weather conditions. Furthermore, direct use of captured CO_2 as a feedstock in the process has the potential to make the MP production carbon-neutral (Pikaar et al., 2017; Linder, 2019).

It is worth noting that scaling up the MP production process requires consideration of the important aspects related to assurance of stability of the growing culture, processing of cells to the final product, guaranty of the process safety, and product quality (Pikaar et al., 2017; Linder, 2019). All these requirements have been already successfully achieved and several commercial companies producing food and feed from microbial biomass exist at the market. One of such success stories is the Finnish start-up Solar Foods which uses microbes and CO_2 as a carbon source for the manufacturing of high-protein ingredient branded Solein which will be available in the market in 2021 (Solein, 2019).

1.2. CO_2 assimilation by hydrogen-oxidizing bacteria

Among various microorganisms, autotrophic hydrogen-oxidizing bacteria (HOB) has been pointed as one of the most powerful microbial actuators of the transition towards sustainable food production. The unique feature of HOB enables it to use the chemical energy embedded in hydrogen gas H_2 to assimilate CO_2 and to build new carbonaceous compounds and energy carriers (Yu, 2014). This feature is advantageous as the efficiency of autotrophic growth of HOB is not hindered by the utilization of expensive plant-derived carbon sources, such as sugars or carbohydrates, required for heterotrophic growth (Nangle et al., 2017). The intrinsic premier product of CO_2 assimilation by HOB is biomass which is high in total protein content, has valuable amino acid content, and availability for proteolytic enzymes, and therefore can be considered as a potential protein source and a meat substitute (Volova and Barashkov, 2010).

The concept of CO_2 assimilation by HOB has been successfully proven by different research groups. For instance, Matassa et al. (2016) evaluated the potential of HOB to upgrade NH_3 and CO_2 under autotrophic conditions into biomass with a protein content of approximately 71%. The research group managed to achieve maximum volumetric productivity of 0.41 g of cell dry weight per liter per hour under the continuous operation of the fermentation process. Studies of Yu et al. (2013) revealed the trends affecting the energy efficiency of CO_2 fixation in accordance with the limitation of essential gas substrates required for HOB growth. It was found out that the efficiency of biomass production is substantially affected by the CO_2 concentration. Biomass was produced with a high energy efficiency of up to 50% with moderate O_2 concentrations signifying the possible overall solar-to-biomass conversion efficiency of 5%. Under H_2 limitation, the CO_2 fixation efficiency declined with time. Obviously, the overall process efficiency of CO_2 fixation by HOB is to a great extent dependent on the mass transfer of the main reactant gasses (CO_2, H_2, and O_2) to bacterial cells suspended in the cultivation medium (Yu, 2014). To the best of the authors' knowledge, the maximum value of H_2 conversion efficiency up to 80% in the continuous operation of the fermentation process is reported in (Matassa et al., 2016).

1.3. CO_2 fixation with hybrid biological–inorganic systems

In order to overcome the inherent constraints related to the low solubility of the main reactant gasses, a scalable electricity-driven CO_2 fixation process in the so-called hybrid biological–inorganic (HBI) systems have been developed by Torella et al. (2015). Overall, HBI systems couple biocompatible catalysts to produce H_2 by in situ water splitting and specific microorganisms that use the derived reducing equivalent as an energy source for CO_2 fixation (Nangle et al., 2017). A combination of water electrolysis and CO_2 fixation by HOB in the HBI system may potentially overcome many of the challenges intrinsic to the traditional gas fermentation process relying on the external supply of the reactants. Besides improved gas transfer to the liquid medium HBI process does not experience selectivity problems between organic compounds over a narrow thermodynamic range, does not suffer from difficulties of performing multi-electron reductions for C–C bond formation and its efficiency is not significantly diminished when utilizing air instead of concentrated CO_2 source (Nangle et al., 2017; Liu et al., 2016). Moreover, the HBI process is presumably beneficial over gas fermentation because it allows avoiding the utilization of separate electrolyzer unit and hence decreases the CAPEX while at the same time increasing the safety of the process as the handling of the explosive H_2 and O_2 mixture is not required.

The HBI process has been first tested on a laboratory scale by Torella et al. (2015). They reported the development of an integrated bioelectrochemical system in which water splitting is performed using earth-abundant biocompatible catalysts and HOB to fix CO_2 into biomass and isopropanol with substantial yields of 17.8% and 3.9% of thermodynamic maximum over 24 h, respectively. In the subsequent studies, Liu et al. (2016) managed to improve the HBI process and achieved CO_2 reduction efficiency of approximately 50% when producing bacterial biomass. It is interesting to note, that the CO_2 reduction efficiency decreased only by a factor of 2.7 and reached 20% when using air instead of pure CO_2. However, utilization of concentrated CO_2 might play an important role in the overall HBI process efficiency as it brings practical advantages such as smaller required flow-rate, leading to e.g. less effect on the temperature balance in the bioreactor than by using air as the CO_2 source. Other chemicals, such as polyhydroxybutyrate (PHB) and isopropanol, were produced with the 24-h maximum efficiencies of 42% and 39%, correspondingly. Finally, the calculated solar-to-chemical efficiencies were 9.7% for biomass, 7.6% for bioplastic, and 7.1% for fusel alcohols.

One notable constraint of the HBI process is the incidental formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) or hydroxyl radicals, at the cathode side. At pH = 7 ROS formation is thermodynamically more favorable than H_2 production at and below potentials of hydrogen evolution reaction (HER). ROS are toxic to HOB and hinder its biological growth affecting overall process energy efficiency. To overcome this constraint, huge efforts have been made to develop catalysts selective for H_2 production instead of ROS production (Nangle et al., 2017). Substantial increase in performance has been achieved when using cobalt-phosphorous (Co-P) as a HER catalyst in studies of Liu et al. (2016). Sluggish kinetics of oxygen evolution reaction (OER) in neutral pH represents another challenge for HBI processes.

However, the problem is once again tackled by using efficient earth-abundant catalysts such as cobalt–phosphate (Co–Pi) alloy developed by Kanan et al. (Kanan and Nocera, 2008) and successfully applied as anode coating for HOB cultivation in (Torella et al., 2015; Liu et al., 2016). After phenomenological and proof-of-concept discovery of the HBI process, the scientific focus has shifted towards the objectives of enhanced energy efficiency, product selectivity, process robustness, and scaling up. Up to date the research in the field has mostly concentrated on single electrolytic cells immersed in the cultivation medium, and multiple cell stack structures suitable for industrial-scale production have not been widely studied (Givirovskiy et al., 2019a).

Herein, the Neo-Carbon Food demonstration setup for the pilot-scale HBI process is introduced (LUT University VTT Technical Research Centre of Finland, 2019). So far the production of value-added commodities with the HBI system has been proven on a laboratory scale. Thus, the main objective of this paper is to discuss topics related to scaling up the HBI system. In situ water electrolysis stack is introduced to enable hydrogen production directly in the cultivation medium to overcome relatively low hydrogen utilization rates reported for traditional fermentation. Laboratory scale cultivations show a very high hydrogen utilization rate for the HBI system (Givirovskiy et al., 2019a). Aspects related to the specific energy consumption of in situ electrolysis are studied and discussed in detail including the practical aspects related to combining hydrogen production into the HBI system with biomass. Further, the integration of the direct CO_2 capture from the air to the HBI system is studied. Finally, the specific energy consumption of the in situ water electrolysis and CO_2 capture are reported.

2. Materials and methods

2.1. Medium

The mineral medium used for the bioelectrochemical cultivation of HOB is based on a Leibniz-Institut DSMZ growth medium number 81 (DSMZ, 2011) with the following changes: (i) The major chloride compounds have been replaced with the corresponding sulfates, to minimize the production of chlorine gas by the electrolysis current. The trace element solution SL-6 of Pfennig (1974), used in the medium preparation was left unchanged both for convenience, and because some bacteria might require chloride as a micronutrient especially at high salt concentrations (Roeβler et al., 2006). (ii) The ferric ammonium citrate concentration was lowered to 5 mg/L from the 50 mg/L recommended by DSMZ, to prevent iron precipitation from the medium during even a few days storage. (iii) Na_2SO_4 was added to increase the electrical conductivity of the medium and lower the required electrolysis voltage. The final mineral medium composition is given in Table 1.

The medium has a pH of 7 and a conductivity of 12 mS/cm^2.

Table 1
Composition of the cultivation medium.

Compound	Amount
KH_2PO_4	2.3 g/L
$Na_2HPO_4*2H_2O$	2.9 g/L
Na_2SO_4	5.45 g/L
$(NH_4)_2SO_4$	1.19 g/L
$MgSO_4*7H_2O$	0.5 g/L
$CaSO_4*2H_2O$	11.7 mg/L
$MnSO_4*H_2O$	4.4 mg/L
$NaVO_3$	5 mg/L
$NaHCO_3$	0.5 mg/L
$C_6H_8FeNO_7$	5 mg/L
$ZnSO_4*7H_2O$	0.5 mg/L
H_3BO_3	1.5 mg/L
$CoCl_2*6H_2O$	1 mg/L
$CuCl_2*2H_2O$	0.05 mg/L
$NiCl_2*6H_2O$	0.1 mg/L
$Na_2MoO_4*2H_2O$	0.15 mg/L

2.2. Pilot plant description and operation

The main parts of the studied HBI pilot are: the bioreactor tank, the direct air capture unit of CO_2, and the in situ water electrolyzer as shown in Fig. 1. The image of the pilot unit is depicted in Fig. 2 while the piping and instrument schematic of HBI system with equipment labels and key specification is given in the Appendix. The equivalent information of the direct air capture setup is given in (Bajamundi et al., 2019).

A stainless steel bioreactor tank is one of the core elements of the system used for biomass growth, accumulation, sampling, and process monitoring. The tank is equipped with the 2-blade stirrer (E-6) operated at 900 rpm to ensure completely mixed conditions. Water jacket and Lauda RP855 circulation thermostat were used to maintain the optimal for HOB cultivation temperature of 30°C. A centrifugal vertical multistage pump (P-1) EBARA model EVMSUL with maximum flow rate of 3 m^3/h, controlled by a frequency converter (ABB ACS355), was used to guarantee constant liquid circulation in the system.

At the beginning of the process, the system was flushed with ethanol for sterilization. Subsequently, the fresh medium with the total volume of 20 L and enriched HOB culture was supplied to the bioreactor tank successively using the Flowrox dosing pump (P-5) through the sterile filtering system (E-5). The semi-continuous operation of the system was set by extracting a predefined amount and supplying the same amount of fresh medium to the reactor with an interval defined by the measured optical density. The 1M NH_4OH and 1M H_3PO_4 solutions were used to maintain the pH neutral conditions during the process.

In order to enhance the hydrogen production rate and energy efficiency, compared with simply immersed electrodes, an in situ water electrolysis stack (E-1), shown in Fig. 2 (c) was designed and implemented. The cultivation medium with microbes was pumped through the electrolyzer stack to maximize the H_2 gas utilization. Electrolyzer stack current was supplied and measured in case of serial connection with a Sorensen DLM (40V/15A) laboratory power source and in case of parallel connection with an Aim-TTi QPX1200SP (60V/50A) laboratory power source while the stack cell voltages were measured with a Keithley 2701 data acquisition system.

Flammability of H_2 represents a concern for the safety of any hydrogen containing process. Furthermore, the energy efficiency of the HBI process is dependent on the value of applied current in the electrolyzer stack by means of hydrogen utilization and voltage–current characteristics of the electrolyzer stack. Thus, it is critical to supply current at values satisfying the condition when the amount of H_2 produced is equal to the amount of H_2 consumed by HOB and no excess H_2 is accumulated in the headspace of the bioreactor. To make sure that explosive gas mixture accumulation doesn't happen even when the production of H_2 exceeds the consumption, air purging was implemented to the bioreactor.

The H_2 concentration in the exhaust gas was analyzed using BCP-H_2 (BlueSens) thermal conductivity detector (TCD) equipped with a two-stage drying solution based on a condensing dryer (E-3) followed by silica gel tank (E-4) to prevent moisture ingress from the gas flow into TCD. Electrolyzer stack current had been shifting in accordance with the collected data of H_2 concentration in the exhaust. The H_2 concentration was maintained under the lower explosion limit of 4% during the whole pilot operation. The ambient hydrogen level is monitored with Honeywell Sensepoint XCL gas

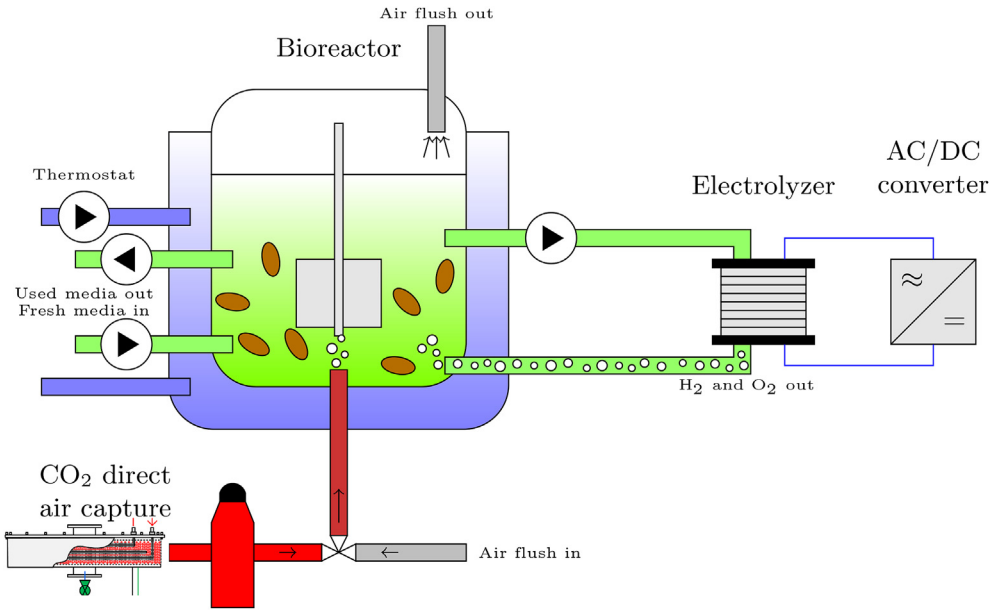

Fig. 1. Schematic diagram of the HBI system with in situ water electrolysis and direct air capture of CO_2.

detector to trigger an alarm and stop operation automatically in case of hydrogen leaks.

The pressure (Tragaf FTP 6.0 A) and temperature (Pt100 RTD + IPAQ C201) transmitters apply 20 mA signal. The pressure and temperature input signals are logged with the EL3058 Beckhoff automation Ethercat module connected to Beckhoff EK9000 Modbus TCP/UDP Bus Coupler. The pH balancing pumps (P-2 and P-3) are controlled over RS-485 (EL6022) and mass flow controllers (MFC-1 and MFC-2) over RS-232 (EL6002) with Beckhoff CX8190 embedded PC communicating with the main automation system over Modbus TCP.

The measurement system control automation of the pilot setup is implemented in a LabVIEW environment. All data is stored online with a 100 ms interval to the LUT measurement database, which can be accessed with the Grafana data observation platform. All the analyses are performed and illustrations plotted with MATLAB software.

2.3. In situ water electrolysis stack

The electrolyzer device was designed as a stack of 2 mm thick electrode plates with zig-zag flow arrangement, shown in Fig. 2(c). The active area of a single electrode is 380 cm^2, and there are ten cells connected in series. So far the electrodes are plain AISI 316L stainless steel, although it has been shown that energy efficiency could be further improved by suitable catalyst coating (Givirovskiy et al., 2019b, 2020). The slit orifices in electrode plates pass the fluid flow between the electrolyzer stages. The orifice locations were chosen based on computational fluid dynamics (CFD) calculations with OpenFOAM software of a few varied cases, so that sufficiently uniform flow velocity distribution over the electrode plates was achieved. The fluid flow velocity distribution is shown in Fig. 3.

Compared with the initial in situ electrolysis stack prototype, described in (Givirovskiy et al., 2019a), the distance between electrodes is decreased to 2.8 mm to minimize conduction losses. The distance is set by using machined spacer rings between the electrode plates. The spacer rings machined of polyacetal (POM) plastic provide also the electrical insulation, carry the preload of the stack assembly, locate the elements in the stack, and electrically isolate the bolts from the electrodes. The electrodes are sealed by planar elastomer seal rings.

Attachment points for electrical connections were laser cut to the electrode plates for electrical connections and monitoring of the individual electrolyzer cell voltages during the test runs. The stainless steel AISI 316L endplates of 10 mm thickness carry the pressure. The design pressure is 8 bar. Welded hose connections are used for the inlet and outlet. The stack is assembled using eight M8 bolts, and the bolt preload is distributed evenly to the stack by the thick washer rings.

The stack arrangement of the electrolyzer device enables reconfigurable arrangement for testing and development of the process, and scalability of the process to industrial scale. In addition, the well-defined alignment of electrode plates allows minimal variation of distance between the electrodes, and thus even electrical current density to the electrolysis process. The measured flow rate in the electrolyzer device was in the range of 0.8 m^3/h-1.3 m^3/h. The flow between the electrode plates is turbulent but close to the laminar condition. Slight turbulence was intended as a design objective to keep the electrode surfaces clean from accumulating biomass and to provide local mixing and slight agitation to the microbes.

2.4. Direct air capture of CO_2

The CO_2 is the carbon source and the main building material for HOB growth. In the present study, CO_2 was captured from air using a DAC unit based on temperature–vacuum swing adsorption. The solid adsorbent in the DAC is a proprietary aminoresin adsorbent. The operation of the DAC unit and the physicochemical characteristics of the adsorbent have been reported earlier in Bajamundi et al. (2019) and Elfving et al., 2017a, 2017b, respectively.

The unit was operated by adsorbing at night taking advantage of lower night temperatures enhancing the adsorption and producing CO_2 during the daytime, such as in the SOLETAIR-project (Vidal et al., 2018). The desorption phase was carried out by using the automated sequence reported earlier (Bajamundi et al., 2019). Typically a 60 min CO_2 desorption time was used for each bed pair, which means the time when the bed pair is subjected to both

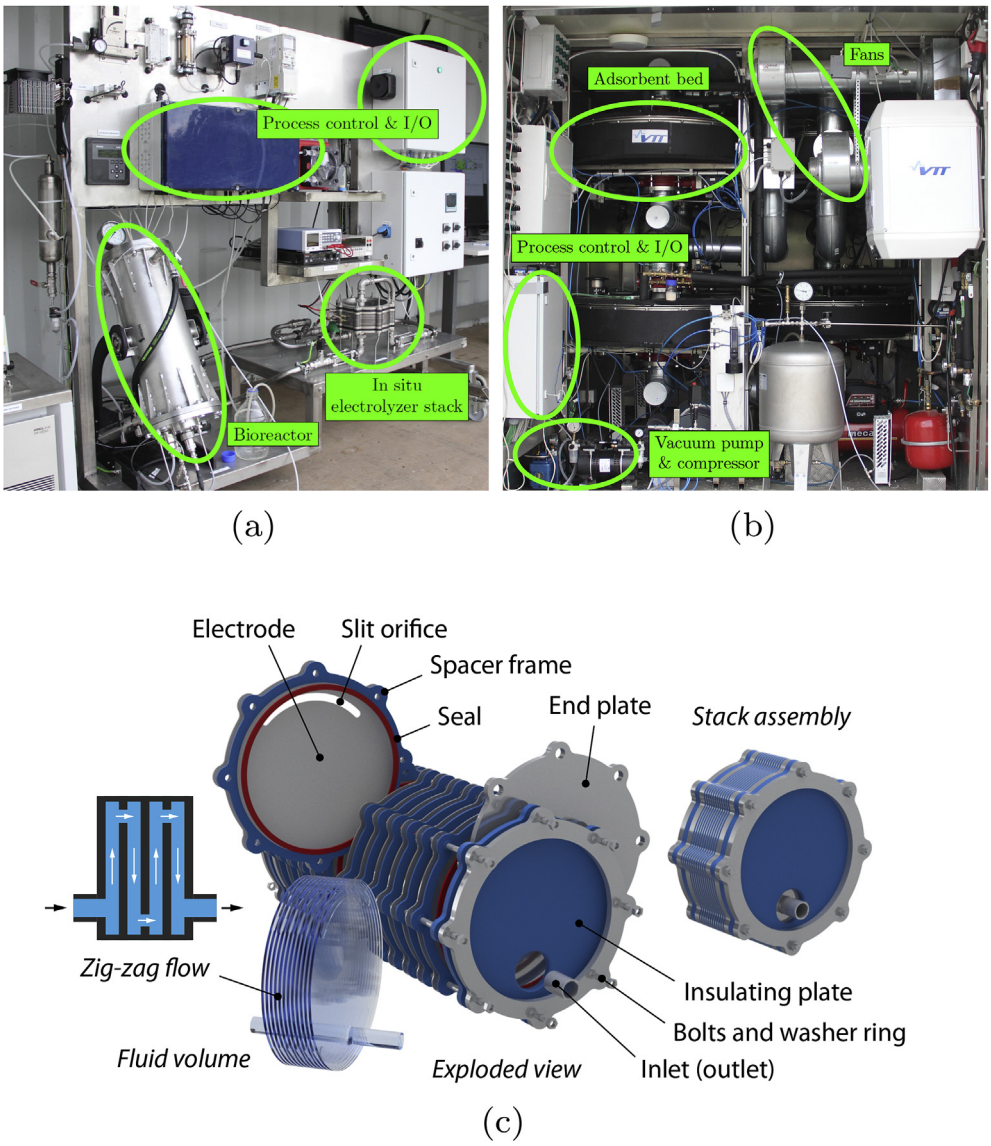

Fig. 2. Neo-Carbon Food setup: (a) Pilot-scale setup for MP production with in situ water electrolysis; (b) Direct air capture unit of CO_2; (c) In situ water electrolysis stack with ten cells in series.

heating at a maximum of 80°C and vacuum. The produced CO_2 gas was stored into an initially empty buffer with volume of 650 L, consisting of one 50 L buffer tank and a 600 L bundle. CO_2 from the buffer was continuously supplied to the bioreactor at a flow rate of 250 mL_n/min and sparged at the bottom of the bioreactor mixed with an airflow of 1 L_n/min.

3. Results and discussion

3.1. Hydrogen production with in situ water electrolysis

The performance of the in situ electrolysis is verified without hydrogen-consuming biomass by measuring the exhaust gas hydrogen content with thermal conductivity detector (TCD) under air flush controlled by a mass flow controller. The test was performed with both serial and parallel connection of the electrolyzer stack cells to compare the performance and especially to detect the amount of stray currents. Measured stack voltages as a function of current are shown in Fig. 4.

Cell voltages are typicaly well above the thermoneutral voltage 1.48 V. Voltage efficiency can be defined by the stack voltage and thermoneutral voltage

$$\eta_U = \frac{U_{tn}\, N_{cell}}{U}, \tag{1}$$

where U_{tn} is the thermoneutral voltage, N_{cell} is the number of cells in series, and U is the stack voltage. The lack of catalyst and the low conductivity of the cultivation medium cause that cell voltages even reach the level of 3 V indicating voltage efficiency to be below 50%. In case of parallel connection all the cells have same voltage, but in case of serial connection the outermost cells have significantly higher voltage than the other cells. This indicates high leakage currents in the serial connected stack. The hydrogen production rate (mol/s) of an electrolyzer stack is linearly proportional to the stack current

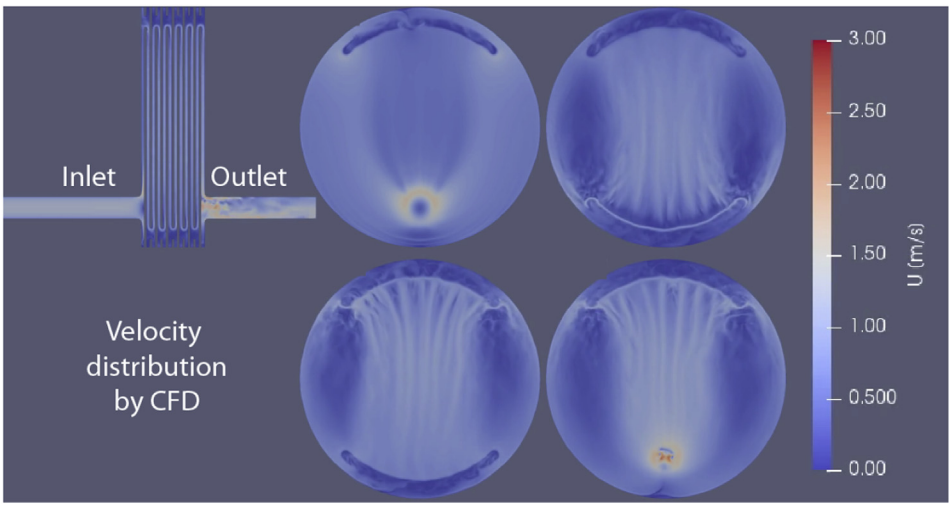

Fig. 3. Fluid flow distribution of the in situ electrolyzer stack analyzed by computational fluid dynamics.

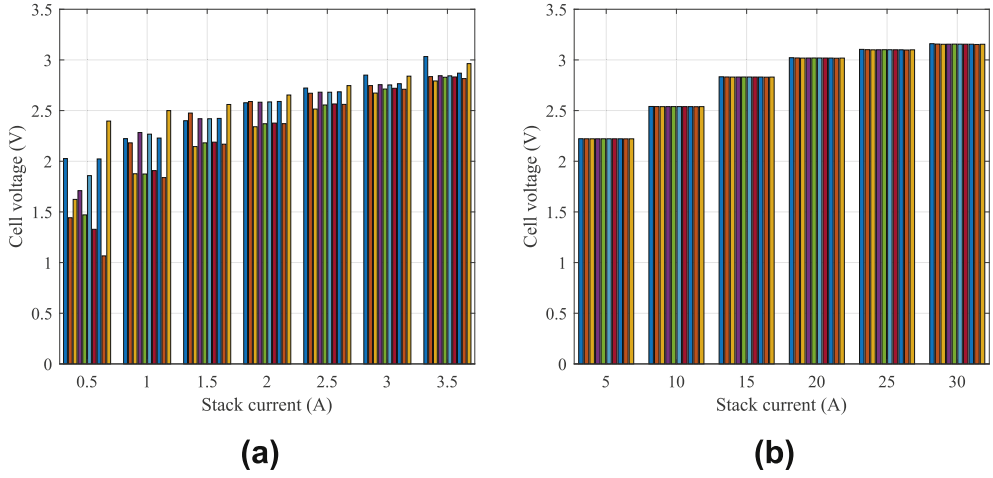

Fig. 4. Individual cell voltages of the in situ water electrolyzer stack as a function of stack current without microbes: (a) Serial connection; (b) Parallel connection.

$$\dot{n}_{H_2} = \eta_F N_{cell} \frac{i}{z F}, \tag{2}$$

where z is the number of moles of electrons transferred in the reaction (for hydrogen, $z = 2$), F is the Faraday constant (9.6485×10^4C/mol), i is the stack current (A), and η_F is the Faraday efficiency, also known as the current efficiency, and N_{cell} is the number of electrolytic cells in series. Faraday efficiency calculated by measured hydrogen flow rate at the exhaust air and stack current is presented in Fig. 5.

In the case of serial connection, the Faraday efficiency is low throughout the current range supporting the assumption of high leakage currents through the flow channels. With a parallel connection, the Faraday efficiency gets values above 80% at currents higher than 15 A. However, the low efficiency at the lowest current levels remains unexplained, but the hydrogen and oxygen gas recombination, other possible side reactions, and measurement inaccuracy are the most probable explanations. As no catalyst is present recombination should be moderate and is not considered as a safety issue in the water solution.

The specific energy consumption E_s of an electrolysis process can be obtained based on the stack voltage, current, and hydrogen production rate

$$E_s = \frac{\int_0^T i(t)\, u(t)\, \mathrm{d}t}{\int_0^T \dot{m}_{H_2}\, \mathrm{d}t}, \tag{3}$$

where T is the time interval under study. The higher heating value (HHV) is the minimum energy required to produce hydrogen gas with a thermoneutral process. The per mass unit HHV of hydrogen gas is 39.4 kWh/kg, which can be assumed to represent the energy consumption of the process with a 100% efficiency. The specific energy consumption of the in situ electrolyzer is given in Fig. 6.

The SEC of the in situ electrolyzer is significantly higher compared with traditional alkaline or PEM electrolyzers, which typically reach values around 50 kWh/kgH_2. The main factors leading to high specific energy consumption even under Faraday efficiencies exceeding 80% are low conductivity of the cultivation medium and the lack of catalyst coating of the electrodes. However, the efficiency of the in situ electrolyzer can be significantly improved by redesigning the stack to avoid stray currents and applying catalytic coatings. On the other hand, even the studied

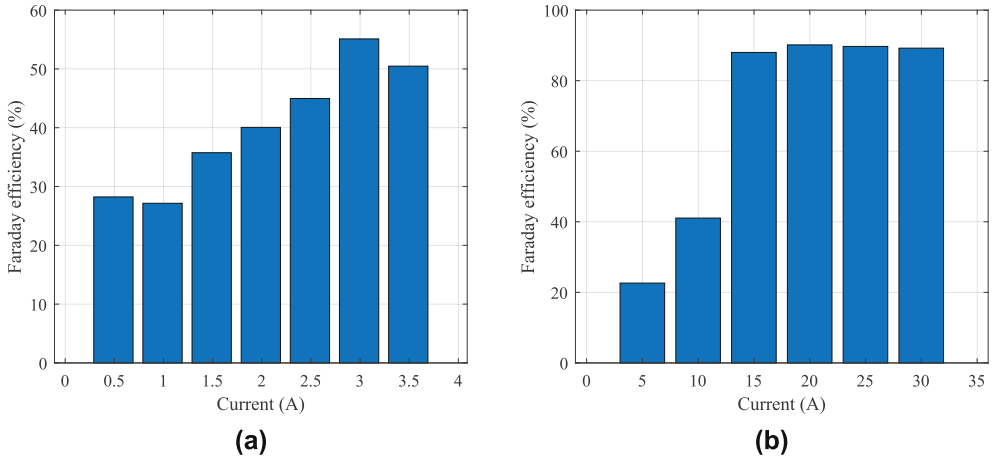

Fig. 5. Faraday efficiencies as a function of current without microbes: (a) Serial connection; (b) Parallel connection.

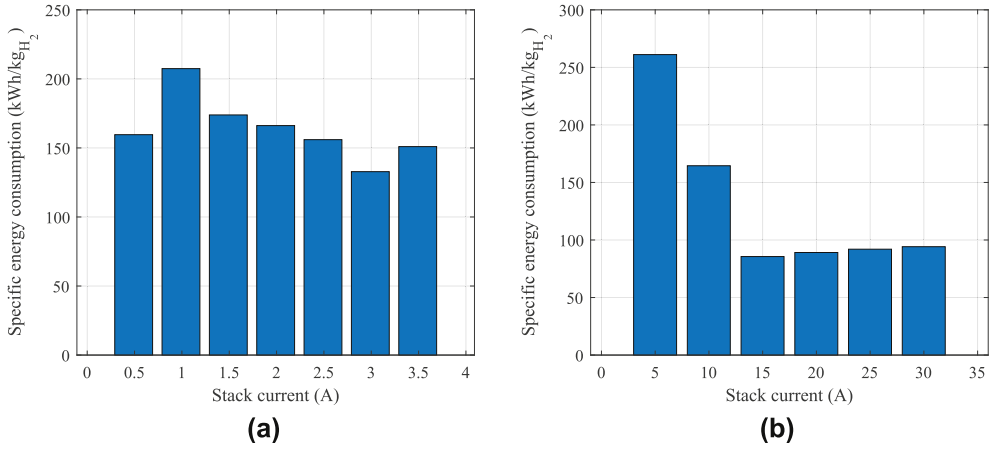

Fig. 6. Specific energy consumption as a function of current without microbes: (a) Serial connection; (b) Parallel connection.

prototype of in situ electrolysis with relatively simple structure and low-cost materials offers a high hydrogen utilization ratio as shown later on. For biomass cultivation campaigns the parallel connection is selected based on the higher efficiency. As scaled up to industrial-scale the parallel connection would require high current with a very low voltage level that is unfavorable for the power supply electronics. Therefore, the stack should be redesigned to minimize the stray currents in case of serial connection.

3.2. Carbon dioxide production

Direct air capture (DAC) is a carbon-negative technology that was first introduced as a process utilizing hydroxide-solutions for chemisorption of CO_2 from air (Jonge et al., 2019; Sanz-Perez et al., 2016). DAC has been reported to be an option for CO_2 enrichment in greenhouses (Rodríguez-Mosqueda et al., 2018), however to the best of authors' knowledge its use in microbial cultivation has not been reported yet. In the present study solid-adsorbent, DAC is used for the HBI cultivation of protein-rich biomass because this type of DAC offers advantages such as small unit size, scalability, and low-temperature demand, often less than 100˚C (Sanz-Perez et al., 2016; Bajamundi et al., 2019). The CO_2 production of the direct air capture unit of 2 kg–3 kg per day (Bajamundi et al., 2019) is well over the approximately 0.49 kg per day (using gas correction factor of 0.74 for CO_2) that was supplied to the bioreactor. Therefore, after the initial filing of the CO_2 buffer, the DAC unit was not operated every day. The specific energy requirements of the DAC were calculated in six typical days of operation and the results are shown in Fig. 7. As it can be clearly seen from Fig. 7 higher produced CO_2 amounts usually also resulted in lower specific energy

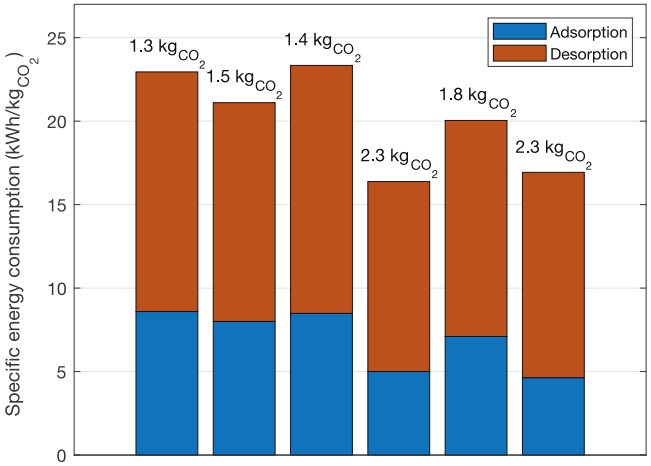

Fig. 7. The specific energy consumption of the DAC unit of six experiments. The produced daily CO_2 amounts are also shown on top of each bar.

consumption (SEC). It is also evident that the contribution of the desorption phase is much higher than the adsorption phase, ranging from 62% up to 73% of the total SEC. The lower amounts of produced CO_2 and higher SEC than reported earlier in (Bajamundi et al., 2019) are partly the result of a shorter desorption phase (60 min vs. 75 min). However, most of the variation can probably be explained with changes in atmospheric conditions.

3.3. Biomass production

The biomass cultivation was tested with several test campaigns. The pilot setup was operated in a semi-continuous mode with periodic extraction of the biomass-rich medium and simultaneous addition of the same amount of approximately 4 L of fresh cultivation medium to the system. The CO_2 captured from the air was continuously sparged from the bottom of the bioreactor through the mineral medium with the experimentally defined flow of 250 mL_n/min while mixed with airflow of $1L_n$/min. In order to saturate the culture with H_2 and O_2, the medium with biomass was pumped through the in situ electrolyzer stack with a constant flow rate of approximately 1 m^3/h over the first test campaign.

The visual comparison of the electrolyzer device opened after several weeks of test runs and the CFD velocity distributions are presented in Fig. 8. The comparison shows a good correlation between the amount of accumulated biomass on the surfaces and the flow velocity distribution determined by CFD.

Further, the electrodeposition of metals from the medium solution onto the surfaces of electrodes and corrosion of the electrode plates at the regions near the edges of the slit orifices was found. The indications of corrosion may be due to the high local flow velocity and abrupt geometry at the slit orifices together with gas mixed or diluted into the fluid, that would favor cavitation. The cavitation induced erosion together with oxygen present in the process may then have been caused the corrosion. A Hitachi S-3400N field-emission scanning electron microscope (SEM) equipped with an energy-dispersive X-ray spectroscopy (EDX) measurements was used to examine the surface of the electrolyzer stack electrodes. It can be clearly seen from Fig. 9 that the surfaces of stainless steel (SS) electrodes after the first run were fully coated. The EDS analysis revealed that most of the attached compounds were metals from the medium solution, such as Mg, Zn, Ni, Fe, Co, and carbon referring to the bacterial biomass. In addition to more powerful pumping with flow rate approximately 1.5 m^3/h, the problem was tackled by switching the polarity every 15 min in the electrolyzer stack with a pair of relays. Electrode material with higher resistance against pitting corrosion should be selected.

The biomass analysis showed heavy contamination proposing most of the biomass to be contaminants instead of the desired HOB. However, most of the hydrogen produced is consumed in the process as the exhaust hydrogen content is much smaller than in the test without microbes with the same current proposing that at least some hydrogen consuming organism is produced. The actual CO_2 fixation cannot be estimated as the CO_2 content of the exhaust gas was not monitored. The most probable reason for contamination is the unsuccessful sterilization of the system despite the sanitation with 2% NaOH and flushing with 70% ethanol at a temperature of 55°C before the autoclaved cultivation medium was added through a sterile filter.

Because of contamination, it is impossible to conduct any analysis of the specific energy consumption of the MP production. In the previous laboratory tests, the hydrogen utilization rate was with in situ electrolysis close to 100%, which is considerably higher than the gaseous hydrogen utilization rate of 80% reported for external hydrogen production (Matassa et al., 2016).

The in situ electrolysis increases the system complexity compared with traditional fermentors making system sterilization more challenging. Furthermore, with in situ electrolysis the proportion of produced hydrogen and oxygen cannot be controlled, which may lead to unfavorable growth conditions. Therefore, the key challenge in the future, in addition to maximizing the hydrogen production energy efficiency, is to avoid contamination.

The production of edible microbial biomass using the HBI process with integrated water electrolysis and biological catalysis is still in the early and developing stage (Nangle et al., 2017). Despite outlined technical challenges and optimization prerequisites, when scaling up a panoptic economical evaluation and life-cycle analysis is required to give a final decision on the viability of the investigating process. Various aspects must be taken into consideration among which are the costs required for renewable electricity for water electrolysis, CO_2 capture costs, expenditures for nutrients and chemicals used for cultivation medium, capital investments, and others. Applied marketing research is of crucial importance to

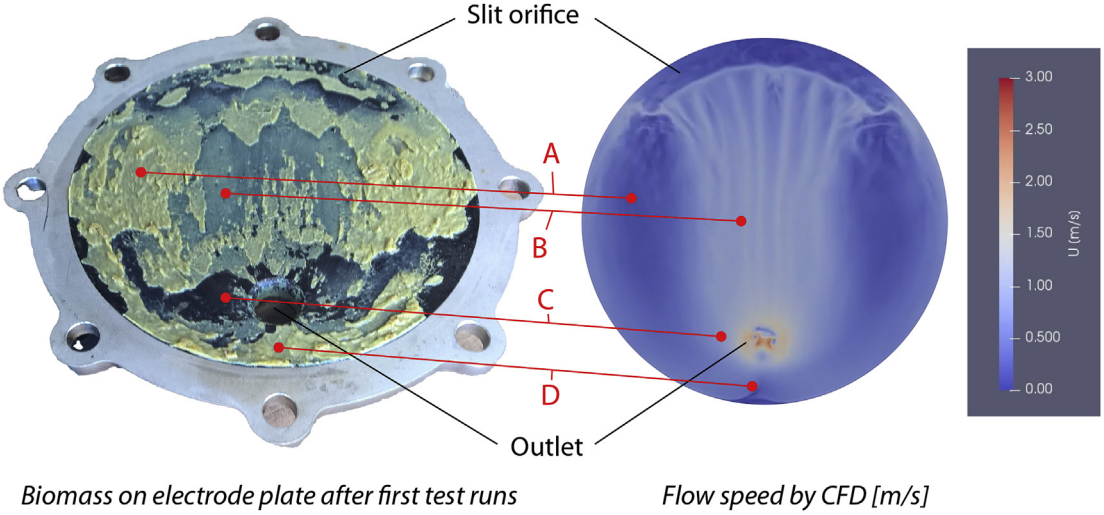

Fig. 8. The amount of accumulated biomass on the electrode plate of the end chamber after first test runs is shown on the left. The visual comparison to the flow velocity distribution by CFD shows thick layer of biomass at areas at low flow velocity (locations A and D), thin layer of biomass at intermediate flow velocity (location B), and clean surface at higher velocity (location C).

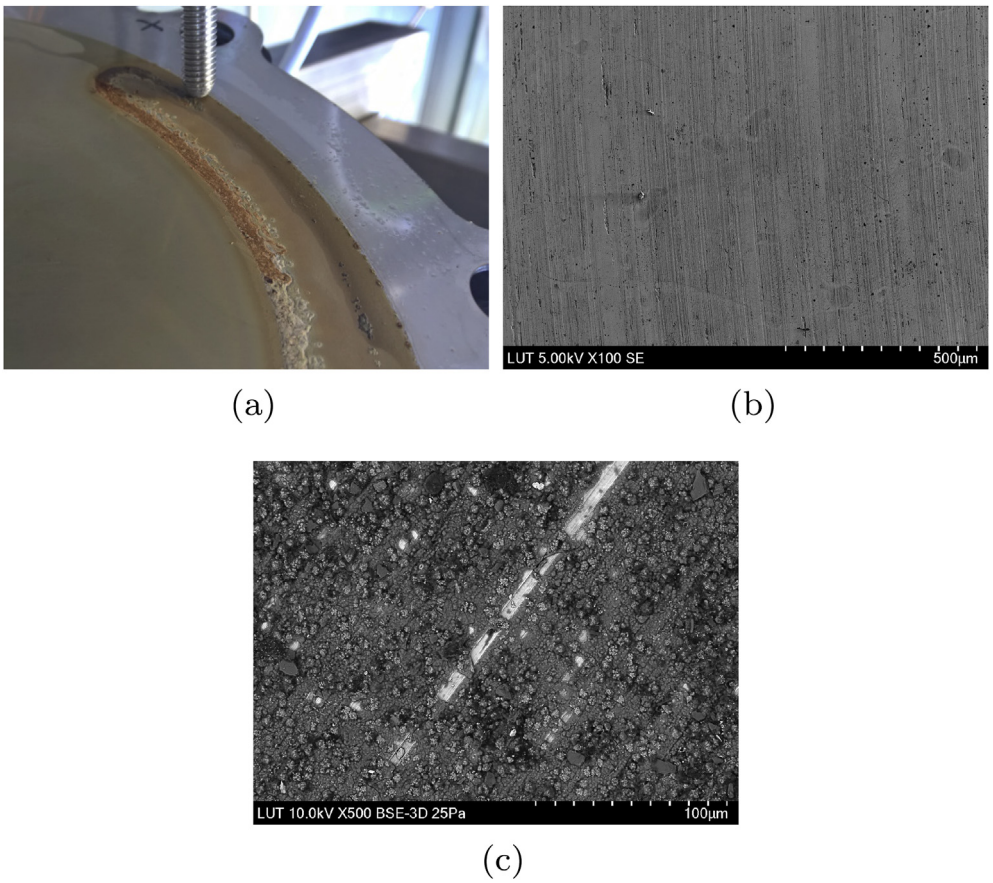

Fig. 9. Stack coating during the first test campaign: (a) Electrode plate coating and corrosion; (b) SEM image of electrode surface (SS) in the beginning of the campaign; (c) SEM image of the coated electrode surface in the end of the campaign.

understand the overall acceptability from regulators and consumers. Regardless, it is already ponderable that the process has significant potential in terms of competing with the purely chemical approaches from CO_2 and provides a viable source of sustainable manufacturing.

4. Conclusions

In the present study, a pilot-scale hybrid biological-inorganic (HBI) process has been introduced to enable sustainable production of microbial protein. The specific energy consumption of in situ water electrolysis and direct air capture of CO_2 have been reported. The major advantage of in situ electrolysis is high hydrogen utilization and no need to handle hydrogen gas in concentrations above lower explosion limit. On the other hand, the efficiency of the hydrogen production is limited and in situ electrolysis also increases the system complexity making the sterilization more challenging compared with external electrolysis.

The key directions of future research will be focused on the: (i) development of microbial culture capable to increase the rate of CO_2 conversion into bacterial biomass; (ii) optimization of electrolyzer stack design in order to mitigate the effect of leakage currents and increase the energy efficiency of in situ water electrolysis; (iii) application of biocompatible earth-abundant electrode coatings and high-surface materials to improve the electrocatalytic efficiency of water splitting and avoid leaching of compounds toxic for bacterial growth; (iv) an extensive economic analysis is essential to evaluate the industrial viability of the HBI production of the protein; (v) assessing whether tailoring the direct air capture process for microbial cultivation can lower the specific energy requirement of produced CO_2, and to support this objective; (vi) studying which is the optimal CO_2 concentration level for maximum growth of the microbial biomass; (vii) studying the optimal current density to maximize the growth. As the test cultivations showed high contamination, the next goal is to achieve a sterile environment for microbial protein growth to further report biomass yield and specific energy consumption of microbial protein production with pilot-scale HBI system. Nevertheless, even now it is possible to conclude that the developed process represents the potential to revolutionize the food and feed production industries, drastically changing their effect on the planet's environment.

CRediT authorship contribution statement

Vesa Ruuskanen: Conceptualization, Methodology, Software, Investigation, Writing - original draft, Project administration, Funding acquisition. **Georgy Givirovskiy:** Conceptualization, Methodology, Investigation, Writing - original draft. **Jere Elfving:** Conceptualization, Methodology, Investigation, Writing - original draft. **Petteri Kokkonen:** Conceptualization, Methodology, Investigation, Writing - original draft. **Aku Karvinen:** Conceptualization, Methodology, Investigation, Writing - original draft. **Lauri Järvinen:** Investigation, Writing - review & editing, Software, Visualization. **Jani Sillman:** Conceptualization, Writing - review & editing. **Miika Vainikka:** Investigation, Writing - review & editing, Visualization. **Jero Ahola:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors 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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Appendix. Piping and instrument diagram with list of equipment

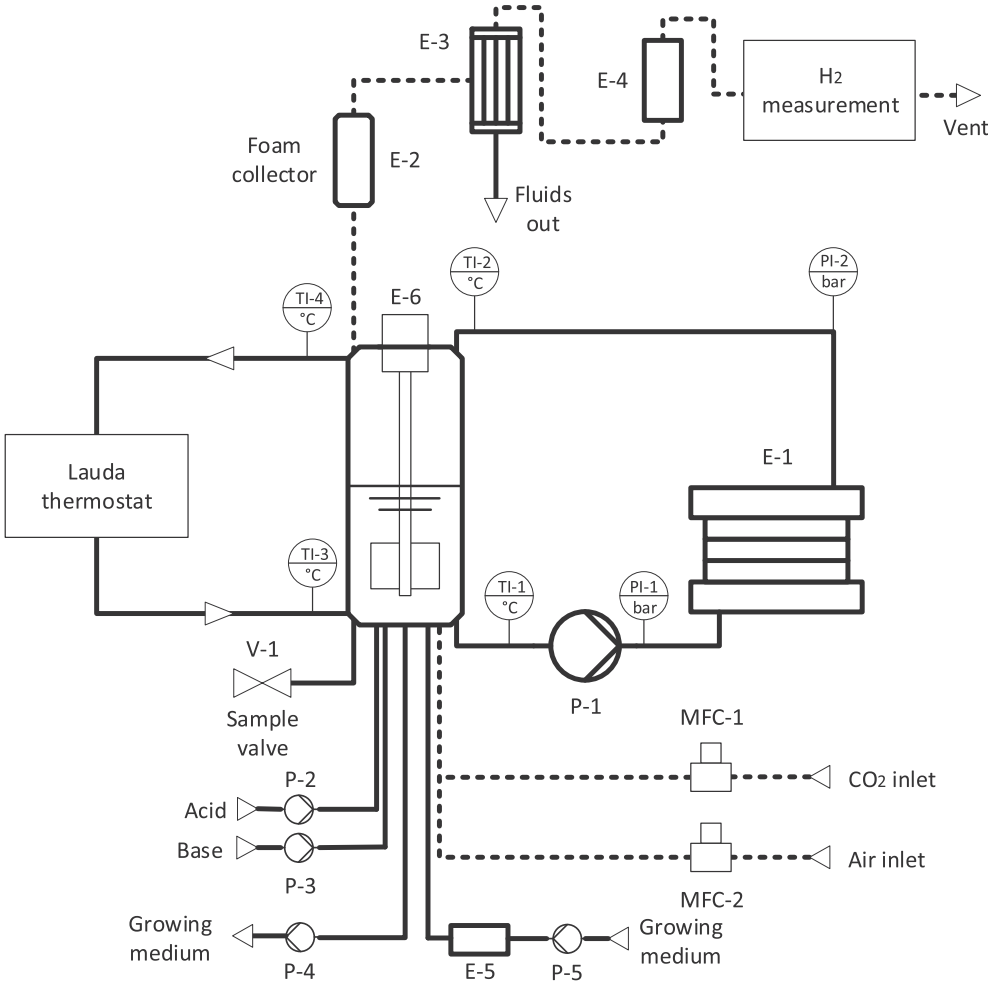

Fig. 10. Piping and instrument diagram of the Neo-Carbon Food setup. Labeled as follows: valves (V), equipment (E), pumps (P) and mass flow controllers (MFC). Gas piping is indicated by dashed lines.

Table 2
List of main equipment with their key specifications. For most equipment the accuracy is given related to reading (RD) and full scale (FS).

Device	Type	Range	Accuracy
Pump P-1	EBARA EVMSUL	0–3 m^3/h	–
Pump P-3	Watson Marlow 400/A	0–30 mL/min	–
Pump P-4	Watson Marlow 400/A	0–30 mL/min	–
Pump P-5	Flowrox LPP-M2-S	0–2.1 L/min	–
Current supply	Aim-TTi QPX1200SP	0–50 A	±0.3% RD ±20 mA
Voltage meas.	Keithley 2701	0–1000 V	±4ppm FS ±10ppm RD
MFC-1 (CO_2)	Bronkhorst MCL-016F	6-300 mL_n/min	±0.1% FS ±0.5% RD
MFC-2 (Air)	Bronkhorst F-201CI	0–5 L/min	±0.1% FS ±0.5% RD
H_2 TCD	BCP-H_2	010%$_{Vol.}$	±0.2% FS ±3% RD
TI-1 … 4	Pt100 RTD + IPAQ C201	0100°C	±0.1°C
PI-1 … 2	Tragaf FTP 6.0	0–6 bar(g)	±0.4% FS
Analog input	Beckhoff EL3058	4–20 mA	±0.3% FS

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